BOOK OF ABSTRACTS

5th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 1–4, 2011
Prague, Czech Republic

Jana Pulkrabová and Monika Tomaniová
Editors




INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

RIKILT WAGENINGEN UR

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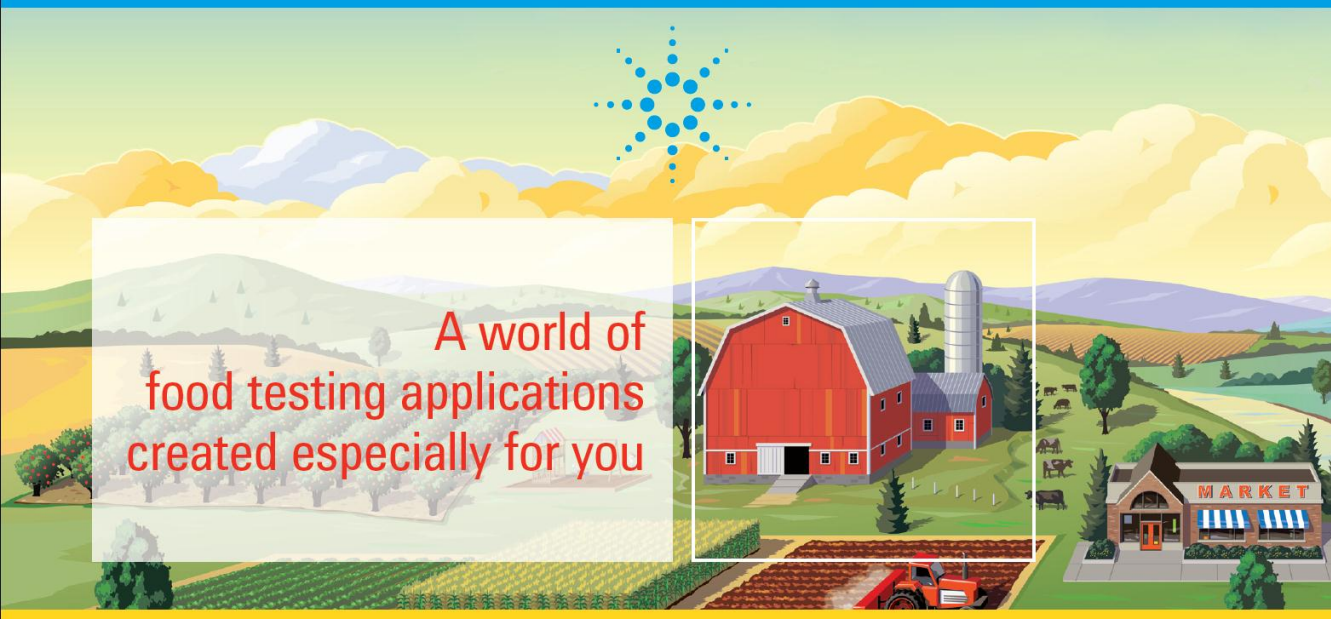

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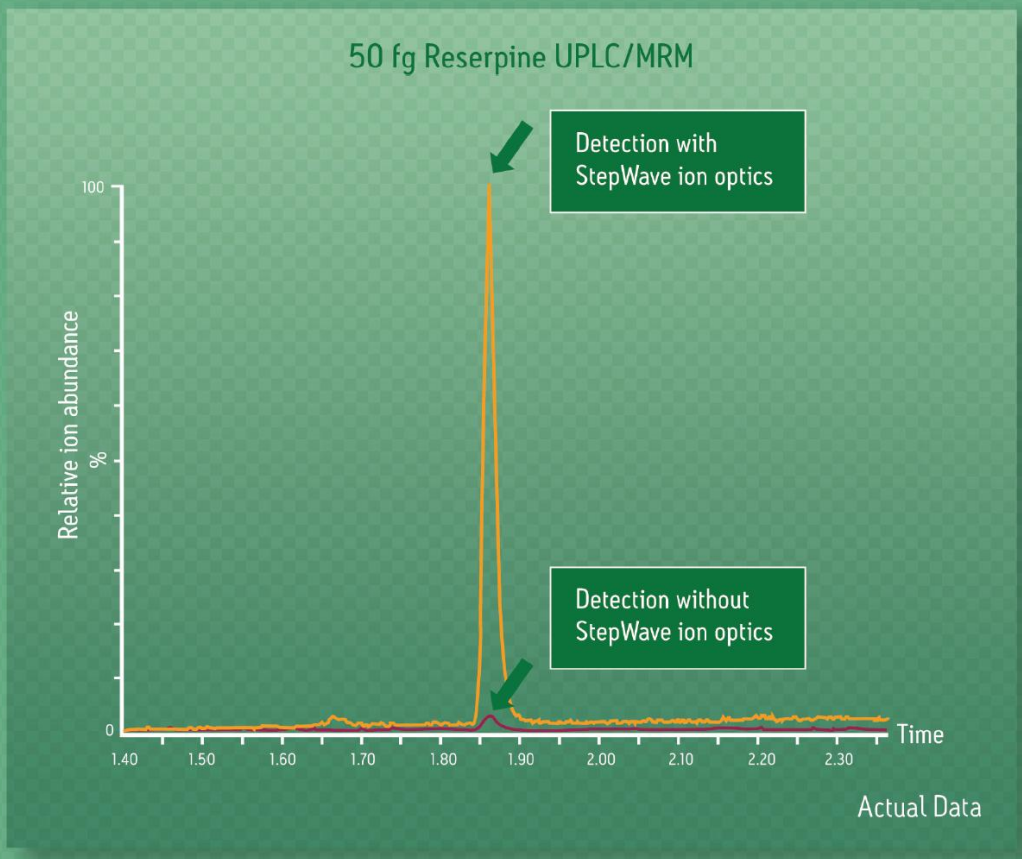

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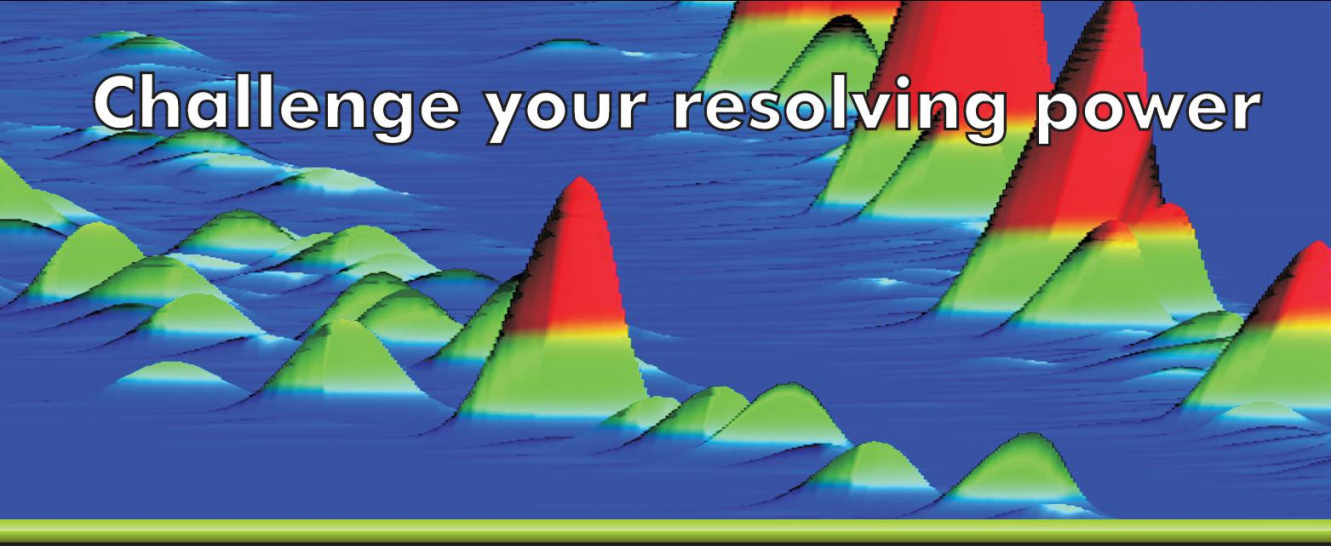

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SEMINARS AND WORKSHOPS

SYMPOSIUM WORKSHOP I, NOVEMBER 1, 2011 (14:00–17:30)

WORKSHOP „YOUNG SCIENTISTS IN EU RESEARCH ACTIVITIES RESEARCH ACTIVITIES AND OPPORTUNITIES FOR COLLABORATION STRENGTHENING“

Registration for the workshop and welcome coffee from 13:30

14:00–14:10 **OPENING AND WELCOME**
***Jana Hajslova**, Institute of Chemical Technology, Prague, Czech Republic*
***Nada Konickova**, Technology Centre AS CR, Prague, Czech Republic*

*Moderator of the workshop: **Franz Ulberth**, EC–JRC–IRMM, Geel, Belgium*

14:10–14:40 **EU RESEARCH IN SUPPORT OF THE KNOWLEDGE BASED BIO-ECONOMY (KBBE)**
***Antonio di Giulio**, EC–DG Research, Brussels, Belgium*

14:40–15:10 **CHALLENGES IN FOOD RESEARCH AND COLLABORATION OPPORTUNITIES OFFERED BY THE JOINT RESEARCH CENTRE (JRC)**
***Franz Ulberth**, EC–JRC–Institute for Reference Materials and Measurements (IRMM), Geel, Belgium*

15:10–16:10 **YOUNG SCIENTISTS´ CAREERS: WHAT ARE THE REQUIREMENTS TO GET EMPLOYED IN ACADEMIA, INDUSTRY AND / OR PUBLIC SECTOR?**
ROUNDTABLE DISCUSSION
*Moderator: **Jana Hajslova**, Institute of Chemical Technology, Prague, Czech Republic*
Panelists representing various sectors:
***Michel Nielen**, Wageningen University, The Netherlands*
***Antonio di Giulio**, Franz Ulberth, European Commission, Belgium*
***Hans-Gerd Janssen**, Unilever, The Netherlands*
***Michele Suman**, Barilla Food Research Labs, Italy*
***Rainer Malisch**, European Union Reference Laboratory (EU–RL), Germany*

16:10–16:30 Coffee break

16:30–16:50 **7TH EU FRAMEWORK PROGRAM – SPECIFIC PROGRAM “PEOPLE” FOR RESEARCHERS´ MOBILITY**
***Petra Perutkova**, Technology centre AS CR, Prague, Czech Republic*

16:50–17:00 **PERSONAL EXPERIENCE OF A YOUNG SCIENTIST: MY MSC AND PHD STUDIES ABROAD**
***Anastasia Meimaridou**, RIKILT–Institute of Food Safety and Wageningen UR, The Netherlands*

17:00–17:20 **PORTAL EURAXESS – A GATEWAY TO RESEARCH CAREER**
***Viktoria Bodnarova**, Euraxess Czech Republic, Prague, Czech Republic*

17:20–17:30 **QUESTIONS / ANSWERS**
CLOSING OF THE WORKSHOP

SYMPOSIUM WORKSHOP II, NOVEMBER 1, 2011 (14:00–17:00)

WORKSHOP ON "INFRARED AND RAMAN SPECTROSCOPY FOR MONITORING OF AGRICULTURAL FOOD AND FEED PRODUCTS" SPONSORED BY FOSS AND BRUKER

Chaired by Pierre DARDENNE and Vincent BAETEN – Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium

The agro-food sector is facing deep and rapid changes. Policy-makers at European and national levels are faced with increasing consumer concerns about food safety and quality issues. These concerns arise in part from previous food safety crises (e.g. dioxin, BSE, melamine) and in part from the health impact of food and feed. Environmental, ethical and animal welfare aspects of agro-food production have also become matters of public concern. Likewise consumers realize that they can make new demands for high quality, healthy and safe food products only if there are methods to assess the compliance to these criteria. The main outcome of these demands is an increased need for appropriate techniques and methods to help producers, retailers and processors to control and to track their products. The agro-food sector is also focused on setting up agricultural production systems that will have a smaller impact on the environment and that will respect specific or traditional practices.

Food safety and quality controls are often performed using reference methods that have limitations. These methods are (i) time-consuming, while the need is for techniques able to give an instantaneous answer; (ii) expensive, while the appropriate safety and quality controls at any crucial step of the food chain require to perform a huge number of analyses; (iii) performed in the laboratory, while the management control has to be at the production level (on-line measurement) or directly at the field level (in-field measurement); (iv) inflexible and single purpose (one method/one parameter), while security and quality control need rapid methods that allow the simultaneous analyses of different compounds; (v) sampling dependent, while the analysis has to be representative of the whole product batch; (vi) not always respectful of the environment (toxic reagents), while the international analytical community looks for minimising the impact of any action on environment or quality of life.

Limitations of reference methods for food safety and quality control have prompted research teams from public centres, universities and private companies to develop new analytical solutions, based on spectroscopic technologies (e.g. fluorescence spectroscopy, near infrared spectroscopy (NIR), mid infrared spectroscopy (MID), Raman spectroscopy). The advantages of spectroscopic techniques are the speed, the ease of use, the reasonable start-up cost, the non-destructiveness and the possibility of on-line or in the field implementation. Spectroscopic methods enable product control at a much higher frequency which will upgrade the food safety and quality control system. The development of robust and flexible spectroscopic instrumentations adapted for on-line/in the field control of the production chain is well suited for the continuous monitoring of processes from raw materials to finished products. Such systems provide real-time analyses with an increased sample throughput. Spectroscopic imaging techniques allow collection of spectroscopic images at single kernel or particle levels. This is of great interest for laboratories that control feed compound or cereals. Other decisive advantages of spectroscopic methods are the ability to determine simultaneously different factors, no use of reagents and reduced sample preparation.

PROGRAM:

Registration for the workshop from 13:30

14:00–14:40 **NIR INFRARED SPECTROSCOPY: 30 YEARS OF EXPERIENCE AT THE SERVICE OF THE FOOD AND FEED SECTORS**
***Pierre Dardenne**, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*

14:40–15:10 **MOLECULAR SPECTROSCOPY TECHNIQUES: TOOLS FOR THE DETECTION OF CONTAMINANTS. SAMPLING AND ANALYTICAL CONSIDERATIONS**
***Vincent Baeten,** Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*

15:10–15:30 Pause

15:30–16:00 **ANALYSIS OF MILK BY NIR, MIR AND RAMAN SPECTROSCOPY: SUCCESS STORIES**
***Ouissam Abbas**, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*

16:00–16:30 **PRESENTATION / DEMO ON NIR/MIR INSTRUMENTATION AND APPLICATIONS**
Foss company

16:30–17:00 **PRESENTATION / DEMO NIR/MIR/RAMAN INSTRUMENTATION AND APPLICATIONS**
Bruker company

VENDOR SEMINAR, NOVEMBER 2, 2011 (13:15–14:15)

INNOVATIVE NOMINAL AND ACCURATE MASS BASED LCMSMS WORKFLOWS AND SOLUTIONS FOR ADVANCED QUALITATIVE AND QUANTITATIVE FOOD ANALYSIS

The Use of LC/MS/MS for the Routine Screening of Food Contaminants Using High Resolution Mass Spectrometry Systems

***Dr. Andre Schreiber**, Food Technical Marketing Manager, AB SCIEX, Canada*

Brief introduction to the TripleTOF 5600 System and the description of workflows, including the software tools (XIC Manager, MarkerView), in the screening of food contaminants will be provided. The quantitation using high resolution in both MS (TOF) and MS/MS (MRMHR) modes will be discussed.

Fighting Background Using Improved Selectivity for Better Quantitation Limits in LC/MS/MS

***Stefanie Kreppenhofer**, Support Specialist, AB SCIEX, Germany*

Detection limits in quantitative LC/MS/MS analysis are often compromised in heavy matrices by isobaric interferences detected in MRM mode. Possible solutions to overcome this limitation include a) high resolution MS, b) multiple steps of MS/MS (MRM^3), or c) Differential Mobility Spectrometry (DMS). SelexION technology based on DMS will be introduced. Examples of all techniques to minimize background will be presented.

Easy Adoption of LC/MS/MS in Routine Food Laboratory

***Brent Lefebvre**, Product Mgr. Food & Environmental, AB SCIEX, Canada*

Cliquid® Software, with its simple four step workflow, and pre-configured iMethod™ Tests are designed to reduce the barriers to adoption of LC/MS/MS in routine laboratory. New iMethods, as well as iDQuant™ Standards Kit for pesticide analysis will be introduced.

Detection of Allergens by LC-MS/MS Using a Multi-Allergen Assay

***Stephen Lock**, Mgr., Applications, AB SCIEX, United Kingdom*

The Codex Alimentarius recommends that eight potential allergens should always be declared on pre-packaged foods: peanuts, tree nuts, eggs, milk, cereals containing gluten, shellfish, fish, and sulphites. Methodology of detection of most of these allergens utilizing LC/MS/MS is presented.

VENDOR SEMINAR, NOVEMBER 2, 2011 (13:15–14:15)

RAPID SCREENING FOR FOOD ADULTERATION AND QUALITY BY DART MS

Ambient mass spectrometry employing a DART ion source for metabolomic fingerprinting/profiling: A powerful tool for beer origin recognition

***Tomas Cajka*, Katerina Riddellova, Monika Tomaniova, Jana Hajslova**
Institute of Chemical Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technicka 3, 166 28 Prague 6, Czech Republic, E-mail: tomas.cajka@vscht.cz

Ambient mass spectrometry (MS) is a rapidly growing area representing an attractive alternative to conventional analytical approaches. Recently introduced ionization techniques, such as direct analysis in real time (DART), desorption electrospray ionization (DESI), or atmospheric pressure solids analysis probe (ASAP), allow direct examination of various types of samples in the open atmosphere and at ground potential. Little or no sample treatment prior to analysis is required. Additionally, time-consuming separation of sample components, which is usually employed by chromatographic methods, can be omitted with ambient MS.
In this presentation, the potential of DART–MS strategy to distinguish beers according to the brand origin will be demonstrated. In a first step, the DART–MS instrumental conditions were optimized to obtain the broadest possible representation of ionizable compounds occurring in beer samples (direct measurement, no sample preparation). In the next step, metabolomic fingerprints/profiles (mass spectra) of a large set of different beer brands (Trappist and non-Trappist specialty beers) were acquired. In the final phase, the experimental data were analyzed using partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), and artificial neural networks with multilayer perceptrons (ANN-MLP) with the aim of distinguishing (i) the beers labeled as Rochefort 8; (ii) a group consisting of Rochefort 6, 8, 10 beers; and (iii) Trappist beers. The data generated by this emerging technique were also compared to those obtained by a “gold standard” represented by solid-phase microextraction (SPME) coupled to GC–TOFMS used for the analysis of beer volatiles.

Acknowledgement: The financial support by the European Commission through the 6th Framework Programme (contract no FP6-FOOD-2004-006942 – TRACE) and the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6046137305) is gratefully acknowledged.

DART mass spectrometry and its coupling with planar chromatography: Identification of flavonoids and phenolic compounds in propolis

***Elena S.Chernetsova*[1,2], Gertrud E. Morlock [1,3]**
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[2] *On leave from Russian Research Center “Kurchatov Institute”, Akademika Kurchatova sq. 1, 123182 Moscow, Russia, and People’s Friendship University of Russia, Miklukho-Maklaya st. 6, 117198 Moscow, Russia*
[3] *Institute of Nutritional Science, Justus-Liebig-University of Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany*

Propolis is a complex product of bees, which has been used in folk medicine for hundreds of years. As flavonoids and phenolic compounds are of primary interest due to their biological activity and positive action on human health, their pattern was investigated in the propolis samples. For the initial screening and identification of marker compounds in the still unknown chemical profile of German propolis sorts, high-performance thin-layer chromatography (HPTLC) was used, and the differentiation between different types of propolis and the assignment of the origin of the propolis samples was performed. Hyphenated techniques, including post-chromatographic derivatization and different couplings of planar chromatography with mass spectrometry, were used for the identification of the components from the characteristic zones of marker

compounds on the plate. The following possibilities of using DART-MS for flavonoids and phenolic marker compounds identification were studied:

1. DART-MS coupled with HPTLC online (analysis directly from the HPTLC plate) or offline (by means of collecting of the extracts from HPTLC zones and further analysis of these extracts), confirmation or identification of propolis components;
2. DART-MS with a benchtop Orbitrap mass analyser, identification of components in selected propolis extracts analysed as liquid samples, as dried spots on a carrier (HPTLC plate), or as extracts from the marker zones of HPTLC plate.

The respective results will be presented and discussed.

Acknowledgements: We are grateful to Irina Scholl for performing the HPTLC experiments during her diploma thesis, to Annette Schroeder and Nadine Kunz (Apicultural State Institute, Stuttgart, Germany) for providing the propolis samples, and to Maciej Bromirski and Olaf Scheibner (Thermo Fisher Scientific, Bremen, Germany) for assistance in analyzing samples using benchtop Orbitrap (Exactive). This work was financially supported by the program Erasmus Mundus Action 2 "IAMONET-RU".

Ultra-Fast DART Screening to the Rescue: Detecting Adulteration of Dietary Supplements and Identifying Residual Pesticides using Direct Analysis in Real Time (DART) High-Resolution Accurate Mass Analysis

***Elizabeth Crawford* and Brian Musselman**
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Herbal supplements ranging from weight loss supplements to natural antioxidant and nutrient supplements are current targets for adulteration and fraud. The need for fast and accurate characterization of herbal supplements, which generally contain complex mixtures of molecules, is growing as the counterfeiting of natural products is becoming more common. Pesticide detection in these consumer products and on the surfaces of fruits and vegetables is also of concern and a rapid screening technique is also needed.
DART mass spectrometry is used as a quick and efficient means of characterization of herbal supplements; to quickly screen and qualify both national and international herbal products for quality and contamination. The next generation DART ionization source, the ID-CUBE™ provides a low cost, simple method of screening with the sample analysis time of 10 seconds per sample. Liquid or solid samples are simply placed on the OpenSpot™ Sample Card; the card is place in the source and heated, producing ions via DART. The operation of this ionization source has been significantly simplified and miniaturized from the current generation DART-SVP ion source making it of greater interest as a tool in a mobile lab setting at border and importation agencies.
Rapid screening of pesticides present in herbal supplements and on the surfaces of fruits and vegetables has also been facilitated by using DART coupled with the high-resolution accurate mass Thermo Exactive mass spectrometer. This screening technique is demonstrated for both gross and residual levels of adulterants and pesticides in consumer commodities.

Evaluating Porous Materials for Sampling Pesticides from Surfaces using Direct Analysis in Real Time (DART)-Mass Spectrometry

Elizabeth Crawford and Brian Musselman
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www.ionsense.com

Rapid screening of pesticides present on the surface of fruits and vegetables has been facilitated by using direct analysis in real time (DART) open air high resolution accurate mass mass spectrometry. These experiments focus on the use of various materials to collect pesticides from large objects including plants and produce commodities by using a vacuum-assisted sampling approach. Evaluation of the efficiency of various polymeric foams, cotton swabs and wire mesh for capture of analytes with and without the use of solvents will also be examined. Suitability of different materials as both sampling and desorption ionization support will be reported.

These experiments build on the original pesticide screening experiments where polyethylene foam was used as both the collection and desorption substrate. Small fruits and nuts were examined for pesticides using "Transmission-mode" DART-MS analysis[1].

1. Edison, S., et al., Rapid Commun. Mass Spectrom., 2011, 25, 127-139

VENDOR SEMINAR, NOVEMBER 2, 2011 (13:15–14:15)

QUALITY ASSURANCE FOR MYCOTOXIN MONITORING IN A HACCP BASED APPROACH – REFERENCE MATERIALS AND PROFICIENCY TESTING

www.r-biopharm.com

Quality Assurance aspects of mycotoxin testing

Ronald Niemeijer [1, 2], ***Sigrid Haas-Lauterbach*** [1], ***Carrie Maune*** [2]
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Mycotoxin testing poses some unique challenges for analytical laboratories. Using solid validated analytical methods are only part of the solution to providing the best analytical results possible. By utilizing some basic tools as part of the overall QA program, laboratories can build additional quality into their systems.

Understanding that sampling contributes more to variability of results than any other component of mycotoxin analysis is a critical concept. This is crucial for raw commodities such as grains. Mycotoxins are not evenly distributed PLUS they may be present at extremely low levels of contamination. Large samples of whole grains must be collected and subsampled properly, the entire probed sample should be ground finely and then mixed well before taking an analytical sample for testing. In addition, the appearance of commodities might be deceiving. Many times great looking grain can have substantial mycotoxin contamination, and likewise products that look like they would likely have substantial mycotoxin contamination my actually not contain much in the way of mycotoxins. A study on single kernels of corn shows this in great detail.

Next, knowledge of the type of samples is crucial. Samples submitted for mycotoxin analysis may be as simple as corn or wheat or as complex as nutraceuticals or complex animals feeds. Knowing your sample will give you insight on potential mycotoxins. For example wheat products are more commonly contaminated with DON and zearalenone and only in some unusual instances are aflatoxin and fumonisin detected. This knowledge can assist with determining which toxins to analyze for especially in samples associated with animal or human health issues. In addition evaluating the matrix that will be analyzed can help determine the best method for the analysis. Many simple matrices such as grains and simple feeds can be analyzed by test kits. In particular in the light of a HACCP based approach rapid test kits can play an important role. We will demonstrate the use of such a rapid test kit, the RIDA® QUICK lateral flow test in combination with the quantitative reader RDA® QUICK SCAN for e.g. testing incoming grains.

Complex matrices typically require analytical methodology such as HPLC, LC/MS or GC and also require multiple steps to remove interferences so that a purified extract with minimal interferences can be utilized for analysis. This will help minimize the possibility of matrix interferences that could be falsely interpreted as the toxin. When unusual matrices are analyzed it is always good practice to analyze a matrix spike to confirm an acceptable toxin recovery through the method. This use of mycotoxin standards to prepare matrix spikes is an excellent tool to measure overall success of the method on an unusual matrix that may not have been specifically validated on any method. In these cases a matrix spike adds an extra quality parameter to the procedure.

Reference materials can serve as a cornerstone to build daily quality assurance data. Utilizing a reference material such as a naturally contaminated grain sample with each sample run provides valuable information about all of the method parameters. When reference materials are used from the extraction step all the way through the method, the reference material provides a complete check on the entire system. It insures extraction was efficient, technician techniques were solid, standards were accurate and instrumentation was running as it should be. Daily documentation of this reference material result can be graphed and used as acceptance criteria for every mycotoxin run.

Technician training and documentation is a critical part of any laboratory and reference materials can also be used as both a training tool and as an ongoing check on analyst capabilities. Reference materials are available in a wide variety of matrices and toxin combinations. Select the combination that most closely reflects the laboratories sample submissions. These reference materials can also be used when method validations need to be completed. These materials are helpful and can provide some real world uncertainty data on methods as they are validated.

Reference materials can be also used in proficiency testing. Trilogy Analytical Laboratory introduced a proficiency testing scheme, Double Check, which is an excellent tool in the quality assurance process of mycotoxin analysis. In this workshop we will demonstrate the use of the Trilogy Double Check proficiency testing.

Quality assurance in a mycotoxin analysis may be more challenging than for other compounds, however with some basic tools mentioned during this workshop the accuracy of the results reported can be assured.

VENDOR SEMINAR, NOVEMBER 2, 2011 (13:15–14:15)

WATERS TODAY. FEATURED: SCIENTIFIC INNOVATION, FOOD AUTHENTICITY, PROFILING & QUANTITATION

Chair: Dr Sandra Rontree, European Headquarters, Waters Corporation

Use of the Xevo TQ-S as applied to the routine and not so routine analysis of animal tissues for residues of veterinary medicines

***Guest speaker: Dr Simon Hird**, FERA, UK*

Even with approved use, it is expected that residues will usually turn up in food supplies. Conditions of use are set to ensure that these residues are not at levels that may cause harm. For many of these compounds, legal action limits/levels in food have been established or their use is banned. Fera delivers two separate UK surveillance programs: the statutory plan covers > 30,000 samples of UK produce each collected for a different analysis, whereas the non-statutory program is much smaller (1400 samples) and focuses mainly on imports. The later requires multiple tests on each sample for which a multi-residue approach is essential.

We have been using three Xevo TQ-S instruments with UPLC to deliver much of the above programs. Case studies will be presented to show how the enhanced sensitivity and additional capabilities is helping us deliver determination of a range of different classes of analytes and many combined as a multi-residue suite.

Profiling of Highly Complex Citrus Juice Samples using UPLC Ion Mobility Time of Flight Mass Spectrometry

***Ramesh Rao**, Director Strategic Marketing, Waters Corporation*

Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties. Such attributes mean many flavonoid-containing plant species may be used as functional foods or phytomedicines. The presence of flavonoids in Citrus juices has attracted attention because of their biological and physiological importance. The use of HPLC-MS and HPLC-MS/MS based methods to profile flavonoids has become more routine. The role of flavonoids compounds as markers is important and is a challenge due to sample complexity. HDMS can provide a route to specific and unambiguous identification. As well as enable the unequivocal distinction of flavonoid isomers.

High definition mass spectrometry has been utilised to profile citrus juice products and this techniques offers some unique advantages to profiling complex mixtures. It is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility (IM) mass spectrometry is a rapid orthogonal gas separation phase technique that technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape and charge, as well as mass. The study undertaken investigates the use of Ion Mobility separation in combination with UPLC (Ultra High Performance Chromatography).

The profiling study undertaken clearly shows the benefits of using HDMS. The results obtained show that isomer/conformational analysis can be performed. It is possible to separate co-eluting analytes and increase peak capacity. This enables single component accurate mass spectra of chromatographic co-eluting components to be obtained. These were used to generate elemental composition information. The enhanced peak capacity enables more information to be extracted from fragmentation studies and the individual MSe fragmentation spectra have been obtained for flavonoid isomers which are co-eluting, from which structural elucidation has been performed.

The enhanced peak capacity brought about by profiling using UPLC HDMS can be visualised rapidly using the MSe data viewer. Co-eluting compounds and unknown isomers can to be resolved rapidly.

VENDOR SEMINAR, NOVEMBER 3, 2011 (7:30–8:30)

INNOVATIVE TOOLS FOR FOOD ANALYSIS WITH HYPHENATED TECHNIQUES

SHIMADZU
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since 1875

Innovative tools for food analysis with hyphenated techniques

***Dr Iva Chvilickova**, Shimadzu Praha o.s.*

Modern food quality and safety requires highly sensitive and flexible analytical instrumentation. This includes hyphenation of technologies like GC×GC(q)MS, MDGCMS and LC×GCMS. Single quadruple MS technology is well established in standard analytical procedures. In GC×GCMS however sampling speed of quadrupole MS systems was too low to provide quantitative analysis over a reasonable mass range adapted to the application. The new Shimadzu GCMS QP2010 Ultra allows 50 spectra (scans) (max 100 spectra/sec, 20 000 amu/sec) per second over a mass range of more than 300 amu which is sufficient for pesticide residue analysis. Each modulated peak has more than 15 data points (scans) which allows precise quantification.

In flavor analysis very often colelutions appear in branches of a chromatogram which favors classical heart cut MDGC analysis. The innovative Shimadzu MDGCMS-2010 allows MS identification in the first and second dimension (flavor profiling). All Method relevant parameters including column dimensions are stored in the method files and regions of the first dimension which has to be separated further are defined as transfer peaks by simple mouse clicks in the stand by (reference chromatogram). No shifts of uncutted peaks are observed due to the unique designed multi deans switch of the MDGCMS-2010.

In pesticide residue analysis of fat containing matrices (fat content larger than 3%) still GPC clean up has to be done prior to GCMS analysis. In off line GPC this need manual operation. In the Shimadzu on line LC-GCMS system all modules are software controlled. Automated sample clean up is therefore easily possible. The system can also be used as a comprehensive LC×GCMS system.

VENDOR SEMINAR, NOVEMBER 3, 2011 (7:30–8:30)

HOW TO DETECT MULTIPLE ANALYTES FROM ONE SAMPLE, INCLUDING ANTIBIOTIC RESIDUES AND BACTERIAL CONTAMINANTS

Part I: How to use your lab resources more efficiently: multi-analyte suspension array affinity assays!

Dr Aldert Bergwerff, Mr Hugh Ballantine Dykes
RnAssays BV | Contact a.bergwerff@rnassays.com | rnassays.com

Part II: Ensure food safety and quality with UNISENSOR rapid, multi-analyte and on-field testing.

Dr Benoit Granier, Mr Olivier Heynen
Unisensor | Contact benoit.granier@unisensor.be | unisensor.be

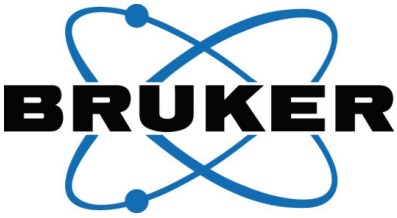
It is long understood that the simultaneous detection of multiple analytes within a single sample in a single analysis run is not only more efficient, but opens new diagnostic approaches as well. Besides reduced analytical costs, less sample handling, fewer sample switching errors and less waste, it allows the profiling of herds, flocks or product batches. For example, declaring a swine herd free from specific (transmissible) diseases and/or free from residues of the most prevalent classes of antibiotics using one identical affordable platform. This is not possible with instrumental high-end techniques such as mass-spectrometry.

The detection of antibiotics in food matrices has been dramatically developed for many years to give users, trust, accuracy and speed. Among the undisputed actors in the field, Unisensor has greatly contributed by providing simple and field tests for rapid detection in milk. *In vitro* approaches have been patented and are today available.

On the other hand, arrays of beads, where each bead can be identified using a flow cytometer, have been produced for the user by RnAssays in such a way that they allow the detection of e.g. *Salmonella enterica* spp., *Trichinella spiralis*, *Toxoplasma gondii*, PRRS virus, *Actinobacillus pleuropneumoniae* serotypes simultaneously. In this modular system, many other pathogens can and will be added to this series. The Residue Plex product line, using the technology developed by Unisensor, targets the simultaneous detection of residues of several families of antibiotics in different analytical matrices. We thus have entered an intriguing next phase in affinity screening testing, providing higher quality data than the conventional assays. Our solutions will set a new standard in assays.

VENDOR SEMINAR: NOVEMBER 3, 2011 (13:15–14:15)

BRUKER – INNOVATION AND TRADITION IN FOOD ANALYSIS



A new complete solution for automated, comprehensive ESI-(Q-)TOF full scan accurate mass screening of pesticides in food with high confidence

***Decker, P.*, Meyer, S**
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Fast and comprehensive full scan accurate mass screening for hundreds of pesticides simultaneously has meanwhile found its way into routine use taking advantage of the high number of possible targets and additionally allowing for unknown evaluation and retrospective analysis. The screening procedure relies on full scan accurate mass data and a target compound database, which basically only needs the information about name (identifier in the result table) and sum formula (accurate mass information) of a target. However, the use of comprehensive databases containing hundreds or even thousands of sum formula/name entries only is anything else than advisable. In the case of pesticide screening in food low signal intensities in highly complex matrix samples have to be evaluated to achieve the required reporting levels, thus leading to a meaningless high number of false positive results. Inclusion of additional information and knowledge therefore is essential to obtain reliable results. The screening solution presented here makes use of multiple levels of confirmation and result rating for maximum confidence in the results. Examples for the workflow and system performance will be given.

Matrix matched standards reveal matrix MRM interferences and minimise false results in pesticide residue analysis of grains and pulses

***Patrick Jeanville*[1], Bruce Peebles[2,3], Katherine Rousetty[2,3], Felician Muntean[1]; Steven Schachterle[1]; Chris Kellog[1], Robert Trengove[2,3]**
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GCMS based analysis of pesticide residues in grains often results in enhanced response and the degree of enhancement is dependent on the type of grain. Some grain matrices cause matrix interference leading to false positives and false negatives, particularly when published MRMs are used without validation.

In this presentation, pesticide and matrix combinations will be shown that demonstrate the failure to accurately monitor particular pesticides in that matrix. The ideal combination of pesticide and matrix where the matrix background shows no interferences, and the combination where the matrix causes false positives or significant limitations in limits of detection and quantification will be discussed and illustrated. In extreme cases the matrix may completely supress the detection of some pesticides due to irreversible binding by matrix components. Published MRMs should be used only after they have been validated to be free from matrix interference in the matrix system under study.

Using GC-QQQ-MS, this approach has been tested in wheat, barley, oats, chick peas, canola and soybeans after a modified QuEChERS extractuion. Some examples of "problem pesticides" in each matrix will be presented.

Use and Qualification of TXRF for Trace Element Analysis of Dietary Supplements and Nutrients

Armin Gross
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Total reflection X-ray fluorescence (TXRF) analysis has proven its capabilities in many different fields. The major advantages of the method are that it allows simultaneous multi-element detection and quantification down to the ppb range, using a highly sensitive spectrometer as the unique Bruker S2 PICOFOX is. Additionally, TXRF analysis shows no matrix effects, requires minimal sample amounts and almost no preparation effort.

The first part of the presentation focuses on the use of TXRF as a tool for investigating product adulteration issues during the food production processes as well as for qualifying phytochemical standards used in the dietary supplement industry. Real-world examples of how TXRF is used to solve quality issues with phytochemical reference standards, identify unknowns and analyze limited sample amounts will be covered.

The second part describes the analysis of liquid nutritional products (LNP). LNP are nutritionally supplemented for patients who are recovering from illness, injury or surgery and for people at risk of malnutrition. Mineral analysis is of crucial importance for product compliance and quality control. Here, TXRF spectroscopy is clearly identified as a suitable and rapid method for the multi-element quantification including macro and micro-nutrients.

NMR-Based Food Quality Screening

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Based on the metabolomics approach, 1H-Nuclear Magnetic Resonance (1H-NMR) screening has rapidly expanded in recent years in the area of food quality control. Indeed, 1H-NMR screening is a fast, multiparametric method, requiring only a small amount of sample and minimal sample preparation.

1H-NMR is a global, non-targeted approach allowing the acquisition of spectral fingerprints. The reliability and reproducibility of 1H-NMR, makes it a technique of choice for profiling of samples, by allowing the creation of statistical models based on authentic reference samples.

This non-targeted method allows the evaluation of numerous parameters linked to quality and authenticity, in only one measurement. Quantification of multiple relevant compounds, as well as classification and verification of the samples is done within minutes. This allows not only to assess the authenticity of the samples but also to detect unknown frauds that would not be detected by conventional targeted approaches.

The full automation of the measurement, including automated data analysis report generation, allows high-throughput analysis and consequently low costs per measurement. A further advantage is that the direct quantification with NMR does not require the use of internal standards.

The achievements of NMR-based screening of fruit juices and the different parameters evaluated will be discussed in detail. Validation results of the method will also be shown. In particular, comparison of NMR quantification results to official methods as well as results of proficiency testing with FAPAS® will be discussed.

Based on the experience on fruit juices, similar screening methods are under development for other food products like wine, edible oil and honey.

In conclusion, NMR is a very cost and time effective method for the simultaneous evaluation of many quality parameters in food.

VENDOR SEMINAR, NOVEMBER 3, 2011 (13:15–14:15)

HIGH-END SOLUTIONS FOR YOUR FOOD ANALYSIS CHALLENGES: SAMPLE PREP – SEPARATION – MS DETECTION

New superfast AND high-resolution LECO TOF MS instrument line: No compromise anymore

Jitka Zrostlíková, Tomáš Kovalczuk, et al.
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Recently introduced and Pittcon 2011 gold-awarded LECO's Folded Flight Path™ (FFP™) TOF-MS technology enables mass resolution of 100,000 FWHM, mass accuracy <1 ppm, and acquisition rates of up to 200 spectra/second - all with fine isotopic abundance measurements to facilitate high-confidence analyte identification. Applications from the field of pesticides and other food contaminants analysis will be shown in this presentation.

High Quality Analysis of Pesticides in Marijuana for Medicine using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, Amanda Rigdon, Frank Dorman
Restek Corporation, 110 Benner Circle, Bellefonte, PA, USA, E-mail: Jack.Cochran@restek.com
The Pennsylvania State University, University Park, PA

At least 17 states in the USA have legalized medical marijuana. Therapeutic benefits include pain relief, control of nausea and vomiting, stimulating appetite, and muscle relaxation. Unfortunately, patients still have no assurances on the safety of the medicine due to potential harmful levels of pesticide residues, and currently, no methods for analysis of these residues in marijuana exist. We used the Quick-Easy-Cheap-Effective-Rugged-Safe (QuEChERS) extraction approach for pesticides in marijuana.

The resulting complex extract required cartridge solid phase extraction cleanup, followed by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). Good recoveries were obtained for the developed method for most pesticides (over 90 were examined), and incurred pesticide residues were detected in some of the illicit marijuana samples used for the work.

Dynamic Headspace - A Powerful Tool for Flavour and Fragrance Analysis

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Accurate qualitative and quantitative analysis of perfumed or flavoured products is essential to the flavour and fragrance industry. Especially when unknown samples need to be analyzed traditional methods of GC analysis often lead to only vague results and often require time consuming and cumbersome sample preparation techniques such as solvent extraction (liquid/liquid, Soxhlet, Likens-Nickerson). The technique of dynamic headspace requires minimal sample preparation, and significantly reduces overall analysis time while also improving data quality. In this work, the dynamic headspace technique is applied to different types of consumer products. The analysis of neat perfume oil is compared with that of consumer products containing the same oil.

VENDOR SEMINAR, NOVEMBER 3, 2011 (13:15–14:15)

NEW INNOVATIVE CHROMATOGRAPHY COLUMNS AND METHOD OPTIMIZATION FOR FOOD APPLICATIONS

SIGMA-ALDRICH®

Use of Ionic Liquid Stationary Phases in the GC Analysis of Food Volatiles

P. Q. Tranchida, C. Ragonese, P. Dugo, L. Mondello

In recent years there has been an increasing interest, amongst researchers operating in the chromatography field, directed to the use of ionic liquids (IL). The latter are a group of low melting-point, non-molecular solvents with differing solvation properties, in relation to the particular cation-anion combination. Gas chromatography, using IL stationary phases, has been reported in the analysis of essential oils, PAHs, chlorinated pesticides and straight-chain saturated fatty acid methyl esters (FAME), etc. The use of IL phases has also been reported in the field of tuned pressure dual-column and comprehensive two-dimensional gas chromatography.

The present lecture is focused on the evaluation of use of a series of IL stationary phases in the analysis of a variety of real-world food samples. The high selectivity of IL phases in specific food applications is fully demonstrated.

Determination of Herbicides at low trace level (ppt), using water sample direct injection in UHPLC/MS/MS couple with RP Amide and F5 Ascentis Express fused core HPLC column

E. Belotti, L. Meni, M. Ruggeri and R. Ferrari

The purpose of the experiment was to test the possibility to inject, without any extraction or purification process samples directly in LCMSMS of drinking water or groundwater. To have a simple and robust system for the rapid recovery and improving the reproducibility of the method to use routinely Furthermore to compare columns of different polarity and selectivity to improve the chromatographic profiles of metabolites of atrazine, in particular the desethyl desisopropyl atrazine.

Intravalidation of multiresidual methods for Mycotoxines in cereals at ppb level using Ascentis Express RP Amide and F5, couple with UHPLC/MS/MS

R. Ferrari, E. Belotti, L. Meni, M. Ruggeri

The determination of multiresidual Mycotoxine in cereals has become nowadays a routine analysis. The optimization of analysis time, the limit of detection and the robustness of the method are important parameters for the validation and certification of an optimum method. This Presentation describes the pairing of innovative UHPLC, Fused Core HPLC column technology and LC / MS / MS optimization for the validation of a fast, efficient and reproducible method, considering the issues of separation and detection of some mycotoxins.

VENDOR SEMINAR, NOVEMBER 3, 2011 (13:15–14:15)

USING ADVANCED TECHNOLOGY TO SOLVE NEW CHALLENGES IN FOOD ANALYSIS

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Our Message is simple – we can address your Food Safety Challenges. Join us at our free seminar at RAFA on 3 November 2011 to find out more.

We will present recent developments and advances in analytical chemistry of emerging food contaminants and residues, ranging from novel techniques for allergens determination to the analytical methods for biologically active flavorings in food.

Feasibility of an Exactive Orbitrap™ system equipped with high collision dissociation chamber for a reliable identification of food allergens

Linda Monaci[a]***, Ilario Losito***[b]***, A. Visconti***[a]
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[b] *University of Bari, Department of Bari, Via Orabona 4, 70126 Bari, Italy*

Potentials of a HPLC-High Resolution Mass Spectrometry method based on a non-hybrid Orbitrap mass analyser as an analytical tool for fast screening of milk allergens in food samples is for the first time presented. The method enables a reliable identification and characterisation of milk allergens in complex food matrices. Detection of milk allergens is based on the identification of unique casein peptide-markers in food extracts undergoing tryptic digestion. The extremely high mass accuracy (≤ 5ppm) and resolution (up to 100.000 FWHM) provided by the Orbitrap technology allows a fast preliminary identification of four peptide-markers of caseins on the base of the accurate *m/z* value of their generated ions. Besides, the availability of a high-energy collisionally activated dissociation cell integrated within the mass spectrometer enables acquisition of peptide MS/MS-like spectra through post-source fragmentation providing the final confirmation of a correct peptide attribution.

The method presented appears to be very promising as a reliable and potentially high-throughput approach to the analytical challenge represented by the detection of trace levels of protein allergen in complex food matrices.

A Solid-Phase Micro-Extraction GC/MS/MS Method for Rapid Quantitative Analysis of Food and Beverages for the Presence of Biologically Active Flavorings

Katerina Bousova[a]***, Klaus Mittendorf***[a]***, Hamide Senyuva***[b]
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In the year 2008 the European Parliament and the Council of the European Union released the European Regulation 1334/2008 (1), which lays down rules on flavorings and food ingredients with flavoring properties for use in and on foods. Among other rules it stipulates the flavoring substances, which have the restrictions and regulatory limits for food. There have been developed a lot of methods for determination the individual flavorings in different matrices, however no one was suitable at use for regulatory purposes. The aim of this work was preparing the method, which will be able these needs complete.

The presented method was developed using automated headspace solid-phase micro-extraction (HS/SPME) coupled with GC-MS/MS to simultaneously determine the presence of seven biologically active flavoring substances whose levels of use in processed foods is controlled by statutory limits. The method can be applied to identify and quantify the presence of 1,2-benzopyrone (coumarin), β-asarone, 1-allyl-4-methoxybenzene (estragole), menthofuran, 4-allyl-1,2-dimethoxybenzene (methyl eugenol), pulegone and thujone at levels ranging from 0.5 to 3000 mg/kg. The method has been optimized and validated for three different generic food types categorized on the basis of composition and anticipated use levels of flavorings and food ingredients. The food categories are: (1) Alcoholic & non-alcoholic beverages; (2) Semi-solid processed foods (e.g. soups, sauces, confectionary etc.) and (3) Solid foods (muesli, bakery products etc.). The method is simple, inexpensive, rapid,

and eliminates the use of flammable and toxic solvents. There is no sample preparation and, using MS/MS, unequivocal confirmation of identification is achieved even in highly complex matrices containing many potential interfering volatiles. The method precision for spiked samples ranged from 2 to 21 % with the greater variability associated with solid matrices. The LODs and LOQs were well below 0.1 and 0.5 mg/kg respectively, in all cases for individual substances fulfilling requirements for enforcement purposes. The robustness of the method was demonstrated in a small survey of retail samples of spirits (4), flavored milks (5), energy drinks (3), liqueurs (5), soups (5), sauces (10), herbal teas (5) and breakfast cereals (3).

During optimization a developing method were investigated various features of food and beverages, which can have a significant influence on the effectual extraction of target compounds on the SPME fiber. Foremost the different content of ethanol and sugar in alcoholic and non-alcoholic beverages was suspected.

Reference:

Regulation (EC) No 1334/2008 of 16 December 2008 on flavorings and certain food ingredients with flavoring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. Official Journal of the European Union. (2008) L 354/34-50.

VENDOR SEMINAR, NOVEMBER 4, 2011 (13:15–14:15)

AGILENT TECHNOLOGIES: FLEXIBLE STRATEGIES FOR YOUR FOOD ANALYSIS

Agilent Technologies

GC/Q-TOF for Target, Non-target and Unknowns: The Benefits of High Resolution, Accurate Mass and Fast Acquisition Rates MS and MS/MS

***Terry L. Sheehan, PhD**, GC/MS Marketing Manager, Agilent Technologies. United States*

***Greg Well, PhD**, Agilent Technologies. United States*

Although GC/TOF systems have been applied to a variety of quantitative and qualitative tasks for target analytes, non-targets and true unknowns (not included in spectral database), the majority of commercial GC/TOF systems are limited to nominal mass information (unit mass resolution) and do not have MS/MS capability. High resolution (R>10K), accurate mass (<1ppm) TOF significantly increases detection selectivity, eliminates many interferences seen with nominal mass data and allows rapid, high efficiency screening of target and non-target compounds. Additionally, accurate mass information on molecular ion and fragment ions greatly increases the qualitative power of the MS for confirming the identity of targets and non-targets. In Q-TOF mode, the application of high resolution and accurate mass to Product Ion spectra provides selectivity against matrix interferences that cannot be equaled by a tandem (triple) quadrupole MS. For the structural elucidation of unknowns, the combination of MS/MS dissociation studies and accurate mass data makes the Q-TOF MS a very useful tool with sensitivity that cannot be equaled by NMR. Although the high cost of Q-TOF technology will limit it use in many routine labs, Q-TOF GC/MS provides invaluable tools for R&D laboratories working in food safety, natural products, and flavor-fragrance.

New Solutions for Food Applications Using Atomic Spectroscopy

***Evrim Kilicgedik**, Atomic Spectroscopy Product Specialist, Agilent Technologies. United Kingdom*

How to choose best elemental analyses technique for food products. The potential implications of metals in foods are increasing interest, both from the point of view of nutritional and health benefits and from the point of view of potential toxicity. Regulated limits for many heavy metals are being reduced and it is now common for the chemical form of the element to be monitored, as well as the total concentration. Agilent's Atomic Spectroscopy instruments offer a solution for these measurements, providing sensitive and accurate trace metal analyses using AAS, ICP and ICP-MS.

How LC and GC techniques enable Organic Mass Spectrometry in target analysis of food safety

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From Mycotoxins to Pesticides and Vet Drugs to POP's, increases in the performance of organic mass spectrometry is enabling new approaches to be explored which increase the scope of what can be achieved with this technique. LCMS and GCMS techniques have always been powerful tools for analysts wanting to increase the amount of information resulting from one injection of a food extract. They also offer the potential to increase the confidence of identification and accuracy of quantification all at the same time. Agilent has some powerful new tools that can leverage these benefits and these will be discussed in the context of some novel food applications.

However in a complex food matrix, producing an extract which contains all targets that are of interest whilst at the same time ensuring that the rest of the matrix does not compromise the analysis, is challenging and the challenge increases as the analyst looks to add yet more targets to a single injection. Two things that can help are (a) expertise in sample prep strategies developed by leading labs around the world (b) new technology to drive sensitivity to new levels hence allowing the possibility to inject less matrix. This presentation will outline how Agilent's collaborations with key food labs and it's unrelenting product development in Chromatography and Mass Spectrometry combine to address these challenges and open up new possibilities for powerful food analysis.

ORAL SESSIONS

(L-1 – L-78)

L-1
EC PRIORITIES CONCERNING AGRI-FOOD RESEARCH AND INNOVATION

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The presentation will focus on the EU 2020 Strategy and its implications in building a sustainable food chain through the promotion of innovation in agri-food systems, increasing food production sustainability, healthy food and food security in Europe and beyond. Europe 2020 - a European strategy for smart, sustainable and inclusive growth - developing an economy based on knowledge and innovation; promoting a more resource efficient, greener and more competitive economy; fostering a high employment economy delivering social and territorial cohesion.

L-2
INTRODUCTION TO EMERGING ISSUES ON NANOPARTICLES IN THE FOOD CHAIN

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Food can contain a number of different materials in the nanosize range (typically 1–100 nm), originating from different sources. Natural food ingredients in molecular form or minerals have usually a size of a few nm or even below. Food can be "contaminated" by nanosized particles through food processing, by nano-particulate agrochemicals or the environment. In recent years however the application of nanotechnology in food production and food packaging has experienced rapid development and has developed into a wide-ranging industrial business. A number of new processes and materials derived from nanotechnology can offer new food products with e.g. improved tastes, reduced amount of salt, sugar, fat and preservatives, increased bioavailability etc. For example the nutritional value of food can be improved by nano-sized nutrients and supplements or encapsulation of nutrients; new tastes/sensations and creamier textures can be achieved by nanostructuring of food ingredients with less or no additional fat. For food packaging applications, such developments have led to new materials with improved mechanical, barrier and antimicrobial properties. Applications of nanotechnologies in food has raises a number of safety, environmental, ethical, and regulatory issues.The main concerns relate to the lack of knowledge with regard to the interactions of nanosized materials at the molecular of physiological levels and their potential effects and impacts on health. The presentation will give a short introduction into the principal issues on the use of nanomaterials in food and feed and related regulatory aspects.

Keywords: nanoparticles, food, applications, regulations

L-3
FOOD CRISES & NEW POPS: CHALLENGES IN ANALYSIS

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Risks for human health from PCBs and dioxins are mainly related to consumption of food from animal origin. During the last decade, repeated cases of contamination of feedingstuffs highlighted the importance of feed as the potential contamination media. Reducing the dioxin uptake by human is thus highly dependent of actions taken to minimize the contamination of all feed materials such as, not only, raw materials, but also recycled products and ingredients (e.g. citrus pulp pellets, recycled fats, mineral clays, choline chloride component, hydrochloric acid related to gelatin production, guar gum thickener, biodiesel-related fatty acids…).
Despite those actions, isolated cases of contamination might still arise. The implementation of continuous monitoring strategies, the enforcement of the maximum-action-target level strategy, as well as the availability of a Rapid Alert System for Food and Feed (RASFF), nowadays allows actions to be taken more rapidly and in a coordinated manner in order to reduce the potential human exposure to a minimum in case a contamination event is reported. This normally translates in so called 'food crises', which now often have much larger impact on our economies than on our health.
In order to be able to timely respond to health threats caused by contaminated food or feed, laboratories have to be endowed with large and efficient analytical capacities. The entire screening-confirmatory approach of the EU relies on the responsiveness of such expert laboratories. They have to be able to handle continuous flows of samples to be screened (and potentially confirmed) for regular monitoring programs, but also to quickly go on alert and accommodate large numbers of suspected samples in case of a contamination event is reported.
Such expert laboratories have to use state-of-the-art technologies, which includes both sample preparation and measurement aspects. One of the major challenges is to combine high level of QA/QC with fast turnover and large sample throughput. Depending on the fact that a method is used for screening or for confirmation, analytical instructions and guidelines are somewhat different but, in each case, requirements are very stringent and specific to the highest level.
Because of all efforts provided by the EU on both analytical and food-feed continuous control aspects, one often says that Europe has one of the highest levels of food safety in the world… So far, however, only a very limited set of analytes is included in those monitoring programs. What about all the other potential harmful molecules present in our food but that we do not look for? What about potential synergic effects of mixture of untargeted toxicants?
Next to the continuous discussions regarding the potential use of such or such alternative tools for specific PCB and dioxin monitoring, it would perhaps be more fruitful for our health to concentrate efforts on the development of analytical approaches that would allow to enlarge the list of target compounds to more 'exotic' (un)suspected persistent molecules… A more proactive and exhaustive approach is probably needed to more appropriately ensure high level of food quality.

L-4
PEPTIDE AND OLIGONUCLEOTIDES APTAMERS AS NEW LIGANDS FOR ANALYTICAL CHEMISTRY

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So far, several bio-analytical methods have used nucleic acid probes to detect specific sequences in RNA or DNA targets through hybridisation. More recently, specific nucleic acids, aptamers, selected from random sequence pools, have been shown to bind non-nucleic acid targets, such as small molecules or proteins. The development of in vitro selection and amplification techniques has allowed the identification of specific aptamers, which bind to the target molecules with high affinity. Many small organic molecules with molecular weights from 100 to 10000 Da have been shown to be good targets for selection. Moreover, aptamers can be selected against difficult target haptens, such as toxins or prions. The selected aptamers can bind to their targets with high affinity and even discriminate between closely related targets. Aptamers can thus be considered as a valid alternative to antibodies or other bio-mimetic receptors, for the development of biosensors and other analytical methods. The production of aptamers is commonly performed by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process, which, starting from large libraries of oligonucleotides, allows the isolation of large amounts of functional nucleic acids by an iterative process of in vitro selection and subsequent amplification through polymerase chain reaction. Aptamers are suitable for applications based on molecular recognition as analytical, diagnostic and therapeutic tools. In this review, the main analytical methods which have been developed using aptamers, will be discussed together with an overview on the aptamer selection process. Recently Aptamers from peptides libraries have been realized with similar techniques in order to obtain structures with much varied receptors points and higher analytical capabilities. Some experimental results will be reported and discussed.

Keywords: Aptamers, biosensors

L-5
FINGERPRINTING / PROFILING: A NOVEL APPROACH FOR A HIGH THROUGHPUT AND COMPREHENSIVE ASSESSMENT OF QUALITY AND SAFETY OF FOOD LIPIDS

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Lipids, the key component of many foodstuffs, are not only a source of essential components such as ω-3 and ω-6 fatty acids, but they are also accompanied by other important lipophilic components represented by vitamins, sterols etc. On the other hand, lipids may contain various toxic and /or anti-nutritive compounds generated from natural precursors as the result of (auto)oxidation processes or thermal processing. Chromatography, together with mass spectrometry, are essential analytical tools in this field. In addition to targeted compositional analysis, non-target fingerprinting / profiling techniques have recently emerged as the novel strategy for assessment of lipids quality and safety. In our study, ambient mass spectrometry employing DART (Direct Analysis in Real Time) and/or ASAP (Atmospheric Solids Analysis Probe) coupled with (ultra)high resolution mass spectrometry (MS) were used for characterization of various food lipids. In this way, not only intact triacyl glycerols but also their oxidation products such as hydroperoxides, epoxides etc., together with reactive polar breakdown products, can be detected. When using a simple sample preparation step, also occurrence of "emerging" processing contaminants 3-MCPD diesters could be documented. To obtain fingerprints of headspace volatiles, solid-phase microextraction–electron ionization high resolution mass spectrometry (HS-SPME–EI-HRMS) was employed. Advanced chemometric strategies represented by linear discrimination analysis (LDA) and neural networks (NN) were used for processing of generated data and quality markers identification.

Keywords: Lipidomics, fingerprinting, profiling

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L-6
TRACEABILITY AND AUTHENTICITY ISSUES: REQUIREMENTS FOR ADEQUATE ANALYTICAL METHODS

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European consumers' behaviour has been undergoing gradual changes. People require not only enough and high quality sanitary products (dietary, hygienic and health standards), but also certification and reassurance of product origin and production methods. In order to preserve quality food products coming from particular geographical areas and to protect consumers against imitations and false information, the European Commission defines via different regulations the labels Traditional Speciality Guaranteed (TSG), Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). Traceability is an essential tool to enhance trader and consumer confidence in the safety, quality and authenticity of the food. It also helps the regulatory authorities to detect fraud and dangerous substances. According to Regulation EC 178/2002, the term traceability means the ability to trace and follow a food, feed, food-producing animal or substance intended or expected to be incorporated into a food or feed, through all stages of production, processing and distribution. Objective criteria that identify and trace back a product are mandatory. Research in this area is partly focused on developing methods to authenticate the food products and on the demonstration of their suitability. This talk will come with some reflections obtained after more than 15 years of research in method development for the authenticity of food products and the participation to about 10 European projects dealing with traceability and authenticity issues. Key criteria as appropriate sampling plan, sampling preparation procedures, full protocol validation and suitable expression of results of proposed analytical methods are necessary in order to fulfil the traceability and authentication issues.

Keywords: authenticity and traceability, food and feed, sampling plan, sampling procedure, validation

L-7
USE OF PROTEIN- AND METABOLITE PROFILING TECHNIQUES ON WHEAT GRAIN IN SEARCH OF BIOMARKERS DISTINGUISHING SAMPLES GROWN UNDER DIFFERENT AGRICULTURAL SYSTEMS

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The increasing popularity of organic farming and organic food leads to a great economic interest in finding discriminating analytical methods to ensure the authenticity of organic labeled products. Using the profiling approach aims firstly, to detect a large number of known and unknown metabolites and proteins and secondly, to identify differences in occurrence or concentration of these biomolecules in wheat of the different agricultural systems. Samples of 11 different wheat grain varieties were taken from the controlled DOK-field trial of the Research Institute of Organic Agriculture (FiBL) and the research station Agroscope Reckenholz-Tänikon (ART), Switzerland. Each variety was cultivated with four plot replications at the same location under organic and conventional conditions, which ensures an equal environmental influence and therefore the comparability of research results. We analysed 11 different wheat varieties of the harvest year 2007 to assess the influence of a diverse genetic pool on the spread of analytical results. Additionally, samples of the wheat variety "Runal" were taken over three harvest years in order to account for influence of seasonal variations. Protein profiling was performed with 2D-gelelectrophoresis and 2D-image analysis was done using Delta2D-software. Proteins were identified via MALDI-TOF-MS/MS and database searches. Metabolite profiles were generated with GC-MS from derivatised methanolic extracts of finely ground whole wheat grains. Employing these techniques on the variety "Runal", we were able to identify 48 metabolites and additionally to detect 245 not identified metabolites (TAGs). In this pool of biomolecules, two metabolites showed significant differences in normalised peak areas in all three harvest years of "Runal". Across all 11 varieties of the 2007 harvest year, 5 metabolites and 11 TAGs with significant differences in peak areas between the cultivation forms were detected, using Student t-tests. PCA performed on data for the individual varieties revealed a clustering according to the cultivation forms. However, PCA of metabolites and TAGs of combined data of all 11 varieties did not result in a clustering. Protein identification is currently in progress. For "Runal" we were able to detect 2 proteins with significant different levels in samples of conventional and organic cultivation forms until now. Based on individual varieties, metabolite profiling has shown promising results with respect to discriminate organic and conventional wheat. Results viewed across all 11 varieties indicated a higher influence of the variety and seasonal effects than the cultivation form on metabolite concentration. Further work will prove, if significant differences of concentrations in individual metabolites and TAGs as well as proteins can be used to discriminate between cultivations forms across multiple wheat varieties.
Keywords: wheat, food omics, organic farming, authenticity

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L-8
MULTIDIMENSIONAL GC (MDGC) AND CARBON ISOTOPE RATIO MS (GC-C-IRMS) FOR THE AUTHENTICITY ASSESSMENT OF CITRUS ESSENTIAL OILS

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Enantiomeric ratios of chiral volatile components represent a useful parameter for the assessment of authenticity of essential oils. However, often the seasonal variations which occur during ripening of the fruits are quite wide, not yet well determined, thus rendering this tool not completely reliable by itself. Assessment of genuineness is thus often carried out by multiple analytical techniques (physico-chemical analyses, GC, es-GC, HPLC) in order to evaluate sufficient parameters to express a secure judgment. More over if distilled oils are analyzed the enantiomeric ratios are subject to variations due to the possible reactions with consequent tendency to racemization of some chiral volatiles. The carbon isotope ratio, on the other hand, is strictly dependent on the plant biochemistry, and on the geographical origin. It is not subject to seasonal variation, nor to the extraction procedure used. However at this time sufficient data on authenticity ranges for all Citrus oils is not yet available. This study has been carried out on numerous samples of different Citrus essential oils by Es-MDGC, Es-GC and GC-C-IRMS, to determine if the combination of enantiomeric ratios with the carbon isotope ratios can be effective to determine the genuineness of the samples studied.

Keywords: enantiomeric ratios, isotopic ratio, citrus essential oils

L-9*
MASS SPECTROMETRY-BASED METABOLOMICS FOR AUTHENTICITY ASSESSMENT OF FRUIT JUICES

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Fruit juices represent a food commodity with a high economic value and large-scale production, which have made them a likely target for adulteration and fraud. The most common procedures in fruit juice adulteration involve the addition of water, sugars, pulp wash, or juice coming from other cheaper fruits, such as apple and grapefruit. Until now, a number of methods have been developed to tackle various aspects of fruit juice authenticity. The most established approaches are based on profiling of carbohydrates, phenolic compounds, carotenoids, amino or other organic acids using chromatographic methods such as high performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to various types of detectors. With regard to targeted nature of all the above mentioned methods, each of them can be successfully applied to monitor one or only few specific fraudulent practices. It should be noted, however, that fraud performers are usually one step ahead of the available testing methods, as new and more sophisticated adulteration strategies are continuously developed. Therefore, analytical approaches and platforms facilitating more comprehensive insight into the chemical composition of fruit juices and its changes, as associated with adulteration, are required. In this study, the feasibility of HPLC–MS technique employing hybrid triple quadrupole/linear ion trap (QqQ/LIT) mass analyzer for metabolomic-based authentication of fruit juices (orange, grapefruit, apple, pomegranate, blueberry, cranberry and their admixtures) representing different price categories, was explored with the aim to detect their adulteration. Complex HPLC–MS profiles, obtained by analysis of diluted juice samples, were treated with automated data mining algorithm and further processed by multivariate chemometric methods (principal component analysis, PCA, and linear discriminant analysis, LDA). Based on LDA classification model constructed with the use of positive electrospray ionization data, reliable detection of or ange juice adulteration with apple and grapefruit juice was possible at 15% addition level. Feasibility of this approach for authentication of other valuable fruit juices, such as pomegranate, blueberry or cranberry, was also demonstrated. In the next phase, accurate single MS and MS/MS mass spectra were acquired for the most characteristic marker compounds employing quadrupole/time-of-flight mass spectrometry (QqTOFMS). Using a combination of experimental data and information available in chemical, metabolomic and mass spectral databases, more than 50% of characteristic markers were identified. Additionally, the applicability of rapid, no separation fingerprinting approaches to control fruit juices authenticity was explored.

Keywords: Fruit Juices, Metabolomics, Adulteration, Liquid Chromatography, Mass Spectrometry

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L-10
PRESERVATION OF PRIMER AND PROBES ON "READY-TO-USE" 96-WELL MICROTITER PLATES: A STEP FORWARD TOWARDS ENHANCING THROUGHPUT OF REAL TIME PCR APPLICATIONS IN FOOD AND FEED TRACEABILITY

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PCR applications in food analysis are used for pathogen detection, to identify and quantify genetically modified (gm) food and feed, for allergen causing foods and also for the identification of fraud using less expensive ingredients substituting high price products. In particular for pathogens and gm foods methods are internationally standardized for many years already. However the application is still time and labour-intensive due to the individual components necessary to combine for a single PCR: primers, probes, sample DNA and master mix containing the polymerase and buffer need to be pipetted before the run can be started. There is the potential for mis-pipetting due to many individual components which may result in significant differences when analyzing the same sample by different laboratories. In order to improve the reproducibility and the harmonization of PCR application we developed a protocol for the pre-coating of DNA on the surface of microtiter plates ready to be used for up to two years storage without the loss of performance. For that purpose three different agents were compared: trehalose, PEG 8000 and gelatine. Out of the three agents trehalose seems to be the most appropriate one. The mode-of-action of trehalose is most probably based on the interaction with hydrogen bonds thus replacing essential water molecules of the surrounding water. Applying the developed protocol we were able to develop microtiter plates specifically designed for the parallel detection of individual allergens and for the parallel screening for GMOs in food and feed. The applicability of the "ready-to-use" microtiter plates have been tested on food samples from the market. In addition to the already labelled ingredients further hidden not declared traces of allergens were detected. This demonstrates their suitability as a tracking and tracing tool in food and feed control.

Keywords: pre-coating, food analysis, PCR

L-11*
AUTHENTICITY AND QUALITY OF SPIRIT VINEGAR: METHODS FOR DETECTION OF SYNTHETIC ACETIC ACID

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Spirit vinegar is extensively used as an acidifying and food preserving agent. It is a traditional component of various food products (e.g. pickles, ketchups) giving them characteristic aroma and taste. On the other hand, it is also possible to use synthetic acetic acid as a food additive (E260). Synthetic acetic acid can be intentionally used (partially or totally) as substitution of vinegar and the addition of synthetic acetic acid is not indicated on the product label. It can be assumed that the products containing pure synthetic acetic acid instead of vinegar differ in taste, aroma and also in content of biologically active substances, because vinegar contains also the specific components derived from raw material and metabolites of fermentation. The identification of synthetic acetic acid in vinegars is of interest to food industry. The objective of this study was to prove differences in the sensory and chemical qualities of spirit vinegar, synthetic acetic acid and their mixtures. The different types of spirit vinegars and synthetic acetic acids were analyzed. The profile of volatiles was specified by HS-SPME-GC/MS and HS-SPME-GC/FID. The results were verified also by the isotopic analysis of acetic acid. The 2H/1H by SNIF-NMR and 13C/12C by IRMS analyses provide information about the botanic origin of acetic acid and enable the detection of adulteration of vinegar by synthetic acetic acid. The differences in sensory qualities were determined by the triangular method. The taste, aroma and general perception were evaluated by the paired preference test. In total eleven samples were analyzed, including six spirit vinegars purchased from the market, three synthetic acetic acids diluted with water and two model samples prepared in the laboratory as the mixtures of analyzed vinegars and synthetic acetic acids. The sensory evaluation was not sufficiently distinctive instrument; the differences between spirit vinegars and synthetic acetic acids diluted with water were not clearly statistically s ignificant (P = 95%). The instrumental measurements (HS-SPME-GC/MS) showed that the samples of spirit vinegars compared to synthetic acetic acids diluted with water differed in the composition of volatile compounds, namely ethanol and ethyl acetate (indicators of the fermentation process). In the spirit vinegars, the concentrations of ethanol and ethyl acetate ranged from 0.09 to 0.34 g/l and from 0.01 to 0.05 g/l respectively. The differences in the sensory and chemical qualities of the samples were demonstrated, however, considering the limited set of the samples the obtained results cannot be generalized yet.

Keywords: spirit vinegar, synthetic acetic acid, authenticity

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L-12
METHODS APPLIED IN ORGANIC FOOD AUTHENTICATION WITH FOCUS ON CRYSTALLIZATION WITH ADDITIVES

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The organic market is continuously growing. Parallel to this growth there is a demand for analytical methods allowing the authentication of organically grown crops. For organic products, the organic production process is regulated by EC 834/2007 and association regulations. Several methods have been applied for authentication of a number of crops, but until now, fast fingerprinting methods, which can be applied within a certification process, are still under investigation. Therefore the aim of this presentation is to present available analytical methods, markers and concepts for their ability to be applied in the documentation of the organic production and for the prevention of fraud. The presentation discusses results from method approaches applied on the differentiation of food samples from organic and conventional farming. Those methods cover e.g. the stable isotopes, using multi-elemental and various key-metabolites analysis. Moreover primary and secondary metabolites are detected to generate the characteristic fingerprints of organic and conventional food samples. The new technique crystallization with additives will be presented in more detail. Reproducible crystallisation patterns, which are characteristic for the sample material investigated, emerge when an aqueous cupric chloride ($CuCl_2 \cdot 2H_2O$) solution is crystallised on a glass dish in the presence of a plant extract. The emerged patterns are evaluated by computer assisted image analysis followed by multivariate statistical tools, such as principal component analysis (PCA). Characteristic method steps and parameters as sample preparation, mixing ratio, evaporation time, region of interest of the patterns were investigated and standardized. This standardized crystallization technique was applied on defined samples from a controlled field trial, harvested in three following years. Wheat samples from organic and conventional farming practices can be differentiated over the three year period.

Keywords: Organic food, authentications, crystallization with additives

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L-13
CUTTING-EDGE ANALYTICAL TECHNIQUES FOR NANOPARTICLES IN FOOD

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Nanotechnology applications for the food sector are intensively investigated and developed at the moment. A number of nanomaterials are already in use as food additives or in food contact materials. Furthermore, approved (bulk) food additives may have a size distribution which extends down to the sub 100 nm range, e.g. fumed silica (E551). At the same time, limited knowledge is available on the potential impact of engineered nanoparticles (ENP) on consumers' health. A prerequisite for toxicological, toxicokinetic, migration and exposure assessment studies is the availability of analytical tools for the detection and characterisation of ENP in complex matrices such as food. Given the huge diversity of ENP for potential use in the food and feed sector in terms of chemical composition, size, size distribution, surface activity/modifications etc. and possible interactions with food matrix components (e.g. proteins) it is a challenging task to develop tailored solutions. While there are a number of established techniques to characterise (inorganic) ENP in their pure state it is crucial for food to develop sample preparation techniques that yield artefact-free samples for imaging techniques or separate the particles from the matrix for subsequent application of suited detection techniques, e.g. mass spectrometry. In the case of imaging techniques electron microscopy in its different forms (e.g. SEM, TEM) seems most promising, especially when coupled to spectroscopic methods such as EDX. Screening approaches for specific applications include the use of biosensors with ENP-specific recognition elements and ELISAs for certain organic ENP. A combination of instrumental separation techniques with specific detectors is often unavoidable for the reliable characterisation and quantification of ENP in the food matrix. Hydrodynamic chromatography (HDC) is a robust flow technique for the separation of ENP from larger particles and matrix components. It has successfully been applied to the measurement of silica NP in food commodities. Field flow fractionation (FFF) offers a higher resolution as compared to HDC and has been used for the separation of different inorganic ENP (e.g. Ag, SiO_2, TiO_2). Differential mobility analysis (DMA) can also be utilised for the analysis of liquid sample extracts when a tailored electrospray ionisation is applied. Detectors that can be combined on- or off-line with these separation techniques include light scattering (SLS, DLS, NTA), UV-DAD and mass spectrometry. In particular ICP-MS is the method of choice for inorganic ENP. Single particle ICP-MS is an interesting approach for the rapid screening for ENP in complex matrices after minimal sample preparation. The selection of an appropriate detector for organic ENP largely depends on the size and composition of the target analyte. Conventional HPLC-MS can be used for fullerenes, while in the case of complex protein based encapsulates MALDI-ToF-MS can be an option.

Keywords: nanoparticles, electron microscopy, separation (FFF, HDC, DMA), mass spectrometry (ICP-MS, MALDI-ToF), screening (biosensor, ELISA)

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L-14
PIXE: A TOOL FOR NANOPARTICLE QUANTIFICATION IN FOOD ANALYSIS

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With the advent of engineered nanoparticles (NPs) produced in high quantities and employed as coatings in food containers or as additives in products, the need to evaluate their potential toxicological effects is necessary. A current necessity for food analysis is to be able to quantify NPs with in a simple manner and with a high sensitivity. A competent tool to make this quantification possible is the use of Particle-Induced X-ray Emission (PIXE) method. This method is based on the quantification of x-rays emitted from a target irradiated with an ion beam. PIXE can be used in conjunction with other ion beam methods such as Nuclear Reaction Analysis (NRA) to provide a wider range of monitoring and quantification. The advantages of this methodology are: multi-element analysis, ppm-level of sensitivity, non destructive, solid or liquid sample analysis, and fast measurements. In this study we will present the scope of the PIXE methodology, our setup and sample preparation method; and will illustrate the sensitivity levels that can be achieved for the quantification of relevant NPs (i.e. SiO2, Ag, Au) in different food matrices ranging from simple to complex matrices (i.e. water vs milk).

Keywords: PIXE, nanoparticles, food analysis, contaminants, residues

L-15
PRODUCTION AND CHARACTERIZATION OF ANTIBODIES AGAINST CROSSLINKED GELATIN NANOPARTICLES AND ITS USE FOR ELISA SCREENING KIT DEVELOPMENT

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Nanotechnologies begin to have more and more applications in the food sector. The nanoparticles are used to provide vitamins and other nutrients in foods and beverages without affecting taste and colour. They are also used to develop new tastes, preserve the texture of the food, control the release of flavours, improve the bioavailability of certain components such as antioxidants, vitamins and control the freshness through nano-sensors. Cross-linked gelatine nanoparticles are a component of nano-sized carriers for delivery of nutrients and supplements in food and related products. This paper describes the production and characterization of polyclonal antibodies in rabbits against the gelatin nanoparticles. Two kind of immunizations were investigated, one with subcutaneously injection and the other with intravenous as first injection. The antibodies were characterized using different enzyme-linked immunosorbent assay (ELISA) format, inhibitor format with the antigen coated on plate for the detection of immune response and sandwich format for the development of the method. The antibodies were purified by protein A column and coupled to Horseradish peroxidase (HRP) for capture/detection system. The antibodies show a good sensitivity and specificity to the nanomaterials without significant cross-reactivity against native gelatin. Currently there are no screening techniques for nanoparticles, except the imaging methods that have already been developed for the detection of nanosilver. These techniques require the use of expensive machinery and sample preparation methods are often laborious. ELISA offers a rapid and low-cost way of screening food, feed and beverages. We have studied prototype ELISA assay for the detection of gelatine based nano-carrier systems. Fruit juice and soft drink are the matrices selected for the assay development. Sample preparation is also an important step for the development.

Keywords: Gelatine nanoparticles, nano-sensor, Antibodies, ELISA screening

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L-16
DETECTION AND CHARACTERIZATION OF ENGINEERED NANOPARTICLES IN FOOD BY FLOW FIELD-FLOW FRACTIONATION COUPLED TO INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

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The analysis of engineered nanoparticles (ENPs) involves detection, identification, quantification and, if possible, detailed characterization. Provision of metrics of importance in food safety studies however, presents the analyst with numerous challenges. Sample preparation methodologies are required for reducing the complexity of the sample matrices with minimum alteration of the virgin ENPs, followed by transfer of the ENPs into liquid suspension compatible with the analytical instruments. Following appropriate sample preparation, field-flow fractionation (FFF) is one of the most promising techniques for separation of particles. In the framework of the NanoLyse project work focuses on the development of FFF-based methods for the quantification of two different types of ENPs, namely metallic (silver) and metal oxide (silica) nanoparticles, in food matrices. Depending on the purification of the sample by the preparation method, suitable detection methods following FFF separation include UV-Vis absorbance, dynamic (DLS) and multi-angle laser light scattering (MALLS) as well as inductively coupled plasma mass spectrometry (ICP-MS), each for different purposes. UV-Vis absorbance could be used the detect AgNPs and SiO_2NPs dispersed in water. Nevertheless, the application for complex samples like food extracts will be difficult since the signal is a complex mixture from light scattering and true absorption which also includes size and material dependent effects. Quantification with high sensitivity could, however, be achieved by FFF coupled to ICP-MS. Silver had a low limit of detection of a few 100 pg/ml because of the absence of interferences in ICP-MS and the low natural abundance of silver. Concerning the SiO_2NPs, difficulties were encountered for the analysis of Si due to the presence of Si in the sample introduction system and due to multiple interferences on the three isotopes of Si. The usage of an inert sample introduction kit for ICP-MS decreased the background level. The use of a collision/reaction cell or dynamic reaction cell was shown to decrease the LOD especially in the presence of a high carbon background. Direct size information could be obtained for AgNPs in the size range of 20 to 70 nm based on on-line DLS measurements. The optimum silver mass concentration for DLS of AgNPs in the given size range was in the order of magnitude of a few 100 ng/ml. For 100 nm SiO_2NP direct size determination was possible for Si concentrations down to of a few µg/ml by using the FFF coupled to a MALLS detector. However, the light scattering signal is not specific for SiO_2NPs and has to be used with care in complex samples. Furthermore, different sample preparation methods were tested and optimized for release of AgNPs and SiO_2NPs from two types of food matrices, namely soup and lean meat. These methods included enzymatic digestion and chemical digestion with nitric acid.

Keywords: nanoparticles, silver, silica, field flow fractionation, sample preparation

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L-17*
IMAGING TECHNIQUES FOR DETECTION AND CHARACTERIZATION OF INORGANIC NANOPARTICLES IN FOOD

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Electron microscopy is widely used for the visualisation of nanoparticles and a promising tool for nanoparticle characterisation in complex matrices. However, the preparation of complex specimen for electron microscopy such as food stuff containing nanoparticles is highly challenging: samples have to be prepared in such a way that they can be introduced into the microscope vacuum chamber while withstanding high beam energies and preserve the sample in its original state to avoid sample alteration and imaging artefacts. Therefore, in this project, we aim to develop methods for low impact sample preparation as well as protocols for imaging of nanoparticles in food products using conventional techniques such as TEM and SEM as well as more advanced tools including cryo-SEM, Environmental SEM and WetSEM (capsules for imaging under liquid conditions). This will help to advance nanoparticle characterisation and detection by electron microscopy and ultimately support the risk assessment and development of novel foods based on nanotechnology. This talk will present an overview of our on-going research looking into the development of methods for sample preparation and imaging of inorganic nanoparticles (e.g. SiO_2, Ag) in different food matrices (e.g. soup and meat) by electron microscopy. This research is part of the NanoLyse project, a European collaborative research project, which is partly funded by the European Commission under the 7th Framework Programme, contract no. 245162. It is dedicated to the development of analytical methods for detection and characterisation of engineered nanoparticles in food. The NanoLyse consortium comprises 10 universities and research centres from Europe and Canada.

Keywords: Nanoparticles, Electron Microscopy, Detection, Characterization

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L-18
NANOPARTICLES IN FOOD: METHODS AND MEASUREMENTS

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Products based on nanotechnology or containing engineered nanoparticles are beginning to impact the food associated industries and markets. As a consequence direct and indirect consumer exposure to nanoparticles is likely. We have developed and applied methods for the detection and characterization of nanoparticles in food, in samples from toxicology, and samples from exposure studies which are an essential part of understanding the potential benefits as well as the potential risks of the application of nanoparticles. Single particle – inductively coupled plasma mass spectrometry (SP-ICPMS) is a method capable of detecting single nanoparticles in sample extracts at very low concentrations and has been used to determine nanoparticles in food, food supplements and toxicology samples. Since sample preparation may influence the results, SP-ICPMS, requiring only limited sample preparation, was used as a screening method for the determination of silver nanoparticles in the animal study. Hydrodynamic chromatography in combination with ICPMS (HDC-ICPMS) has been used as a confirmation method to characterize nanoparticles in several food items and in samples from digestion model studies with actual food items containing silica nanoparticles. Analysis of several food items showed that nano-sized silica is present in a number of food products in use by the majority of us. Some food supplements also contained nanoparticles of a different nature, including silver and gold. The digestion model and an animal exposure study were used to determine the fate of silica and silver nanoparticles in food following digestion. The preliminary results of these studies suggest that nanoparticles may survive the stomach and are available for uptake in the intestines. In the animal study silver nanoparticles were found in liver tissue indicating that, depending on the particle size, there may be an actual uptake of silver nanoparticles from the food into the body.

Keywords: nanoparticle, food, toxicology, risk assessment

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L-19
DEVELOPMENTS IN THE APPLICATION OF FLAME RETARDANTS AND CONSEQUENCES FOR THE ANALYSIS IN FOOD

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Every year tens of thousands of people die due to fire. The use of flame retardants (FRs) is one option to at least delay materials to catch fire. This delay offers people either to extinguish the initial fire, to timely call the fire brigade, or to escape. Since the mid 1980s the use of brominated FRs (BFRs) has become popular. Examples are polybrominated diphenylethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A). The high production volumes in combination with the persistent and bioaccumulative properties of many of these BFRs have finally led to restrictions in use. Penta-and OctaBDE have been labeled as 'official' persistent organic pollutant (POP) under the Stockholm Convention on POPs. As a consequence there is a growing market demand for alternatives for PBDEs. The substitution of PBDEs by other FRs will have consequences for the analysis of contaminants in food. Some users have principle or market-driven reasons not to use any BFRs anymore. Those have started to use alternative FRs such as phosphorus FRs (PFRs) or metal-based FRs. Others will simply apply other BFRs, or mixtures of BFRs and PFRs. Also, chlorinated FRs (CFRs) such as dechlorane Plus are still being used. The consequences for the analytical chemist are relatively complex. There is a need for screening of more substances and at lower levels. For example, PFRs may show a less bioaccumulative behavior, but may be more toxic. Chlorinated PFRs do bioaccumulate substantially and have been reported in fish already. Other BFRs such as decabromodiphenylethane have been reported in fish already. The European research project ENFIRO currently addresses the production of alternatives for BFRs that are environmentally safe. The current shortlist comprises several metal based FRs and some PFRs. Sensitive and selective gas chromatographic or liquid chromatographic techniques in combination with mass spectrometric detection are needed for their analysis.

Keywords: brominated, chlorinated, phosphorus flame retardants

L-20
DETERMINATION OF THE 15+1 EU PRIORITY POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN CHOCOLATE BY LIQUID CHROMATOGRAPHY HYPHENATED TO DOPANT ASSISTED TMOSPHERIC PRESSURE PHOTO IONISATION TANDEM MASS SPECTROMETRY

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The upcoming amendment of Commission Regulation (EC) No 1881/2006, setting maximum levels for certain contaminants in foodstuffs as regards polycyclic aromatic hydrocarbons (PAHs), will introduce maximum levels for PAHs in cocoa butter based products. In this context, maximal levels will be introduced for benz[a]pyrene and the sum of the future four EU marker PAHs (PAH4, i.e. benz[a]anthracene, benzo[a]pyrene, benzo[j]fluoranthene, and chrysene). Upon implementation of this piece of legislation, analytical hurdles regarding e.g. sample cleanup and selectivity of the assays are to be foreseen due to the complexity of the chocolate matrix. Preliminary experiments in the current study showed that cocoa butter and chocolate extracts contain a large number of fluorescent substances, hampering specifically fluorescence detection based (U)HPLC methods. In this context, the current investigation reports the development and in-house validation of an analysis method based on liquid chromatography hyphenated to atmospheric pressure photo ionisation tandem mass spectrometry (LC-APPI-MS/MS) for the determination of the 15+1 EU PAHs in chocolate. Depending on the analyte, the method was characterised by repeatability relative standard deviations of about 4.0% to 7.0% and intermediate precision relative standard deviations between 4.3% and 8.5%. The expanded measurement uncertainty of the determination of benzo[a]pyrene by this method was below 15.0%, whereas trueness was evaluated by applying certified reference material and a suitable proficiency test material. In addition to methodological issues, method development and validation data, the current presentation will, in conclusion, highlight results from the analysis of chocolate samples collected in the period 2005–2010 in different EU countries.

Keywords: PAHs, Chocolate, LC-APPI-MS/MS, Occurrence

L-21
MONITORING PERFLUORINATED ALKYL SUBSTANCES IN FOODS – CURRENT METHODS AND QUALITY PERSPECTIVES

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In recent years, perfluorinated alkylated substances (PFASs) have received increasing attention from scientists and policy makers. PFASs are persistent and are found in the environment and thereby enter the food chain. PFOS (perfluorooctanesulfonate) and PFOA (perfluorooctanoic acid) are the most well-known PFASs, but the group of PFASs is expanding rapidly. Other compounds belonging to this class have different chain lengths and/or functional groups (1). PFOS accumulates in fish, whereas PFOA is more water-soluble. In 2008, EFSA has completed a risk assessment on PFOS and PFOA (2) and established TDIs for PFOS (150 ng/kg bw/d) and PFOA (1500 ng/kg bw/d). The European commission recently called for monitoring PFASs in foods (3). However, the analysis of food is challenging, because of the low levels, generally in the low pg/g range (4). These calls for very sensitive methods, which also need to deliver reliable data (accuracy, precision). Food samples are often extracted using medium polar solvents (acetonitrile, methanol), combined with a clean up step using Envicarb and/or KOH saponification and solid phase extraction. Instrumental analysis is generally performed by liquid chromatography-triple quadrupole or time-of-flight mass spectrometry. A recently developed promising extraction approach is based on extraction by tetrahydrofuran-water. These food methods reach maturity, but their intercomparability still needs to be proved. Earlier intercomparison studies showed poor overall performance, yet at a higher (and easier) concentration level (6). Some improvement of data was observed since then. Several other challenges are still ahead and need attention in the near future. These include development of appropriate sample storage conditions and the separation and quantification of branched isomers (7). In addition, there's an urgent need for certified or standard reference materials and ongoing intercomparison studies. When these challenges are met, high quality food contamination data becomes available for a reliable assessment of the human dietary exposure and risks.

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Keywords: PFOS, perfluorinated alkyl substances, analysis, quality assurance

L-22*
ANALYSIS OF 18 PERFLUORINATED COMPOUNDS IN BIOLOGICAL MATRICES BY ON-LINE TURBO FLOW-LC-MS/MS

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Perfluorinated compounds (PFCs) have been manufactured since 40s and are persistent and bioaccumulative due their properties. The aim of the present work has been: I) to develop an on-line methodology turbo-flow extraction to analyze 18 PFCs in biological samples; II) to assess the presence of PFCs in 60 serum samples from unhabitated area as Barcelona and non unhabitated area as Greece; III) to assess the presence of PFCs in non invasive matrices as human hair and from different donors. 150 µl serum or urine sample was taken and introduced into PP eppendorf with 150 µl ACN, shaked 1min and centrifugated at 4000 rpm, 10 min, in order to precipitate the proteins from samples.100 µl of supernatant was introduced into PP insert vial. 0.25 g of human hair was introduced into a 15 ml PP centrifugue tube with 5 ml of ACN. The mixture was introduced in an ultrasonic bath for 15 min and the resulted extract was directly analyzed. The extraction and separtation was carried out in a Thermo Scientific Aria TLX-1 system.Different extraction columns were tested during optimization. The final methodology included two extraction columns in tandem: Cyclone and C18XL.The injection volume was 20 µl at turbulent flow of 1.5 ml/min water pH 3.4. The loop elution (250 µl) was performed with water (pH 3.4):methanol (2:8). Separation was carried out in a LC-column Hypersil GOLD PFP (50×3) and an extracolumn was used after LC pumps in order to remove the contamination from the pumps. Triple mass spectrometer for analysis, equipped with a Turbo Ion Spray source (negative mode) and single reaction monitoring. The method was validated regarding limits of detection between 18–1114 ng/L. The CCα error and CCβ error were calculated according ISO11843 (CCα=39 to 2030 ng/L and CCβ=53 to 2690 ng/L). Recoveries were established between 50-169% by spiking experiments at three different levels in every blank matrix. The applicability of the method was tested in 60 cord blood samples from contaminated and non-contaminated areas. Samples showed levels between 0.09-26.99 µg/L, corresponding the highest ones to perfluorohexanesulfonate (PFHxS). The most ubiquitous patterns were observed for perfluorooctanesulfonate and perfluorooctanoic acid. 30 urine samples were analyzed. Results showed the presence of 44% of PFCs at levels ranging from 0.12–39.27 µg/L even though most of the samples were below MLOQ. Perfluorobutanoic acid was found in 100% of analyzed samples at highest levels (52.72–1495.68 µg/L) which could indicate that this is a degradation product from longer chains. 20 human hair samples were analyzed and perfluorodecanoic acid (PFDA), PFHxS and PFOSA were found at positive concentrations ranging from 1–88 µg/Kg showing PFDA the highest levels.

Keywords: perfluorinated compounds, biological matrices, turbo-flow, liquid chormatography, mass spectrometry

L-23
MULTI-RESIDUE MONITORING OF ENVIRONMENTAL TOXICANTS IN ANIMAL-DERIVED FOOD DURING COOKING BASED ON COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY

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It is well-established that food-producing animals are exposed to various environmental toxicants like dioxins, brominated flamme retardants, polycyclic aromatic hydrocarbons and pesticides and that these compounds entering the animals are transferred to edible tissue, thus representing a chemical human health hazard. In contrast, little is known about the influence of current processing steps like cooking on the food content in these toxicants. The later issue requests the multi-residue determination of a wide spectrum of toxicants which may be present at trace level in complex food matrices. Nowadays, comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-MS/TOF) is probably the most promising technique to cope with this analytical challenge. The present study aimed 1/ to develop a multi-residue method based on GC×GC-MS/TOF to determine 155 toxicants that may be found in meat including polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyl (PCBs), polybrominated diphenyl ethers (PBDEs), polyaromatic hydrocarbon (PAHs), organochlorine (OCs) and organophosphorus (OPs) pesticides and 2/ to investigate the consequence of cooking on minced-meat spiked with these compounds. In a first step, the separation power and the sensitivity of the GCxGC-MS/TOF method were optimized on a mix of the 155 toxicants in hexane. Silanized glassware and analyte protectants were used in order to prevent known activitation phenomena all along the analytical process. Several column sets and modulation parameters were benchmarked. The best set-up included a BPX-5 (30m×0.25mm i.d.×0.25µmdf) x BPX-50 (1mx0.1mm i.d.x0.1µmdf) column set, a 5 second modulation period and a 100 Htz acquisition rate. In a second step, extraction, clean-up and concentration procedures were investigated on minced-meat spiked with the 155 toxicants. Pressurized liquid extraction, freezing-lipid filtration and vacuum concentration were chosen and optimized to achieve the best reproducibility and recovery rates. The performance of the developed method was assessed in terms of separation efficiency, linear range, limit of quantification (LOQ), limit of detection (LOD) and reliability. Finally, the GC×GC-MS/TOF-based method was applied for studying the influence of some standard cooking processes on minced-meat content in environmental toxicants. The paper discusses the toxicant behaviour during cooking on the basis of their physico-chemical properties.

Keywords: GCxGC-TOF/MS, meat cooking, environmental toxicants, pressurized liquid extraction, PCDD/Fs, PBDEs, PCBs, PAHs, OCs, OPs

L-24
APPLICABILITY OF GC-MS/MS FOR DETERMINATION OF PCDD/FS AND PCBS IN FEED AND FOOD

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For the analysis of PCDD/Fs and dioxin-like PCBs (DL-PCBs) in food and feed, screening and confirmatory methods can be applied according to EU regulations. Screening methods can comprise e.g. GC-MS, GC-MS/MS methods or bioanalytical screening methods, confirmatory methods are defined as GC-HRMS methods. The objective of this evaluation is to check the ability of GC-MS/MS systems for analyzing PCDD/Fs and DL-PCBs in feed and food at the level of interest. Important criteria for application of a GC/MS method for screening and possibly also for confirmation are: - Availability of applicable detection methods, - Criteria for identification and - Definition of working range and limit of quantification (LOQ) Therefore the results of GC-MS/MS measurements (TSQ Quantum XLS Ultra Triple-Quadrupole GC-MS/MS, Thermo Scientific, Austin, USA) were compared with routine GC-HRMS measurements using DFS High Resolution MS and MAT95XP (Thermo Scientific, Bremen, Germany) using the identical food and feed extracts. The GC-MS/MS system is in principle applicable for the PCDD/F and dioxin-like PCB analysis in food and feed samples. A good correlation between the results of GC-MS/MS and GC-HRMS could be observed for the analyzed matrices, higher deviations occurred only in the range below 1 pg WHO-PCDD/F-TEQ/g fat depending on limit of quantification and amount of fat applied for clean-up. A calculation of the limit of quantification (LOQ) from the signal-to-noise ratio, as defined in EU regulations and performed for GC-HRMS, was not possible due to the very low noise levels for GC-MS/MS. Therefore criteria for the calculation of the LOQ from the lowest calibrated level were developed. A modified clean-up with a reduced number of clean-up steps to serve as faster screening method was applied in order to test the robustness of the GC-MS/MS systems. The comparison between the normal and the reduced clean-up showed no significant increase of the baseline noise in GC-MS/MS mass traces, but in some cases additional interfering peaks occurred.

Keywords: GC-MS/MS, PCDD/F, PCB, Food, Feed

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L-25*
FORMATION OF DIOXINS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS IN COOKING OIL FUMES

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Introduction Polychlorinated dibenzo-p-dioxins dibenzofurans (PCDD/Fs, dioxins) and dioxin-like polychlorinated biphenyls (dl-PCBs) are ubiquitous and persistent compounds with a well known potential toxicity. Although human exposure to PCDD/Fs and dl-PCBs can occur by various routes, cooking processes are the most direct routes. Previous studies indicated that PCDD/Fs and dl-PCBs could be produced during cooking beef with soybean oil and organic chlorine-containing flavorings (sucralose or chloropropanols). Deep-fat frying, which widely used domestically and commercially, is one of the popular food preparations under consistent high temperature. Although volatile aldehydes and polycyclic aromatic hydrocarbons (PAHs) were detected in various cooking oil fumes, there has been no report on the generation of PCDD/Fs and dl-PCBs in different cooking oil fumes to our knowledge. Thus, this study was carried out to investigate the formation of PCDD/Fs and dl-PCBs in olive, peanut and soybean oil fumes under high temperature with the addition of sucralose.
Materials and Methods Cooking oils (extra virgin oil, peanut oil or soybean oil) 50 g with sucralose 5 g were heated respectively at 245°C for 15 min. An air pump, with an air flow rate of 25 L/min, was turned on during heating and left running for an additional 10 min during cooling to trap oil fumes. Approximately 20 g XAD-2 resin was used for each sample to absorb the oil fumes. Experiments were run in duplicates. PCDD/F and dl-PCB congeners were analyzed by isotopic dilution HRGC/HRMS.
Results and Discussion The concentrations of PCDD/Fs and dl-PCBs in oil fumes collected during heating were recalculated based on the weight of the original oil to correct for this weight loss. When the oils were heated without addition of sucralose, the levels of PCDD/Fs and dl-PCBs in oil fumes were relatively low. Interestingly, the generation of PCDD/Fs and dl-PCBs in oil fumes was almost at the same level for the three oils under study, even the smoke point of extra virgin oil (195°C) is relatively lower than peanut oil and soybean oil (above 230°C). CB 105, CB 114, CB 118, CB156 and CB189 were the major congeners in twelve dl-PCBs congeners. CB 126 contributed preferentially to the TEQs, which made up more than 80% of the total TEQ. CB 169 was the secondary contributor to the total TEQ. 1234678-HpCDF, OCDF, 1234678-HpCDD and OCDD were the four most abundant congeners for the seventeen 2378-PCDD/Fs congeners and 2378-TCDF, 23478-PeCDF, OCDF and 12378-PeDD were the top four toxic contributors for the TEQ of PCDD/Fs. PCDFs levels were much higher than PCDDs, which indicated that the formation of PCDFs was more favorable than PCDDs in this study. The total concentrations of PCDD/Fs were dramaticaly higher than dl-PCBs, which made up nearly 90% of the total concentrations of PCDD/Fs and dl-PCBs. Correspondingly, PCDD/Fs TEQ contributed nearly 99% of the total PCDD/Fs and dl-PCBs TEQ.

Keywords: Cooking, Oil fumes, PCDD/Fs, Dl-PCBs

L-26
THE USE OF "OMICS" APPROACHES IN DEORPHANIZING THE KEY AROMA COMPOUNDS RESPONSIBLE FOR AROMA PERCEPTION OF ROASTED HAZELNUTS

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Roasting is the key process converting raw hazelnuts into a semi-manufactured product with a characteristic aroma. In a previous study, the heat-induced aroma generation in Italian hazelnuts of the variety Tonda Romana was studied by means of the molecular sensory science approach revealing that the pairs 2- and 3-methylbutanal (malty), 2-acetyl- and 2-propionyl-1-pyrroline (roasty, popcorn-like), 2,3-pentandione and 2,3-butandione (buttery), 2-furanmethanethiol and 2-thiophenemethanethiol (coffee-like) as well as 5-methyl-(E)-2-hepten-4-one and 3-methyl-4-heptanone (fruity, hazelnut-like) are among the most important aroma-active compounds. However, further sensory experiments indicated that different aromas are obtained by changing the roasting regime as well as the hazelnut variety. Hence, the aim of the present study was to analyze the heat-induced aroma generation by means of targeted and untargeted approaches, and to correlate the data with the overall sensory impact of the respective samples. The targeted analysis of hazelnuts' Sensometabolome comprises the identification and quantitation of key odorants across a larger sample set. The development of a new comprehensive quantitative approach on basis of GC×GC-TOF-MS in combination with stable isotope dilution assays will be presented in detail. Recombination experiments finally verified the effectiveness of the Sensomics approach to understand the generation of hazelnut aroma on a molecular basis. Then, by means of GC×GC-TOF-MS, the heat-induced changes of the total volatile hazelnut metabolome were investigated employing the so-called COMMA approach. This way, marker compounds are located by application of an untargeted comparative analysis, and the data are correlated with the overall aroma as well as with the outcome of the Sensomics approach. The results showed that a combination of both methods is a useful tool in understanding and controlling the aroma generation during hazelnut roasting.

Keywords: Roasting, aroma, hazelnuts, omics approach, GC×GC-TOF-MS

L-27
ION MOBILITY SPECTROMETRY: A NEW GREEN ANALYTICAL TECHNIQUE FOR DETERMINATION OF VOLATILE COMPOUNDS IN FOOD SAMPLES

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The conventional chromatographic techniques provide quality information but in almost all cases these techniques require tedious previous sample treatment steps and sometimes also provide more information than needed by customers. Therefore, vanguard analytical techniques play an important role in the analysis of food samples because they can also provide quality information in an easy and rapid manner. Ion Mobility Spectrometry (IMS) is a vanguard technique since it can provide useful information of gaseous compounds present in a sample in a few seconds at ambient temperature and pressure. Moreover, IMS presents attractive features among which its sensitivity, versatility, low cost, and portability can be highlighted and they are very important in analytical chemistry field. Furthermore, other important advantage is related to the non-use of organic solvents in the development of the analytical methods, and for this reason IMS can be classified as a green analytical technique. According to the potential of this technique, usefulness of IMS in different food analysis fields has been demonstrated. Firstly, characteristics ion mobility spectra for volatile compounds present in fat samples were used to authenticate the feeding regime of Iberian pigs [1], more than 95 % of the Iberian pigs were correctly predicted according to feeding regimes. In a second project, volatile amines present in fish samples were also determined by IMS to carry out safety or quality control. The results obtained testify to the good precision and robustness of the proposed method [2]. Thirdly, white wines were classified according to their origin using a gas phase separator which was coupled to the IMS device using a flow injection system [3]. Finally, a headspace system was on-line coupled to the IMS to know the quality of an olive oil [4]. The results obtained provide very promising perspectives for the use of IMS to differentiate olive oils samples according to their quality instead of using the classical analytical procedure. Based on all of these results, IMS can be qualified as a new powerful green analytical tool for quality and safety control of food samples.

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Keywords: Ion mobility spectrometry, green analytical technique, Iberian ham, fish, wine and olive oil

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L-28*
RAPID AND SIMULTANEOUS ANALYSIS OF XANTHINES AND POLYPHENOLS AS POTENTIAL BITTER TASTE MARKERS IN BAKERY PRODUCTS BY FOURIER-TRANSFORM NEAR INFRARED (FT-NIR) SPECTROSCOPY

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Several reports in the literature deal with the development of “electronic tongues" for the determination of soluble compounds responsible to elicit different gustative perceptions: the most travelled route is based on an array of sensors operating in solution. Different categories of compounds present in relatively high concentration (hundreds of ppm) like xanthines (caffeine, theobromine, theophilline) or polyphenols (cathechins, epicathechins), are considered responsible for the bitter taste of coffee\cocoa\chocolate based bakery products. Commonly, these molecules are detected using high performance liquid chromatography (HPLC) procedures that are too expensive and time consuming for quality control purposes. To overcome this problem, we developed an analytical strategy based on rapid and non-invasive FT-NIR measurements that can be performed directly on the homogeneously ground solid product. A real example is here reported concerning biscuits evaluation. As first step, the concentration of main xanthines and polyphenols has been quantified on a hot methanol/water (70:30) extract of reference products, using dedicated HPLC-MS investigations. Then, reference products have been mixed in opportune proportions, calculating the final concentration of xanthines and polyphenols inside each mix and using them to generate calibration curves on the FT-NIR instrument, recording about 100 spectra. Calibration models were obtained by using partial least squares (PLS) regression with an external validation technique. Values of the standard errors of prediction (3 ppm and 77 ppm for polyphenols and xanthines, respectively) were comparable to the values of the standard errors of cross-validation. Coefficients of determination indicated a good predictivity of xanthines and polyphenols levels in the PLS calibration model (r^2 xanthines = 0.97, r^2 polyphenols = 0.96) and a similar satisfying discrimination among different contents in the PLS validation models (r^2 xanthines = 0.96, r^2 polyphenols = 0.96). A real validation phase of the generated predictive model was executed by a comparison of further data HPLC-MS vs FTNIR responses, recorded on unknown biscuits: concentration differences between found and predicted levels were generally below 5%. This methodology is able to work directly on solid products and has the potential to be expanded on other categories of gustative molecular markers, like sugars in the case of sweet taste perception.

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Keywords: FT-NIR Spectroscopy, Bakery Products, Bitter Taste, Xanthines, Polyphenols

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L-29
ANALYTICAL AND SENSORY METHODS FOR THE DETECTION OF OFF-FLAVORS

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The odor active fraction of a product (food or non-food) is normally the smallest part of the product but the most important one for accepting or rejecting it. Due to the fact that the volatile fraction can consist of a large number of different substances having different chemical structures, polarities and concentrations over several orders of magnitude makes the identification of the "key compounds" can be a really tricky game. To solve the puzzle a combination of several techniques is required to target the relevant substances. The first approach includes a multitude of sensory methods i.e. where human panelists act as analytical instruments judging the odor of a product. The second approach to odor analysis involves analytical instruments and since odor-active compounds are volatile per se, gas chromatography (GC) is the method of choice. However, no analytical instrument can deliver the most important information –whether a compound is odor-active or not. Additionally, for odor analysis several challenges have to be accounted for when employing analytical instrumentation. First, most odor-active compounds can have sensory threshold in very low concentration ranges down to the nanogram per kilogram range. Thus, one needs to develop methods which have their detection and quantification limits in the same range. This involves concentration steps and the risk of contamination and/or analyte losses. Second, in most cases the "quest" for these small concentrations is often heavily disturbed by non-odor-active volatiles which are present in several magnitudes higher concentrations, often above the maximum GC column capacity, and one has to separate these components from the compounds of interest before the GC analyses, especially when bearing in mind the needed concentration step to reach the sensory threshold values. Several examples for the successful identification of odor active substances by the combination of sensory and analytical methods will be given in the presentation.

Keywords: Off-flavor, sensory analysis, gas chromatography-olfactometry, comprehensive GC×GC

L-30*
PTR-TOF-MS ANALYSIS OF FLAVOUR PROFILES: A NEW TOOL FOR CLASSIFYING APPLE CLONES

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Proton Transfer Reaction-Mass Spectrometry, in its recently developed implementation based on a time-of-flight mass spectrometer (PTR-ToF-MS) has been evaluated as a possible tool for rapid non-destructive investigation of the volatile compounds present in the flavour profile of apple cultivars and clones. Clone characterization is a cutting-edge problem in technical management and royalty application, not only for apple, aiming at unveiling real properties which differentiate the mutated individuals. We show that PTR-ToF-MS coupled with multivariate and data mining methods may successfully be employed to obtain accurate varietal and clonal physical fingerprinting. In particular, we studied the VOCs emission profile of five different clones belonging to three well known apple cultivars, such as Fuji, Golden Delicious and Gala. In all three cases we set classification models able to distinguish all cultivars and some of the clones considered in this study. Furthermore, in the case of Gala we also identified a set of compounds contributing to such clone characterization. Beside its applicative relevance, no data on the volatile profiling of apple clones are available so far; our study indicates the viability of a metabolomic approach for apple volatile compounds based on rapid PTR-ToF-MS fingerprinting.

Keywords: proton transfer reaction-mass spectrometry, apple (malus domestica), cultivars, clones, flavour

L-31
RECENT PROBLEMS ENCOUNTERED IN THE ANALYSIS OF FOODS FOR THE PRESENCE OF LOW LEVEL FOOD ALLERGENS

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Presently, there are three analytical platforms commonly used for the analysis of food allergens, ELISA, mass spectrometric and PCR based methods. Recently, we have encountered a number of issues when comparing the results of these methods. They include, problems associated with the comparability of ELISA based kits, the impact of differing sample preparation techniques and unexpected false positive results. While these issues may appear to be common analytical problems encountered during the analysis of foods, in the case of food allergens, they are significantly more difficult to solve. Since these issues can have a significant impact on the interpretation of analytical results, we have spent a considerable amount of time diagnosing these problems in several recent cases. The conclusions from those studies show it is often necessary to obtain results from multiple analytical platforms to determine accurate allergen levels in foods.

Keywords: Allergens, ELISA, mass spectrometry

L-32
FOOD ALLERGENS PROFILING WITH AN IMAGING SURFACE PLASMON RESONANCE-BASED BIOSENSOR

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Food allergy is a growing health concern, which currently affects approximately 4% of adults and 8% of infants. For consumer protection purposes, food producers are required by law to disclose on the product label whether a major allergen is used during the production process. The commonly employed allergen monitoring methods are highly laborious, time-consuming, and often expensive when screening for multiple allergens. Here, we utilize imaging surface plasmon resonance (iSPR) in combination with antibody array for rapid, quantitative, and multianalyte food allergens detection. We demonstrate how the use of this technology provides a complete allergen profile within short measurement time and with adequate sensitivity. The successful applicability of this approach is demonstrated by analyzing cookies and dark chocolate products from different manufacturers. This newly developed method opens the door to automate and highthroughput allergen analysis, ultimately aiming at providing the consumer with safer food.

Keywords: multiplex immunoassay, allergens screening, imaging Surface Plasmon Resonance

L-33*
MULTISCREENING OF SEVEN ALLERGENS WITH MASS SPECTROMETRY AND COMPARISON WITH COMMERCIALLY AVAILABLE ELISA SYSTEMS

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Allergens are recognized as a major health issue with approximately 8% of children and 2% of the adult population affected. Symptoms occur immediately and can affect the skin, the respiratory and the gastrointestinal tract and may lead to systemic anaphylaxis. More than 160 foods have been shown to evoke a reaction, however only eight of them account for more than 90% of all allergic reactions. In the European Union directive 2007/68/EC lists a total of 13 food allergen groups that are mandatory to label if used as an ingredient. Despite this regulation, total avoidance might be difficult for the allergic consumer, as cross-contamination, e.g. due to the manufacturing on the same production line, occurs. Allergen risk management remains an important issue and analytical methods for the detection of undeclared allergens are needed. Two analytical methods are mainly used for allergen detection: antibody based ELISA and PCR. ELISA test have relatively analysis time and easy handling, however they are not capable of multiplexing. When a sample needs to be analyzed for more than one or two allergens, analysis time and cost increase significantly. Another issue is the influence of processing on the allergen. Processing might destroy the epitopes leading to false negative results. PCR methods have the disadvantage that the DNA is detected and not the allergic protein itself. This might not correlate with the amount of allergenic protein. The presentation will focus on a new multiscreening approach based on triple-quadrupole mass spectrometry. It is capable of simultaneously detecting seven allergens (milk, egg, soy, peanut, hazelnut, walnut, and almond). After extraction the allergens are digested with trypsin and separated by HPLC and analyzed in multiple reaction mode. The selection and the validation of the peptide marker are shown. To evaluate the influence of processing on the detection method, spiked flour samples and incurred bread reference material containing the seven allergens have been produced and analyzed. Results were compared with commercially available ELISA test kits. Both methods were capable of detecting peanut, hazelnut, walnut and almond in processed and unprocessed samples. MS could also detect egg in the processed samples. With the exception of one kit, egg could not be detected with ELISA.

Keywords: food allergens, mass spectrometry, multiplexing

L-34
DEVELOPMENT AND VALIDATION OF A DUPLEX REAL-TIME PCR METHOD FOR THE SIMULTANEOUS DETECTION OF CELERY AND WHITE MUSTARD IN FOOD

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Celery and mustard are known to elicit severe allergic reactions in sensitized persons, including the oral allergy syndrome (OAS), symptoms affecting the skin, the gastrointestinal tract, the respiratory system as well as life-threatening reactions like anaphylaxis. Both allergies are rather common in Central Europe. Celery (celery root: Apium graveolens var. Rapaceum; leaf celery: A. g. var. Secalinum; celery stalks: A. g. var. Dulce) and mustard (white or yellow mustard: Sinapis alba; black mustard: Brassica nigra; brown or oriental mustard: Brassica juncea) are frequently used as ingredients in sauces, spices, sausages and other meat-products as well as in convenience products. Within the European Union, the presence of potentially allergenic celery and mustard in foodstuffs has to be declared according to the EU legislative 2007/68/EC. The aim of the present study was to develop and validate a duplex real-time PCR method allowing the simultaneous detection of traces of celery and white mustard in food. The duplex real-time PCR method was developed starting from two previously published singleplex assays for the detection of celery and white mustard [1,2]. Primers and TaqMan probes were targeted at the Apium graveolens NADPH-dependent mannose-6-phosphate reductase mRNA and the Sinapis alba mRNA for MADS D protein, respectively. The optimized duplex assay allows the detection of DNA from celery root, leaf celery and celery stalks as well as DNA from white mustard. The method does not show any cross-reactivity with 64 different biological species, including various members of the Brassicaceae and Apiaceae family. The limit of detection (LOD) in serially diluted extracts from celery root, leaf celery, celery stalks and white mustard was found to be 10 pg. The PCR efficiency was 99.4% for celery root, 108.3% for celery stalks, 96.5% for leaf celery and 99.0% for white mustard. In both raw and brewed model sausages containing known concentrations of celery and white mustard, the LOD was 0.005% (50 mg/kg) celery and 0.001% (10 mg/kg) white mustard. The PCR efficiency was 90.0% (celery) and 101.1% (white mustard) in raw model sausages and 85.8% (celery) and 91.2% (white mustard) in brewed model sausages. The duplex real-time PCR method was applied to verify correct labeling of commercial food products.

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Keywords: allergen, celery, white mustard, real-time PCR, duplex assay

L-35
ALLERGENS TESTING BY ELISA KITS BENEFITS FROM A STANDARDISED CALIBRANT

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The problem of allergens analysis using ELISA kits from different manufacturers giving significantly different results is widely acknowledged. The effect on proficiency testing results, with different assigned values having to be generated for different kits, has been documented previously [1]. In response to this problem, some experimental FAPAS proficiency tests aimed to establish whether the use of a standardised calibrant could be used to normalise the complete data set without recourse to differentiation. Three recent FAPAS proficiency tests (2776 peanut, 2778 soya and 2781 gluten) sent out three test samples, instead of the usual two. As in a normal allergens test, one of the samples was blank. The other two samples were spiked (at different levels in 2776 and 2781). The spiked samples for 2778 were prepared at the same level. The proficiency tests then followed the normal pattern, with z-scores issued on the basis of differentiation by kit manufacturer and for each test sample. Further analysis of the data was undertaken after the completion of the tests. The ratio of the submitted results for the two spiked samples yielded complete data sets which could be tested for normality of the distribution. Where the raw data for each individual test sample was clearly non-normal and multi-modal, the ratio data yielded a much more normal and symmetrical distribution. The use of one of the test samples as a single-point calibrant has some limitations but the principle of applying a standardisation clearly works. The development of internationally-recognised sets of certified reference calibration standards for use by allergens testing laboratories would greatly benefit the analysis.

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Keywords: Proficiency testing, allergens, calibration standard

L-36
RECENT PROGRESS IN RAPID METHODS FOR FOOD QUALITY AND SAFETY CONTROL

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The presence of potentially hazardous chemicals in food remains a major concern among consumers. Recent food contamination incidents, e.g. the fraudulent addition to animal feed in Germany of fatty acids meant to be used for technical purposes, leading to high levels of dioxins in eggs, certainly contribute to fears about the safety of food. Currently, a variety of analytical test methods is used to help ensure the safety of food and feed in Europe, both for goods produced in the EU and imported from third countries. Many of these methods are tedious and time consuming and require sophisticated and expensive instrumentation. The CONffIDENCE project aims to further improve food and feed safety in Europe and beyond by the development of faster and cost-efficient methods for the detection of a wide range of chemical contaminants in different food and feed commodities. These methods will not only save precious time in ever faster production cycles, but will also permit more food/ feed samples to be monitored due to the lower costs per test. In combination with the broadened spectrum of detectable residues and contaminants the CONffIDENCE project will significantly increase food safety in Europe. Within CONffIDENCE, rapid and simplified multi-methods have been developed for: • persistent organic pollutants: PCB's, brominated flame retardants, PAH's • perfluorinated compounds: PFOS, PFOA, FOSA • pesticides: dithiocarbamates, paraquat • antibiotics: tetracyclines, sulphonamides, quinolones, chloramphenicol, tylosin, malachite green • coccidiostats: lasalocid, monensin, narasin, salinomycin, nicarbazin and diclazuril • heavy metal speciation: inorganic arsenic, methylmercury • alkaloids: ergot, pyrrolizidine and tropane • marine biotoxins: PSP, DSP, ASP, palytoxin and tetrodotoxin • mycotoxins: DON, zearalenone, fumonisins and T-2/HT-2 in products such as seafood, fish feed, cereal-based food and feed, dairy products, vegetables, honey and meat. A balanced mix of novel multiplex technologies has been utilized, including lateral flow devices, flow cytometry with functionalized beads, optical and electrochemical biosensors, metabolomics-like comprehensive profiling, ambient MS and NIR hyperspectral imaging. Currently, most methods have been in-house validated and in the final phase of the project small-scale collaborative studies will be organized. Moreover, the simplified methods will be applied in impact demonstrators that contribute to exposure assessment and validation of hazard models. The consortium consists of 16 partners from 10 European countries, representing 8 research institutes, 5 universities, 2 large food and feed industries and 1 SME. CONffIDENCE has started in May 2008, has a duration of 4 years and is coordinated by RIKILT – Institute of Food Safety, The Netherlands. In the presentation, key results from CONffIDENCE will be presented.

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Keywords: food, chemical contaminants, rapid methods

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L-37
A NOVEL SOLUTION FOR THE RAPID CONTROL OF MULTIPLE PESTICIDE RESIDUES IN TEA

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Current analytical approaches used for the determination of gas chromatography (GC)-amenable pesticide residues in complex matrices with low moisture such as tea are time consuming, require high consumption of solvents, and only a limited number of target analytes is involved. During the isolation step, troublesome bulky coextracts (mainly caffeine) are typically co-isolated. When not removed from the extracts, these compounds can cause serious problems during both GC separation and mass spectrometric (MS) detection. Therefore, streamlining of sample preparation strategy for this type of matrix is an essential part during the pesticide residue analysis. In addition, detection step is also crucial to avoid potential interferences with target masses of particular analytes. In this study, we present a novel solution for the rapid control of GC-amenable multiple pesticide residues (>100) in green and black tea. During the optimization of the sample preparation, a rapid and flexible control of the coextracts isolated from tea (in particular caffeine) was introduced by means of ambient mass spectrometry employing a direct analysis in real time (DART) ion source coupled to a time-of-flight mass spectrometer (TOFMS). In the case of detection of target analytes, various mass analyzes were critically assessed: (i) triple quadrupole (QQQ), (ii) high-resolution TOF, (iii) high-speed unit-resolution TOF, and (iv) high-speed high-resolution TOF. Both sample preparation and MS detection were optimized/selected with the expectation of enhanced speed, high accuracy, and improved selectivity of the analysis.

Keywords: pesticide residue analysis, complex matrices, tea, gas chromatography, mass spectrometry

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L-38
INORGANIC ARSENIC DETERMINED BY SPE SEPARATION AND AAS DETECTION – A NOVEL SPECIATION APPROACH

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Arsenic (As) is a naturally occurring element, which is found at concentrations in the mg/kg range in marine animals. The element is bioaccumulated from seawater. It has a very complex chemistry and more than 50 naturally-occurring arsenic containing species, both inorganic and organic forms, have been identified in marine animals. The organic forms are mainly considered to be non-toxic, whereas inorganic arsenic is highly toxic and exposure may lead to severe adverse effects including cancer. An accurate estimation of inorganic arsenic exposure is therefore highly relevant for evaluation of food safety. However, so far most of the occurrence data collected in the official EU food control are still reported as total arsenic. A simple and inexpensive method for determination of inorganic arsenic in marine based food and feed by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE) has been developed and validated. The SPE separation is based on the different charges (pKa values) of the arsenic species at specific pH, which allow selective elution of organic arsenic compounds (e.g. MA, DMA and AB) and inorganic arsenic in the form of As(V). The sample is heated with a hydrochloric acid and hydrogen peroxide solution (20 minutes at 90°C with 0.06 M HCl, 3% H_2O_2). Hereby the sample is solubilised and As(III) is oxidised to As(V). Inorganic arsenic is selectively separated from other arsenic compounds using strong anion exchange SPE. The procedure include first pre-condition of the column, then loading of the buffered samples (pH 5.0–7.5), washing with 0.5 M acetic acid and finally elution of the sample from the column by 0.5 M HCl. The concentration of arsenic is determined by HG-AAS using external standards. SPE method development and sample extraction was evaluated using a selective HPLC-ICP-MS detection method. No degradation or conversion of organic arsenic species such as AB, MA or DMA were observed under the chosen extraction conditions. The results obtained by SPE-HG-AAS and HPLC-ICP-MS were not significantly different (95% confidence). The method was validated by spiked and naturally incurred marine samples. The limit of detection was 0.08 mg/kg and the in-house reproducibility standard deviations were less than ≤13% for samples containing 0.2 to 1.5 mg/kg inorganic arsenic. The method has furthermore been tested in a collaborative trial on marine feed and food with a satisfactory result and is now in the process for CEN approval as a future European standard method.

Keywords: Inorganic arsenic, speciation, solid phase extraction, atomic absorption spectroscopy, validation.

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L-39*
HIGH-THROUGHPUT GC-MS/MS ANALYSIS OF BFRS (INCLUDING EMERGING COMPOUNDS) IN FISH/SEAFOOD

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Gas chromatography coupled to triple quadrupole mass spectrometry (GC–MS/MS) represents a powerful tool for a highly sensitive and selective determination of various groups of environmental contaminants. In this study, analytical method for identification and quantification of different groups of brominated flame retardants (BFRs) in fish muscle tissue was developed and validated. Not only routinely analyzed polybrominated diphenyl ethers (PBDEs) but also polybrominated biphenyls (PBB) and other alternative BFRs (e.g., decabromodiphenyl ethane and bis(2,4,6-tribromophenoxy)ethane) which are suggested for the monitoring by the European Food Safety Authority (EFSA), were on the target list.
In the first phase, the analysis of solvent standard was carried out and selective transitions for all compounds were chosen. Subsequently, injection parameters and chromatographical separation on different capillary columns (e.g., HP5, Rxi–1614) were optimized to obtain the best chromatographic resolution and quantification limits (LOQs) of all target analytes. The possible thermodegradation of highly brominated PBDEs, especially decabrominated BDE209, had to be taken into consideration and therefore relatively short oven temperature program (< 19 min) with injection in programmable temperature vaporization mode (PTV) was applied.
Finally, fish muscle tissues with different fat content (e.g., trout and salmon – 2 and 14% fat (w/w), respectively) were spiked with all target compounds at two different concentration levels (1 and 5 μg kg^{-1}). Extracts were prepared using ethyl acetate; transfer of non-polar analytes from the aqueous suspension to ethyl acetate layer was supported by addition of inorganic salts. Co-extracted lipids contained in crude organic extract obtained by partition, were subsequently removed on a silica minicolumn. This approach enabled to process six samples in less than one hour; moreover, the volume of an extraction solvent and consumption of other chemicals can be significantly reduced compared to classical Soxhlet extraction based procedure. The recoveries of all target analytes were in the range 73–109% and repeatabilites (expressed as relative standard deviation, RSD) did not exceed 15% even at lower spiking level. Under optimized GC–MS/MS (EI) conditions using Agilent 7000 triple quadrupole the limits of quantification (LOQs) were 0.005–0.5 μg kg^{-1} (higher values were achieved for higher brominated BFRs). Further decrease of LOQs might be obtained by large volume injection (LVI) and by chemical ionisation (CI) that will be tested in the following experiments.

Keywords: GC–MS/MS, BFR, fish

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L-40*
MULTIPLEX SCREENING OF PERSISTENT ORGANIC POLLUTANTS IN FISH USING SPECTRALLY-ENCODED MICROSPHERES

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Persistent organic pollutants (POPs) are food contaminants of a global public health concern and known to be carcinogenic and endocrine disruptors. Their monitoring is essential and an easy-to-use, rapid and affordable multi-analyte screening method with a simplified sample preparation can be a valuable tool prior to instrumental analysis. For this purpose, a flow cytometric immunoassay (FCIA), based on a spectrally-encoded microbeads suspension array technology, was developed for the multiplex detection of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and the emerging polybrominated diphenyl ethers (BDEs) in buffer and fish extracts. The sensitivities of the assays in the 3-plex FCIA format were similar to the individual FCIAs for the marker compounds benzo[a]pyrene, 1,1'-Biphenyl, 3,3',4,4'-tetrachloro- (PCB77) and benzene, 2,4-dibromo-1-(2,4-dibromophenoxy) (BDE47) in buffer with IC50 values of 0.4, 20 and 2 μg L^{-1}, respectively. Apart from the three markers, we could detect at least 14 other POPs. Extracts of fish with different fat contents, prepared with a simplified extraction procedure had an insignificant influence on the overall 3-plex FCIA performance, with the exception of some impact on the PAHs detection. The performance of the 3-plex FCIA, in combination with the simple extraction procedure, is adequate for regulatory control in accordance to the required limits.

Keywords: Multiplex immunoassay, Flow cytometry, POPs, PCBs, BDEs, PAHs, QuEChERS

Acknowledgement: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° KBBE-211326 and the Dutch Ministry of Economic affairs, Agriculture and Innovation. We thank Prof. Dietmar Knopp for providing us the antibodies and conjugates, Prof Jana Hajslova for her creative input, Lucie Drabová for helping in the preparation of the fish extracts and Dimitris Mintzas for the preliminary investigation of the singleplex FCIAs.

L-41
MEASURING BIOTOXINS AND THE ANALYTICAL CHALLENGES STILL AHEAD

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The measurement of small molecular weight contaminants present in foods has increased in importance over the past 20 years for a wide variety of reasons. The range of chemicals measured has markedly increased and the sensitivity of the methods has markedly improved. In some instances such as pesticides and drug residues it can be argued that the method development phase is very mature and only minor modifications are now occurring to the very well established mass spectrometric procedures. Biotoxins, i.e. those chemicals produced by plants, algae, fungi and bacteria still pose a significant range of challenges with regards accurate detection and quantification. Regulators still struggle to determine which of the thousands of potential biotoxins present pose the greatest threats to the consumers and how to balance these risks against assuring a constant and economically viable food supply chain. The algal toxins, which bioaccumulate in many aquatic species, are one such case. Many biotoxins have been identified and shown to cause acute poisonings in animal models and as a result of human exposures; however they are all not regulated due to difficulties in obtaining reference standards to perform important acute and chronic toxicity studies. Some biotoxin families contain multiple congeners which all have different toxicity profiles and in some cases different classes of toxins can be found in single samples and the nature of how these may interact synergistically is far from understood. The use of animal based testing methods for algal toxins long served the public to protect them from exposure however due to a combination of ethical and performance related concerns these are being deregulated in many parts of the world. The presentation will address how the multiple challenges of measuring algal biotoxins is being addressed by the use of innovative technology platforms and outline the need for regulatory authorities to permit such methods to be used in monitoring programmes to better safeguard consumers.

Keywords: Biotoxins, detection, biosensors

L-42*
DEVELOPMENT OF QUANTUM DOTS-BASED LATERAL FLOW IMMUNOASSAY FOR DETECTION OF CHLORAMPHENICOL IN MILK

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One of the important tasks for ensuring food safety is detection of veterinary drugs. Chloramphenicol (CAP) is an antibiotic intensively used in livestock and poultry farming for therapy of bacterial infections and due to this accumulated in foodstuffs of animal origin. Majority of countries have been established maximum residue levels of CAP for different foodstuffs. Instrumental methods (chromatography, ELISA, etc.) are generally used to detect drug residues. These methods are sensitive, but are not suitable for rapid screening. In contrast, lateral flow (immunochromatographic) tests are labor-efficient rapid assays. Commercial immunochromatographic strips are based on gold or latex particles. The aim of our investigation was to develop and apply at first time quantum dots (QDs)-based fluorescent strips for food control on the example of CAP control in milk. Invitrogen water-soluble QDs with emission peak at 625 nm were covalently coupled with anti-CAP monoclonal antibodies. To produce lateral flow strip, CAP-bovine serum albumin conjugate was immobilized onto MDI (India) nitrocellulose membrane as test line, and goat-anti-mouse antibody - as control line. The obtained QD–antibody conjugate was added onto a macroporous pad of the strip. Pure and CAP-spiked cow milk samples were diluted to 20% to eliminate the matrix effect. After their contact with the strip QD-labeled antibodies migrated along the membrane by capillary forces and competitively interact with native and conjugated CAP. The bounded QDs in test and control zones were detected under excitation by UV-light, thus, one or two red fluorescent lines could be seen. Limit of CAP revealing in milk by this assay with visual detection of test line disappearance is 5 ng/mL. In the case of using portative reader, the working range of quantitative assay is 0.25-2 ng/mL. The analysis time is 20 min. The storage of test strips during 6 months at RT does not lead to reliable change of their characteristics. The obtained results demonstrate efficiency of QDs in rapid tests application for dairy foodstuffs.

Keywords: chloramphenicol, milk, quantum dots, lateral flow assay

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L-43
DETERMINATION OF PYRROLIZIDINE, TROPANE AND ERGOT ALKALOIDS IN HONEY, FEED AND CEREALS AND DETECTION OF ERGOT CONTAMINATION IN CEREALS

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The European FP7 project CONffIDENCE is dedicated to the development of inexpensive detection methods for contaminants in food and feed. Among other topics attention is given to methods for the determination of various types of alkaloids as well as alkaloid-containing sclerotia, which may contaminate cereal grains. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They are produced as secondary metabolites by various organisms, mainly plants. Alkaloids are often physiologically active and poisonous. They may display hepatotoxic, carcinogenic, teratogenic and mutagenic effects. Alkaloids can be found in food of plant origin (e.g. cereals, herbs), animal origin (e.g. honey, eggs, milk) and in animal feed. In the project multiplex lateral flow immunoassays are developed to determine three different families of alkaloids (pyrrolizidine alkaloids (PA) in honey and feed; tropane alkaloids (TA) in feed; and ergot alkaloids (EA) in cereals and feed). In addition a near infrared (NIR) hyperspectral imaging spectroscopy method is developed, with which ergot kernels(the fruit bodies of the fungus Claviceps purpurea) can be detected as botanical impurities “in line” in sample streams of cereal grains. In the CONffIDENCE work package “Alkaloids” several steps are distinguished, of which several have been (largely) completed. They are divided over the two main tasks: 1. Multiplex dipstick development. The steps include the procurement of pure alkaloids for method development and conjugate preparation, the production of well-characterized antibodies, the preparation and characterization of test materials for prototype experiments and method validation, and the development of a simple and uniform protocol to rapidly extract samples for the multiplex dipstick determinations. Ongoing activities involve prototype testing, method extension and refinement, and inter-laboratory method transfer. The work will be continued with in-house and small scale inter-laboratory testing to validate the methodology, and then rounded of with impact demonstration through the use of dipstick assays for safety assessment on-farm and in the feed chain. 2. NIR imaging method development. The steps include the setting up and testing of the NIR hyperspectral imaging system combined with some chemometric tools in plane scan camera format, transfer the system into line scan camera format combined with a conveyor belt, followed by in-house validation. This work has been completed. Coming activities involve the installation of the system into an industrial setting for further testing and validation in practice with grain samples of the 2011 harvest.

Keywords: alkaloid, method, analysis, immunoassay, infrared
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L-44
FOOD SAFETY ISSUES, WITH FOCUS ON CONTAMINANTS - THE IMPORTANCE OF QUICK BUT RELIABLE ANALYTICAL RESULTS FOR AN EFFECTIVE ENFORCEMENT OF EU LEGISLATION

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General principles and objectives The EU legislation on contaminants in food fulfils two essential objectives: the protection of public health and removal of internal barriers to trade within the EU. Enforcement of EU legislation on contaminants in food In the EU we have a comprehensive set of feed and food safety legislation on contaminants in food to protect public health. But legislation is only effective in protecting public health if the enforcement is effective and if legislation is uniformly applied across the EU The establishment of uniform sampling and analysis procedures in that respect is of major importance Regulation (EC) 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules provides the regulatory framework for sampling and analytical procedures. This Regulation provides that sampling and analysis methods used in the context of official controls shall comply with relevant Community rules or, (a) if no such rules exist, with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation; or, (b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols. In case such methods do not exist, validation of methods of analysis may take place within a single laboratory according to an internationally accepted protocol. The Commission can take specific measures as regards a) methods of sampling and analysis, including the confirmatory or reference methods to be used in the event of a dispute; (b) performance criteria, analysis parameters, measurement uncertainty and procedures for the validation of the methods; (c) rules on the interpretation of results. EU-RL/NRL networks have been established for several contaminants and are of major importance for the effective application of feed and food safety legislation. The need for co-operation, support and assistance for in many cases complex analysis is self evident. Several enforcement approaches strategies can be followed including the use of screening methods eventually in combination with confirmatory methods etc. In the presentation specific attention will be paid to the different enforcement approaches and the requirements for sampling and analytical methodology used in these approaches and the importance for the risk manager to have quick but above all reliable analytical results to take a decision on appropriate control measures.

Keywords: contaminants, enforcement, screening methods, confirmatroy methods

L-45
GREEN ANALYTICAL METHODS IN FOOD ANALYSIS

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Nowadays, the number and complexity of analytical methodologies required to control the safety and authentication of foods involves the use of a lot of chemicals, energy and economical efforts and provides some collateral risks for both, operators and the environment, due to the use of toxic reagents and solvents and the generation of dangerous wastes. Because of that, efforts are required for greening the analytical practices to avoid the aforementioned deleterious effects. The replacement of toxic reagents by innocuous ones, the miniaturization of analytical procedures and their automation, together with a strong reduction of the analytical steps and the consideration of waste miniaturization and detoxification, as a part of the methodologies their-selves, could contribute to improve the safety of the food analytical procedures and to avoid environmental dangers. However the key factor is to maintain the main analytical figures of merit of the procedures and to save money. So, the ethical compromise with the environment and the new economic opportunities offered can assure the successful implementation of green methods. In this communication we will review the main tools for greening our daily practices at the laboratory from the use of remote and non-invasive methodologies suitable to be employed without any sample preparation, to the development of portable instrumentation and sensors to be used without a previous chemical sample treatment. Special attention will be paid to the miniaturization, automation and on-line waste treatment in food analysis. The aforementioned alternative methodologies will be evaluated using the strengths, weaknesses, opportunities and threats (SWOT) methodology. In short on greening our food analysis practice we will contribute to the sustainability of our business and prevention of pollution effects of analytical practices.

M.de la Guardia and S. Armenta "Green Analytical Chemistry: Theory and Practice" Elsevier 2011. M. de la Guardia and S Garrigues (Eds) "Challenges in Green analytical Chemistry RSC 2011

Keywords: greening analytical chemistry, waste detoxification, innocuous reagents, miniaturization, automation

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L-46
LC/MS ANALYSIS OF GLUTEN PEPTIDES DERIVED FROM SIMULATED GASTROINTESTINAL DIGESTION OF DIFFERENT WHEAT VARIETIES: QUALITY AND SAFETY IMPLICATIONS

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Gluten content of wheat is highly variable, depending on the plant genetics and the growing conditions. Beside short peptides, gastrointestinal digestion of gluten also produces longer ones, since the high proline content of gliadins (16-26%) and glutenins (11-13%) makes them very resistant to the degradation by digestive proteases. In the present work, a method for the extraction of the prolamine fraction was applied to different wheat varieties, followed by a simulated gastrointestinal digestion of the gliadin extracted. The peptide mixtures generated were characterized by LC/MS, and most abundant peptides were identified by low- and high-resolution multiple stage MS techniques and through synthesis of authentic standards. These peptides were also semiquantified in the different samples against a suitable internal standard. The peptide mixtures were found to be highly variable, according to the different content and type of gliadins present in wheat varieties, with strong differences among the varieties tested, both qualitatively (the sequences of the peptides generated) and quantitatively (their amount). The greatest difference was found between common and durum wheat varieties. Peptides present only in the former varieties were identified, and used as molecular markers for identifying and quantifying the presence of common wheat when added to durum wheat samples. Most of the peptides identified were also already known to be pathogenic for people affected by celiac disease, an autoimmune enteropathy triggered by gluten proteins, which develops in some genetically susceptible subjects after gluten consumption. Some samples belonging to defined varieties showed a lower amount of celiac-related pathogenic peptides upon digestion, due to a lower gliadin content. Albeit not safe for celiac patients, the use of these varieties in the formulations of baby food could be of great help for lowering the spread of the disease, since the prevalence of celiac disease seems to be promoted by an early exposure to a large amount of gluten peptides.

Keywords: gliadin, simulated gastrointestinal digestion, peptides identification, wheat varieties, celiac disease.

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L-47
EXPLOITING HIGH PRESSURE CONDITIONS IN COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY AS A NOVEL APPROACH IN FOOD ANALYSIS

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Nowadays, the need for more powerful and discriminating analytical techniques is deemed as essential taking into account the increasing complexity of the samples to be analyzed, and the specificity of information required. As a consequence, in order to achieve the desired level of accuracy and reliability of analytical data, the analysis of complex mixtures requires the combination of both powerful separation techniques and sensitive detection. To this regard, an ever increasing trend is towards the implementation of UHPLC (ultra high pressure liquid chromatography) methods for high efficient analyses. In fact, for some applications, one-dimensional liquid chromatography (1D-LC), due to the limited resolving power, often fails in providing enough separation power. A potential alternative could be represented by comprehensive two-dimensional LC (LC×LC), investigated in the last two decades, where all the sample is subjected to two distinct analytical separations. In this contribution we report some applications in the field of food area exploiting the high throughput and performance that can be attained with current UHPLC technology for challenging LC×LC separations

Keywords: Food analysis, comprehensive liquid chromatography, UHPLC, mass spectrometry

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L-48*
A NEW PROCEDURE TO DETERMINE POLYMERIC PROANTHOCYANINDINS IN PLANT FOODS

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Proanthocyanidins (PA) or condensed tannins are oligomers and polymers of flavan-3-ol and flavan-3,4-diols widely distributed in plant foods. Apart from their known properties associated with astringency and colour in foods, recent research has addressed their bioavailability and health-related biological properties, particularly with regard to the prevention of chronic diseases and gastrointestinal disorders (1). Proanthocyanidin determination is a major concern in food analysis, due to the complexity of their structure, what makes really difficult to find a suitable analytical procedure that could lead to the acquisition of accurate and reproducible results. The content of proanthocyanidins (PA) in foods is usually determined by HPLC analysis of aqueous-organic extracts (2), which address only PA up to 10 monomers length. However, appreciable amounts of polymeric PA that remain in the residues of extraction usually are not considered for analysis. The objective of this work was to develop a new procedure to achieve a complete quantification of food PA. Samples are treated sequentially with acidic methanol/water (50:50 v/v, pH 2) and acetone/water (70:30). Monomeric and oligomeric PA are determined in the extracts by HPLC with fluorimetric detection (2). Dry residues are treated with butanol/HCl (97.5:2.5 v/v) with 0.7 g of $FeCl_3$ at 100°C for 60 min (3, 4) to yield anthocyanidin monomers with absorbance at 555 nm, catechin-anthocyanidin complexes with xanthylium chromophores absorbing at 450 nm (5), and a residue including phlobaphene powder. The coloured compounds are detected spectrophotometrically and an estimation of the phlobaphenes formed is carried out by gravimetric determination. To our knowledge it is the first time the determination of polymeric PA includes three hydrolysis products, including phlobaphenes. We have applied this new procedure, using polymeric PA concentrate (6) as standard, to estimate PA content of some common plant foods. PA content range between 50–3000 mg/100 g for anthocyanin (555 nm) 50–1000 mg/100 g for catechin-anthocyanin compounds (450 nm) and 500–2000 mg/100 g for phlobaphenes. We can conclude proanthocyanidin content in plant foods is much higher than literature data. In fact, USDA Database for the Proanthocyianidin Content of Selected Foods only addresses values between 10-500 mg/100g for most of plant foods. In summary, food PA determination requires measurement of these compounds present in aqueous-organic extracts, and also of the hydrolysis products of the residue.

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Keywords: determination, polymeric proanthocyanidins, plant foods, phlobaphenes.

L-49
INVESTIGATION OF THE INFLUENCE OF HOUSING SYSTEM ON THE CHEMICAL COMPOSITION OF EGGS: A METABOLOMICS APPROACH

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As far as consumption of eggs by humans is concerned, chicken eggs are increasingly recognised as an important source of nutrients. There are many factors that influence the purchase and consumption of eggs from the various systems. Besides the supposed improvement of animal welfare, many consumers in Europe believe that eggs originating from free range or organic farms taste better, have a higher nutritional value and can be beneficial for human health. The price of eggs is also dependent on the housing systems in the production and increases from cage to barn to free range to organic. In the last couple of years several cases of fraud became public, where eggs from a cheaper production/housing system (cage or barn) were sold as eggs from a higher housing system (free range or organic). At the moment, the authenticity of eggs can only be assured by paper trailing and verification assessment. Analytical strategies for guaranteeing quality and uncovering adulteration have been developed for organic eggs, where the absence of added carotenoids (not added in organic production, added in other productions for extra color in egg yolk) can be verified by HPLC-DAD methods. The aim of our study was to elucidate the differences among commercial eggs from different housing systems (cage, free range, barn, and organic), based on natural chemical egg features, using various spectroscopic and chromatographic techniques on different parts of eggs, shell, egg yolk and egg white. 1800 eggs were sampled from 60 farms, within which the four housing systems were equally represented. Another 400 eggs were sampled from 40 farms in order to validate the results found in the main study. Different parts of eggs were analysed with modern techniques such as LIBS, NIR, Raman, Fluorescence, XRF, ICP-MS, LC-MS and GC-MS. In addition to the complete study information and setup, the results of metabolomics approach in egg yolk will be presented. Potential markers were found for organic eggs (other that carotenoids) and also for cage eggs. The results were confirmed by sampling additional eggs in a different season.

Keywords: eggs, housing systems, metabolomics, authenticity, markers

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L-50*
APTAMERS FOR FOOD SAFETY AND QUALITY ASSURANCE: SELECTION OF THE APTAMERS AGAINST LIVE BACTERIAL CELLS

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Aptamers are biomolecular ligands composed of nucleic acids. They can be selected to bind specifically to a range of target molecules such as proteins, bacterial cells, viruses and smaller molecular targets such as organic dyes. They can subsequently be exploited in a fashion analogous to more traditional biomolecules such as antibodies. Aptamers can be chemically synthesised. Therefore, in contrast to antibodies, no ethical issues are involved in aptamer production. The potential of the aptamers and the need for development of new aptamers with specificity against pathogenic micro-organisms will be discussed. In this study the selection method for aptamers against live bacterial cells was developed and aptamers were successfully selected. The specific aptamers were fluorescence labelled and the binding was demonstrated by measuring the fluorescence and by visualising the samples under the fluorescence microscope. These fluorescence aptamers were used to detect the live bacterial cells not only in buffer conditions but also in yogurt samples that were spiked with live bacterial cells.

Keywords: Aptamers, SELEX, foodborne pathogens, pathogen detection

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L-51
BACK-TRACING POULTRY EXPOSURE TO RAPIDLY METABOLIZED ENVIRONMENTAL TOXICANTS BASED ON VOLATILE COMPOUND METABOLIC SIGNATURES IN EDIBLE TISSUES

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We investigated the feasibility of using volatile compound signatures of liver, fat and muscle in poultry to detect previous dietary exposure to different types of environmental toxicants. Five groups of broiler chickens were fed a similar diet either non-contaminated or contaminated with polychlorinated dibenzo-p-dioxins/-furans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) or polycyclic aromatic hydrocarbons (PAHs). The liver, fat and muscle of each chicken were analysed by solid-phase microextraction – mass spectrometry (SPME-MS) for volatile compound metabolic signature and by gas chromatography – high resolution mass spectrometry (GC-HRMS) or gas chromatography – tandem mass spectrometry (GC-MS/MS) to quantify environmental toxicant residues. The results show that the volatile compound metabolic signature could clearly differentiate the non-contaminated chickens from those contaminated with PBDEs or PAHs. The results were particularly striking for PAHs because they showed a clear metabolic response in the liver although these rapidly metabolized toxicants are undetectable in this organ by the targeted reference analytical method. In contrast, the rough metabolic signature obtained by SPME-MS did not enable us to evidence previous exposure to slowly metabolized compounds such as PCDD/Fs and PCBs, the residues of which are clearly detected by targeted reference methods. Finally, the paper will discuss how the present finding might pave the way to a new generation of food safety methods which are not based on the measurement of environmental toxicant residues or their parent metabolites.

Keywords: Non targeted approach, environmental toxicants, poultry-derived food products, volatile compounds

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L-52*
QUANTIFICATION OF FURANIC COMPOUNDS PRESENT IN ESPRESSO AND AROMATIZED ESPRESSO COFFEE SAMPLES USING SPME–GC/MS

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Coffee is, besides a tea, the most consumed drink in the world(1). In literature, there are several studies related to the harmful compounds present in this drink, especially furanic compounds(2). In recent years, studies of the presence of furan in foods have increased due to it's mutagenicity and possible carcinogenicity(3). Furan and furan derivatives have been associated with the flavor components of foods. Coffee represents the food with more content of furanic compound(4). From all volatile compounds present in coffee, furan represents the major part, in particular 4 compounds: 2-furfuraldehyde, 2-furanmethanol, 5-methylfurfuraldehyde and 2-furanmethanol acetate. There are some studies related with the toxic effect of 2-furfuraldehyde and 5-methylfurfuraldhyde, but there are just a few studies about 2-furanmethanol and 2-furanmethanol acetate, which we believe can be harmful for humans. The goal of this work is to quantify these four furan compounds in 6 types of espresso coffee (3 normal espresso coffees 100% Arabica and 3 aromatized coffee samples). For these purpose, a SPME/ GC-MS method was used. Quality parameters of the HS-SPME method such as linearity, limit of detection and quantification were established. Good linearity, between 0.01 and 93 ppb, with correlation coefficients (r2) higher than 0.99 was obtained using seven different water solutions. Limits of detection (LOD) and quantification (LOQ) based on a signal-to-noise ratio (S/N) of 3:1 and 10:1, were also determined. The precision of the automated HS-SPME method was achieved analyzing three water standard solutions spiked at one concentration level of 10 ppm. Relative standard deviations (RSD %) lower than 5% were obtained. Results show that espresso coffee presents a lower number of volatile compounds, compared with aromatized espresso coffee samples. The volatile compounds were grouped in nine families showing that there is a large variety of of these compounds in the different coffees. Related to the quantification of the furanic compounds, the lowest furan compound present in all samples was furan (maximum of 1.6 ppb), and the biggest compound present was 2-furanmethanol (maximum of 1.23 ppm). Statistical analysis was made with one-way ANOVA test with 95% confidence interval, showing significant differences in all compounds present in the six coffees, except for 2-furanmethanol. In future work, it will be interesting to analyze one way to remove these compounds from coffee, once they are not vital for his aroma and could be a large step in the decrease of these compounds both in coffee and in processed food.

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Keywords: espresso coffee, furanic compounds, SPME, GC-MS
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L-53
RAPID DETECTION METHODS FOR FOOD SAFETY AND DEFENSE WITH SPECTROSCOPIC AND IMAGING SYSTEMS

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The U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) has been conducting food safety research with spectroscopic and imaging systems including monochromatic, multispectral, and hyperspectral imaging systems (HIS). With a monochromatic camera, hairline cracks in shell eggs were rapidly detected. Images of multiple eggs captured at atmospheric pressure and when subjected to a small rapid negative pressure can be used detect and identify cracks. For poultry carcass inspection, a prototype real-time HIS has been developed to detect both diseased and fecal contaminated carcasses in a processing plant. The system utilizes a single hyperspectral imaging platform, configured as a real-time multispectral imager, with distinct software-controlled wavelengths and algorithms for each application. For pathogen detection, a hyperspectral imaging technique was developed to detect and differentiate Campylobacter serotypes growing in agar plates from five contaminants often found in commercial poultry carcass rinses. A protocol for imaging the bacteria growing in the media and processing their hyperspectral images was developed with spectral libraries to discriminate the pathogens from the background microflora. This research is also being extended to image spread plates with known mixtures of multiple pure bacteria. Similarly, a HIS is being used to develop a rapid screening method to classify the "big six" non-O157:H7 shiga-toxin producing Escherichia coli (STEC) growing in agar media. On spot-plates, the method can easily discriminate between serotypes O26, O45, O121, and O145, while correctly classifying most of the O103 and O111serotypes (~90%). Further work is continuing to develop spectral libraries of background microorganisms and to classify the organisms on spread plates. On a nano-scale level, Surface Enhanced Raman Scattering (SERS) is being explored for pathogen detection in pure solutions. A nano-metal substrate for SERS and a 785 nm laser were used to detect Salmonella typ himurium in pure cultures at concentrations as low as 100 CFU/ml. Once detected, there is also a need to determine if the pathogens are still viable. In a companion study with Fourier Transform Infrared Spectroscopy (FTIR), discrimination of live Salmonella typhimurium and Salmonella enteritidis from dead cells was achieved at higher concentrations. Lower detection limits are being determined. Finally, a hyperspectral microscope imaging (HMI) method is being developed for foodborne pathogen detection at microscopic levels. An Acousto-Optic Tunable Filter (AOTF)-based HMI method has been used to characterize the spectral properties of biofilms formed by Salmonella enteritidis and by E. coli on stainless steel surfaces. Spectral signatures of these pathogens are currently being collected. Thus, USDA-ARS is currently exploring multiple spectroscopic and imaging methods to screen and/or detect various pathogens at the macro, micro, and nano-levels.

Keywords: hyperspectral imaging, optics, spectroscopy

L-54
ADVANCED PATHOGEN DETECTION SYSTEMS

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In recent years, explosion of research activities on biosensor-based technologies show great promise in rapid and sensitive detection of pathogens and toxins for food safety and food defense applications. Though many of them are designed to detect single organism or a toxin at a time, recent breakthrough in research activities demonstrate that high throughput screening of multi-pathogens in an array format is possible. Sensor technologies including light scattering sensor, cell-based biosensor, fiber-optic sensor, and microfluidic biochip show promise in their ability to detect and identify pathogens or toxins in real-time or near real-time from products and will be discussed (1–4). High throughput screening strategies will not only aid in reducing the cost per testing but also will provide results for the presence or absence of pathogens/toxins or even unknown or genetically altered organisms in samples. This is particularly attractive for food defense applications where the presence of intentionally administered select agents or genetically altered organisms in food or beverages must be detected very quickly.

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Keywords: Biosensor, Pathogen, Toxin, Detection, Identification

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L-55
RAPID ANALYSIS OF FOOD ADDITIVES AND CONTAMINANTS: APPLICATIONS WITHIN A REGULATORY FRAMEWORK

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As the time and distance from “farm to fork” increases, so does the potential for food contamination. While increased education and inspections can assist in reducing food contamination (intentional or accidental), increased testing also plays a critical role in improving the safety of food. Analytical methods, which enable authorities to increase the number of samples tested or analyze for a larger number of contaminants during routine surveillance are desirable. Ideally, a single approach that can detect targeted analytes and highlight potential unknown analytes in a variety food matrices would be utilized. However, until such methods are developed a multi-pronged testing approach to help insure the safety of the food supply is essential. As part of this multi-pronged approach we have been evaluating, direct analysis in real time (DART), turbulent flow chromatography, and portable mass spectrometry as tools for analyzing more samples using less resources and time. DART-MS, which can be performed without analyte extraction and limited sample preparation, is well suited for the analysis and characterization of food contact materials. Recently, we have been evaluating the ability of DART to identify primary aromatic amines in nylon kitchen utensils. While it is clear that DART can detect the PAAs directly from nylon utensils, correlating the response with specific migration limits (SML) is not straightforward. The ability of DART to screen for potential violative samples and results of a recent US survey will be presented. Turbulent flow chromatography, which allows for a rapid online cleanup of complex matrices, has been successfully applied to small molecule analysis in biological samples, but only a limited number of applications to food matrices have been reported. Our work has focused on developing methods for the determination of melamine and cyanuric acid in infant formula. The methods entail limited sample handling and the analysis time is nearly 15 times faster than traditional LC-MS methods. However, the shortened analysis times and limited chromatographic separation can produce challenges with coeluting isobaric interferences, carryover and substantial ion suppression from matrix effects. Much modern food testing relies on mass spectrometry for the analysis of additives and contaminants. Therefore, the development of portable mass spectrometers would provide inspectors with up to the minute information beneficial in trace back or food outbreak investigations and evaluating imports at ports or borders. To this end, we have been testing a prototype miniature mass spectrometer purchased from Purdue University. Ease of setup and operation, instrument accuracy, precision and robustness have all been tested. Additionally, rapid, straightforward sampling techniques such as “paper-spray” and ambient pressure low temperature plasma have been evaluated.

Keywords: DART, Turbulent Flow, Rapid Analysis

L-56
IMPROVED PESTICIDE ANALYSIS WITH GC-MS WITH SUPERSONIC MOLECULAR BEAMS

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An estimated 3000 chemicals are being used as pesticides worldwide (including banned pesticides and other hazardous chemicals). In view of international food trade this large number implies that pesticide analysis should not be treated as target compounds analysis. Thus, current mass spectrometry instrument development is challenged to provide a one system that will be capable of analyzing as many as possible pesticides in full scan mode, in the needed instrumental sensitivity and selectivity in complex agricultural matrices, and in a short amount of time for effective pesticides screening. In order to make a step forward towards meeting the above challenges, a new type of GC-MS with supersonic molecular beam (SMB) interface and its fly through ion source (Supersonic GC-MS) was designed and constructed. It is based on the coupling of a supersonic molecular beam inlet and its dual cage fly-through electron ionization ion source with an Agilent 7890 GC + 5975 MSD (also named 5975-SMB). The GC eluting molecules are mixed with helium make up gas, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, collimated into a SMB, pass a fly-through electron ionization ion source where they are ionized by 70 eV electrons and mass analyzed. The use of short columns (i.e. 4 meters) and high column flow rates such as 16 ml/min lowers pesticides elution temperatures by over 100ºC, thereby enabling the elution of standard as well as thermally labile "LC pesticides", combined with the provision of enhanced molecular ions to all pesticides and fast analysis with under 8 minutes full analysis cycle time. Our proposed 5975-SMB based universal method of pesticide analysis includes the following main attributes: A) Use of full scan together with times programmed SIM for selected main targeted pesticides. SIM provides sufficient instrument sensitivity, and it can be time programmed the same as MS-MS parent ion, while the full scan enables universal analysis hence should ensure against false negatives; B) The use of PTV injector, short column and high column flow rate and the fly through ion source enable the analysis of extended range of pesticides including many of those that currently require LC-MS for their analysis; C) The ions that are selected for SIM include the molecular ion, additional one isotopomer ion and only one high mass fragment since the selectivity against matrix interference is significantly improved for high mass ions hence the elimination of the two low mass fragment ions from the screening should significantly reduce matrix interference; D) The use of short column and high column flow rate enables column temperature programming at 50°C/min right from the start hence analysis cycle time of 8 minutes which is five times faster than commonly employed. In this presentation the various elements and features of improved pesticide analysis with the 5975-SMB will be described, discussed and demonstrated.

Keywords: Supersonic Molecular Beams, GC-MS, Pesticide Analysis

L-57
HIGH THROUGHPUT MONITORING APPROACH FOR MULTIPLE VETERINARY DRUG RESIDUES IN ANIMAL TISSUES

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Multiclass, multiresidue methods (MMMs) have been commonly used in pesticide monitoring programs for more than 45 years, but this concept was not commonly considered for veterinary drug residue monitoring until recently when LC-MS became widely accepted and available in regulatory labs. In our lab, we evaluated 6 MMMs from the scientific literature for UHPLC-MS/MS analysis of more than 120 high priority veterinary drug analytes in beef kidney and muscle tissue. A main conclusion is that all the methods achieved acceptable results for qualitative screening identification purposes below U.S. tolerance levels for nearly all of the analytes. In terms of quantification, each method gave between 70-120% recoveries with <20% RSDs (18 replicates at 3 levels) for about 70% of the drug analytes in spiked samples. We compared results of incurred samples to devise and validate a new streamlined method to achieve the most broadly acceptable results in the fastest time, which still entails sample cleanup to maintain instrument ruggedness. This new method, which may be given the moniker “Goldilocks” if the analytical community agrees, uses 2 g homogenized tissue sample plus 10 mL of 4/1 MeCN/water for extraction by vigorous shaking for 5 min using a high-capacity shaker. After centrifugation for 2 min, the extract is transferred to a centrifuge tube containing 500 mg C-18 and 10 mL hexane (saturated with MeCN), which is shaken for 1 min and centrifuged. The hexane is removed by aspiration and 5 mL of extract is evaporated to remove the MeCN and brought up to 1 mL in water. Injection of 10 µL is made in a 10 min UHPLC-MS/MS method to detect the 120+ targeted drugs at half-tolerance levels or lower in the US. Extensive multi-day, multi-analyst, multi-reagent validation was conducted, and the method was successfully transferred to the USDA Food Safety Inspection Service (FSIS) for implementation initially as a lab-based screening method. It also met the FSIS’ LC-MS/MS identification criter! ia for > 80% of the analytes. No true false positives or false negatives were obtained in the analysis of at least 20 different samples of each matrix type. Validation criteria for quantification was met for about 70% of the analytes, but a second determinative method would still be required for confirmation and enforcement actions in any case. This new MMM achieves sample throughput of 60 pre-homogenized samples/day for a single analyst at a cost of $3 for materials/sample. The implementation of this method will considerably improve the FSIS National Residue Monitoring Program in the US.

Keywords: veterinary drugs; residues; beef; LC-MS/MS

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L-58
NATURAL TOXINS IN PLANTS AND FOODS: FROM TARGET ANALYSIS TOWARDS METABOLOMICS

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Despite huge research investment on mycotoxins (poisonous, low molecular weight, secondary metabolites of moulds), prevention and control remains difficult and the food industry continues to be vulnerable to problems of contamination. Continued research has led to some understanding of fungal metabolism, but has also highlighted the complexity of fungal/plant interactions. When analytical work is undertaken to monitor foods, a significant fraction of bound or masked mycotoxins can remain undetected. The toxicological fate of these substances is largely unknown. Recognising these significant gaps in current knowledge there are great efforts underway to investigate the metabolism of mycotoxins by plants, microbes and animals. In addition, during the last couple of years, research interests have increasingly shifted towards the role of specific gene modifications as a means to understand pathogen-plant interactions at a molecular level and to consquently reduce the level of contamination of foods with natural toxins such as mycotoxins. Mass spectrometry based analytical methods (GC-MS, Q-TOF, LC-MS/MS) have been key for the quantification of natural toxins in plants and foods and for the investigation of the metabolism of these toxic compounds in body fluids such as serum and urine. Metabolite profiling represents an extremely useful tool that finds applications in many aspects of drug discovery, food safety issues and disorders of cells and organisms. One example is a multi-analyte method which has recently been developed capable of quantifying 270 fungal metabolites, respectively, in cultures and grains. Metabolomics or metabolome analysis has been introduced to designate the set of all low-molecular-mass compounds, i.e. metabolites, synthesized by an organism. In contrast to mere metabolite profiling, metabolomics always shows fitness for a functional genomics context. An example for successful research in this area was the finding that the ability of wheat to detoxify the relevant mycotoxin deoxynivalenol into its non-toxic glucosidic form can directly be linked to recently identified resistance genes. This paper will summarize the expertise as well as the required latest state-of-the-art analytical instrumentation available to successfully perform high-level research in the area of mycotoxins and other fungal and plant metabolites relevant to the food and agricultural industry.

Keywords: mycotoxins, fungal/plant metabolism, metabolomics, masked mycotoxins, mass spectrometry

L-59
EFSA CONTAM PANEL'S RISK ASSESSMENT ON MYCOTOXINS: INFLUENCE AND CHALLENGES OF THE ANALYTICAL METHODS

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European Food Safety Authority (EFSA) carries out risk assessments on food and feed safety at the European level. As the risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation. Thus EFSA supports the European Commission (EC), European Parliament and EU Member States in their risk management decisions. EFSA's remit covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In the process of developing its scientific opinions, EFSA's Scientific Panels and Committee have crucial roles. The experts of the Scientific Panels and Committee from all over Europe and the world contribute to the scientific opinions. The EFSA Panel on Contaminants in the food chain (CONTAM Panel) carries out risk assessments in the area of chemical contaminants in food and feed, namely process and environmental contaminants, natural toxicants, mycotoxins and residues of unauthorised substances. In order to assess the risk for public and/or animal health and to prepare the related scientific opinions, the CONTAM Panel first collects and scrutinises available scientific information on the contaminants, their occurrence in food and feed, exposure to humans and animals, toxicokinetics and toxicity. It then establishes health based guidance values for contaminants, compares the estimated exposure levels to the established health based guidance values (humans) or to the identified no-observed-adverse-effect levels (animals), and finally concludes on the risk for humans and/or animals. This presentation outlines the CONTAM Panel's recently published scientific opinions on mycotoxins in food and feed. Mainly the scientific opinion on risks for public health related to presence of zearalenone in food is presented*. EFSA received the request for the opinion on zearalenone from the EC in 2010 and allocated this mandate to the CONTAM Panel. To address all the aspects of the mandate, the CONTAM Panel set up a working group on zearalenone to prepare the scientific opinion. The key parts of the scientific opinion on zearalenone are presented and discussed. These include: terms of reference from the EC, uncertainties of the risk assessment of zearalenone, occurrence and dietary exposure, hazard identification and characterisation, risk characterisation, main conclusions and recommendations of the CONTAM Panel. In addition, the role of the analytical methods in the context of the risk assessment is discussed, especially the reliability of the analytical results in relation to the conclusions on the dietary exposure of the risk assessment is outlined. The challenges linked to the analytical methods and the analytical results, which are encountered during the preparation of the risk assessment, are also presented. The scientific opinion on zearalenone in food is used as an example risk assessment to highlight the importance of the analytical aspects. *www.efsa.europa.eu

Keywords: EFSA, risk assessment, mycotoxins, methods

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L-60*
ASSESSMENT OF EXPOSURE TO THE FUSARIUM TOXIN DEOXYNIVALENOL: A BIOMARKER APPROACH

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Mycotoxins are toxic secondary metabolic products of several fungal species and a serious hazard for human and animal health. According to FAO up to 25% of all grains worldwide are contaminated by these toxins, hence they are an important issue for food safety. So far human exposure to the major mycotoxin deoxynivalenol (DON) is estimated from dietary average intakes or by measurement of the parent toxin in urine after hydrolysis with β-glucuronidase [1]. These approaches are very time, work and cost intensive. The determination of suitable biomarkers offers an elegant alternative approach to assess mycotoxin exposure. They can be useful to examine bioavailability, toxicity and metabolisation patterns and enable inferences on mycotoxin contaminations in ingested food. About 90% of the ingested DON is metabolized to the glucuronide conjugate (DON-GlcA) in humans. We developed a fast, simple and sensitive LC-MS/MS method for the direct quantification of this important metabolite in human urine samples beside the parent toxin DON [2]. In the study at hand the recently developed method was used to analyse urine samples of a volunteer after an eight day duplicate diet experiment. During the first and last two days the individual consumed only food items proven to contain no DON to obtain blank background samples. On the four days in between the volunteer ingested weighted food portions, previously analysed for their mycotoxin concentration levels. By this the total daily uptake of DON could be determined and, by measuring the subsequent urine samples, the urinary excretion rate and the metabolisation pattern was examined. Results are discussed in the light of the tolerated daily intake limit (TDI) established by the Scientific Committee on Food [3].

[1] Meky et al 2003, Food Chem Toxicol 41 (2): 265-273
[2] Warth et al 2011, Anal Bioanal Chem 401: 195–200
[3] SCF 2002, SCF/CS/CNTM/MYC/27 Final

Keywords: Deoxynivalenol glucuronide, Mycotoxin biomarker, Exposure assessment, Human urine, LC-MS/MS

L-61
HIDDEN FUMONISINS: A STEP BEYOND THE ANALYTICAL ISSUE

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Hidden fumonisins have received great attention in the last years as they have been frequently found in maize products in addition to the free forms. A number of studies showed that thermal treatments could give rise to covalent bond formation involving fumonisins and food constituents such as sugars, starch or proteins (Seefelder et al 2003; Kim et al. 2003; Park et al. 2004). Nonetheless, recent results showed the hidden fumonisin occurrence also in mild-treated products and in raw maize, indicating that other planta-related masking mechanisms should be taken into account (Dall'Asta et al. 2009a). In particular, as supported by experimental evidence, a non covalent interaction such as complexation or physical entrapment seems to take place between fumonisins and corn proteins. This kind of behavior may also be at the base of the difficulties in obtaining comparable and reproducible results using different analytical methods (Dall'Asta et al.2009b). Such interactions, indeed, may be differently broken during the extraction process, on account of different experimental parameters applied during extraction, thus leading to different recoveries of the analytes. Recently, the release of these hidden forms upon gastrointestinal digestion has been demonstrated by in vitro models (Dall'Asta et al. 2010), opening a serious problem regarding risk assessment: since hidden fumonisins are actually supposed to contribute to the overall toxicity, consumers may be, as a matter of fact, concretely exposed to a higher risk than that evaluated by routine methods. In this presentation, the state of the art about hidden fumonisin occurrence in raw maize and in corn-based products will be described and the lack of knowledge in this field will be pointed out. In particular, new data about hidden fumonisin occurrence in raw maize will be presented and critically discussed. Finally, a feasible masking mechanism will be proposed on the basis of experimental evidences obtained by a multi-technique approach.

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Seefelder W, Knecht A, Humpf HU. J Agric Food Chem. 2003, 51, 5567-5573.

Keywords: hidden fumonisins, masked mycotoxins, maize

L-62*
LC-MS MULTI-MYCOTOXIN ANALYSIS EMPLOYING QUECHERS LIKE SAMPLE PREPARATION PROCEDURE

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Rapid, simple and cost-effective analytical methods with performance characteristics matching regulatory requirements are needed for effective control of occurrence of mycotoxins in cereals, seeds, fruits, and processed food products to which they might be transferred. The aim of our work was to develop, optimize and validate a generic isolation method not only covering wide range of target mycotoxins differing with their physico-chemical properties, but also being applicable to different types of food and feed matrices. As it turned out, the QuEChERS-based extraction/purification procedure employing partition of aqueous acetonitrile by added inorganic salts was very suitable for this purpose. Determination of more than 50 analytes (aflatoxines, trichothecenes, zearalenone, and ochratoxin A together with their metabolites, sterigmatocystin, fumonisins, enniatins, beauvericin, patulin, tentoxin, meleagrin, paxilline, ergot alkaloids, Alternaria toxins, stachybotrilaktam, mycophenolic acid, penicillic acid, gliotoxin, penitrem A, roquefortin C, etc.) in cereal matrices (maize, wheat, barley), other dry matrices (spices and dried silages), fatty matrices (poppy seeds, peanuts), and high moisture matrices (fruity baby-food) was enabled by employing of QuEChERS-based approach. Optimization of the QuEChERS-based method and particular modifications enabled for the each single matrix will be discussed. As determinative steps, two alternative mass spectrometric approaches exploiting (i) high resolution orbital ion trap (Exactive, Thermo Fisher Scientific), and (ii) high sensitive linear ion trap (QTRAP 5500, AB SCIEX) . In both cases, ultra-high performance liquid chromatography (U-HPLC) was employed for separation of analytes. Detection potential of both of the methods will be assessed.

Keywords: Mycotoxins, QuEChERS, ultra-high performance liquid chromatography, mass spectrometry

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L-63
SCREENING OF PLANT TOXINS IN FOOD AND BOTANICALS USING LC WITH FULL SCAN HIGH RESOLUTION (ORBITRAP) MASS SPECTROMETRY

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Plant toxins are secondary plant metabolites that exhibit acute or chronic toxicity. Plant toxins can be inherently present in our food as constituents of edible crops, aromas, food supplements and (traditional) herbal medicines. In other cases they end up in our food through contamination or adulteration. Compared to other types of food toxicants, relatively little attention has been given to plant toxins despite the fact that several incidents with serious health implications have occurred. The European Food Safety Authority (EFSA) has increased concerns regarding plant toxins and compiled a compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern [1]. More than 600 substances are included in the compendium. For risk assessment and quality and safety control, there is a need for analysis methods to determine these plant toxins in a wide variety of complex matrices. Especially in case of contamination, where it is often not a priori known what to look for, this is a very challenging task. With this work, for the first time, a generic method for simultaneous detection of high numbers of plant toxins from various chemical classes, in a variety of food and feed matrices is presented. LC with full scan high resolution (Orbitrap) MS was chosen as analysis technique to address the high number of analytes, the high complexity of the matrices, and the limited availability of reference standards. The potential of the technique was systematically investigated through a selection of 150 substances mentioned in the EFSA Compendium, representing various toxin classes [2]. The sensitivity was tested using fixed LC-MS conditions. Ion suppression effects and selectivity were evaluated using crude extracts from representative and relevant matrices. The applicability of the method is demonstrated by analysis of a variety of real-life samples, purchased on the market or from cases of intoxication. These included honey, herbal tea, food supplements, poppy seeds, traditional Chinese medicines (TCM), and herb-based feed additives. Plant toxins that were detected included various pyrrolizidine alkaloids, grayanotoxins, opium alkaloids, strychnine, ricinine (marker for ricin), aconitine, aristolochic acid and cardiac glycosides (e.g. digitoxin, digoxin).

[1] EFSA 2009. Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. EFSA Journal 2009; 7(9):281. [100 pp]
[2] H.G.J. Mol, R.C.J. van Dam, P. Zomer, P.P.J. Mulder, Screening of plant toxins in food, feed and botanicals using LC with full scan high resolution (Orbitrap) mass spectrometry, Food Additives and Contaminants, 2011, accepted for publication.

Keywords: Plant toxins, Screening, Food supplements, Honey, High resolution mass spectrometry

L-64*
PYRROLIZIDINE ALKALOIDS – CURRENT TRENDS IN ANALYSIS OF HONEY AND MATERIALS OF PLANT ORIGIN

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Pyrrolizidine alkaloids (PA's) are a group of plant toxins synthesised by more than 6000 plant species and numbering more than 350 individual compounds. Among them only 1,2 unsaturated necine esters are considered to be toxic. They can be found in different products such as honey, bee pollen, herbal preparations, and forage. Main toxic effects of these compounds are hepatic veno-occlusive disease (VOD) and hemorrhagic liver necrosis. Acute intoxications are well described for animals, mainly horses, but also some human cases have been reported. In 2007, the European Food Safety Authority (EFSA) has published an opinion on PA's. The list of compounds to be monitored in feed has been drafted, to represent major PA containing plant families. Legislative limits are currently under discussion. To our knowledge, there is no fully validated analytical method available to detect all relevant PA's in food or feed. Additional complexity is derived from the fact that N-oxides of PA's can be formed in the plant itself. These derivatives exhibit similar toxicity and therefore, they also need to be controlled. Currently, several analytical approaches have been proposed. Individual PA's can be detected using LC- or GC-MS techniques. GC-MS methods require additional N-oxide reduction step prior to analysis. Another approach, so-called sum parameter method, involves complete hydrolysis of PA esters to necine bases and allows group-specific determination of PA's by GC-MS. Screening methods, such as ELISA, have also been developed. This presentation gives a short overview of the above methods highlighting advantages and limitations of each approach. Furthermore, due to the absence of a harmonised analytical methodology, potential challenges for a proficiency test (PT) provider include characterisation of the materials and evaluation of results, depending on the approach chosen by a participating laboratory. With this respect, preparation of reference materials, such as honey and dry plant material, to be used for PT is discussed.

Keywords: Pyrrolizidine alkaloids, Honey, Feed, LC-MS

L-65
RECENT TRENDS ON THE ANALYSIS OF PHYCOTOXINS: THE PERSPECTIVE OF THE EUROPEAN UNION REFERENCE LABORATORY FOR MARINE BIOTOXINS

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The development of analytical methodologies for the efficient control of phycotoxins has been a priority for scientists working in the analytical field, since the contamination of seafood due to the presence of naturally occurring phycotoxins is a major concern worldwide. This concern is not due solely to their broad distribution, but also because of new and emerging phycotoxins. Although there remains a need for more accurate toxicological data, to support risk assessments these emerging toxins may present a serious health threat, thus there is an urgent need for their study and further control. There is also a new trend to move from biological assays to more modern and chemical methods. Before the development of modern analytical methods, mouse bioassays provided a useful tool for the control of most of the known phycotoxins, but the drawbacks of these biological assays prompted a search for analytical alternatives. For the saxitoxins the first approved alternative instrumental method was based on precolumn oxidation liquid chromatography with fluorescence detection (AOAC OMA 2005.06), providing both the first opportunity in 50 years to allow replacement of a controversial MBA, also more sensitive detection in the prevention of paralytic shellfish poisoning. Other efforts have developed additional alternative methods for detecting the saxitoxins, including other LC methods and also screening methods, for example functional and immunochemical assays. These are very challenging but exciting times for the control of phycotoxins, particularly in the EU, as it begins the big step, mandated by EU regulations, of moving away from the MBA for lipophilic marine toxins, being replaced by a physicochemical method based on a chromatographic separation prior to mass spectrometric detection. This approach to managing lipophilic toxins in shellfish represents a very important analytical advance for an efficient and sensitive detection of multiple classes of lipophilic toxins.The European network of marine b iotoxins, under the coordination of the EURLMB, recently accomplished the duty of validating this methodology. Still, many challenges remain. The search for improved and implemented analytical methodologies is still a requirement to ensure seafood safety due to emerging toxins worldwide. Harmonization and validation of these new methods will then be required. Also, as in many other areas in the field of food contaminants, there remains a need for additional reference materials, particularly for emerging toxins, even though significant advances have been made in this particular subject over the last several years. Working further in this direction will require a concerted worldwide effort. An overview of these issues, with a focus on the present situation in the EU will be presented and the future trends and needs, as well as the recent developments and efforts carried out in this direction at the EU Reference laboratory will be discussed.

Keywords: phycotoxins, legislation, analysis, LC-MS/MS

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L-66
EVOLVING TO THE OPTOELECTRONIC MOUSE FOR PHYCOTOXIN ANALYSIS IN SHELLFISH

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Despite ethical and technical concerns the in vivo method, or more commonly referred mouse bioassay, is employed globally as a reference method for phycotoxin analysis in shellfish. This is particularly the case for the global monitoring of paralytic shellfish poisoning (PSP) and emerging toxins. As an alternative to the mouse bioassay, a HPLC-FLD method has been developed for PSP toxin analysis but due to difficulties and limitations in the method this procedure has not been fully implemented as a replacement. Similarly, the detection of the diarrheic shellfish poisoning (DSP) toxins is moving towards LC-MS analysis and amnesic shellfish poisoning (ASP) toxin domoic acid is performed by HPLC analysis. Although alternative methods of detection to the mouse bioassay have been described, to date each analytical method is specific for a particular toxin and its chemical analogues, with each group of toxins requiring separate analysis by different extraction procedures and analytical equipment. An ideal scenario for the monitoring of phycotoxins in shellfish would be to evolve to multiple toxin detection on a single bioanalytical sensing platform, i.e. 'an artificial mouse'. Surface plasmon resonance technology has been displayed as a highly promising bioanalytical tool. This technology offers rapid real time detection requiring minimal toxin standards which is crucial because of their limited availability. A micro-fluidic immobilization device and prototype multiplex SPR biosensor designed for the detection of up to 16 molecular binding interactions in a 4 line by 4 channel array on a single chip has been utilised. This dual system was evaluated in its ability to be fit-for-purpose for the simultaneous detection of three key phycotoxin groups. Domoic acid, okadaic acid and saxitoxin calibration curves in shellfish were achieved in separate flow channels with detection limits of 4000, 36 and 144 µg/kg of mussel respectively. These limits designed on achieving detection below the regulatory action levels. This detection system exhibits enormous potential for multiple phycotoxin screening as alternative to the mouse bioassay with the additional benefit of being able to distinguish between toxin families on a single analysis. Validation data will be presented.

Keywords: marine toxins, biosensing, multiplexing, validation

L-67
DNA-APTAMERS FOR MYCOTOXINS: APPLICATION OF OCHRATOXIN A APTAMER TO WHEAT ANALYSIS

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Aptamers are single-stranded oligonucleotides that are mainly selected using SELEX (Systematic Evolution of Ligands by EXponential) enrichment and are able to discriminate target molecules with high affinity and specificity, even in the case of very closely related structures. Because of their in vitro selection and production, the technology of aptamers is emerging as a viable alternative for use in a broad range of applications including affinity chromatography, lateral flow devices and biosensors. Aptamers have been produced for several targets, including peptides, proteins, drugs, whole cells, and, recently, for mycotoxins, e.g. ochratoxin A (OTA) and fumonisin B1 (FB1). The DNA aptamer with high affinity and specificity to OTA was used as oligosorbent for the preparation of aptamer-based solid phase extraction (SPE) columns. The procedure for the preparation of SPE columns was standardised after evaluating the effect of different parameters, such as oligosorbent volume, column size and breakthrough volume. SPE columns packed with 300 µl oligosorbent (24 nmol aptamer) were successfully used for the clean-up of durum wheat extracts prior to OTA determination by high performance liquid chromatography (HPLC) and fluorescence detection (FLD) in unprocessed durum wheat. The SPE-columns showed a linearity of the dose-response curve in the range of 0.4–500 ng OTA. Average recoveries from wheat samples spiked at levels of 0.5-50 ng/g ranged from 74% to 88% (relative standard deviation

Keywords: DNA-aptamer, ochratoxin A, wheat, SPE columns, fumonisin B1

L-68
CHALLENGES IN TARGETED AND NON-TARGETED ANALYSIS OF PESTICIDE RESIDUES

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Modern pesticide multiresidue analysis is based on streamlined, yet effective sample preparation, such as the QuEChERS sample extraction and clean-up approach, followed by determination of a wide range of analytes in the sample extract using gas and liquid chromatography combined with mass spectrometry (GC-MS and LC-MS). The advancements in the GC-MS and LC-MS instrumentation allow analysis of hundreds of pesticides at very low levels in one analytical run, which is usually conducted using a targeted approach, in which analyte-specific conditions (e.g. MS/MS transitions and collision energies) are set in the acquisition method. The targeted analysis can, however, only determine whether pesticides included in the target list are in the sample or not. Considering the number of pesticides and typically unknown origin of the tested samples, a non-targeted approach seems like an intriguing option for pesticide and other chemical contaminant testing. The non-targeted MS approach is based on full-scan MS data acquisition, typically using a high-resolution, high-mass accuracy time-of-flight or orbitrap MS for increased selectivity. Also, the data processing should be non-targeted utilizing database or library searching. But the MS part is not the only piece of the puzzle in the non-targeted testing. Such as the targeted analysis of pesticides has many challenges, including analyte extractability, stability or matrix effects, the non-targeted analysis faces the same challenges and other issues that require special considerations. This presentation will take a closer look at those challenges and considerations that should be taken into account when analyzing pesticide residues using targeted and non-targeted approaches.

L-69
INFLUENCE OF MATRIX EFFECTS IN QUALITATIVE ANALYSIS BY LC- MS. PROBLEMS AND SOLUTIONS

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Qualitative analysis is nowadays an important tool for routine laboratories, as screening methods are more and more demanded, given the current need to detect illegal or misused compounds, whose presence is not expected in the samples. In this sense, the use of quick and simple screening methods that allow positive identification within a wide range of compounds is the final goal. To do this work different strategies can be applied, TOF-MS, TOF-MS/MS, triple quadrupole or QqQ-ion trap. The application of these different approaches presents advantages and disadvantages and typically the laboratory has to find the best cost-effective way. All of these approaches are commented extensively in this work. Although quantification is not the aim in this kind of analysis, matrix effects can also affect automatic identification of the compounds, as the ionization suppression reduces the detectability of analytes, resulting in an increment of the number of false negatives. Nowadays, the appearance of new generation analytical systems in the market make possible dilution of the samples, as highly sensitive instruments are available. Sample dilution is an easy and effective method to reduce interfering compounds, obtaining this way the injection of less matrix load into the chromatographic system, and so, to diminish matrix effects, although in this case sensitivity is a key factor, given that it implies a reduction in the amount of analytes. All this new developments can allow important advantages and solutions to the pesticide residue control.

Keywords: Pesticides, Matrix effect, Food analysis, Fruits and Vegetables, Dilutions

L-70
ION MOBILITY-TIME-OF-FLIGHT MASS SPECTROMETRY AS A NEW TOOL FOR THE SCREENING OF PESTICIDES RESIDUES IN FOOD

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In recent years, Time-of-Flight (ToF) analysers have been very popular in the field of qualitative analysis for pesticide residues analysis. This growing interest in ToF systems is linked to the intrinsic characteristic of full scan mode, offering the convenience to record an unlimited number of target compounds. Nevertheless, the analytical power of these mass spectrometers can be reduced when the analytes are present in the matrix at very low concentrations. In these conditions, the presence of co-extracted matrix components and in some cases the impurities of reagents used, prevent the possibility of unambiguous identification of the pesticides. Even if you use instruments with higher sensitivity or higher resolving power to overcome the problem, some pesticides remain problematic. We propose a new approach to this issue: the use of Ion mobility (IM) coupled to mass spectrometry (MS). IM is a powerful analytical tool for the separation of complex samples. This technique separates ions on the basis of their size/charge ratios and their interaction with a buffer gas. Ion mobility mass spectrometry experiments were performed on a Synapt G2 spectrometer (Waters, Manchester, UK), which is basically a Q-ToF instrument, except that the collision cell is replaced by an ion mobility cell. The instrument will monitor the time required for an ion to travel through the mobility cell: the drift time. After careful optimization of the IM cell and ToF parameters, spiked samples (leek, orange and pepper) at concentration levels of 10, 50 and 100 µg/kg were analysed with an UPLC hyphenated to the Synapt G2. This presentation focuses on the data obtained which demonstrates the feasibility of this new approach for the comprehensive screening of hundreds of pesticides in complex matrices. We put the emphasis on two major advantages of IM: first, how it can separate the target ions from the matrix interferences consequently improving the signal-to-noise ratio of characteristic ions. Secondly, the use of the drift time as an additional criteria for identification purpose.

Keywords: Ion mobility, Q-ToF, comprehensive screening, pesticide residues

L-71
QUANTITATION OF 3-MCPD ESTERS AND GLYCIDYL ESTERS VIA STABLE ISOTOPE DILUTION ASSAYS IN EDIBLE FATS AND OILS

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Free 3-MCPD is a well-known food constituent, which is supposed to have carcinogenic potential. In recent years, 3-MCPD fatty acid esters have been reported in food, e.g. in refined edible fats and oils. Due to the fact that after consumption a cleavage of the esters to free 3-MCPD is thinkable, efforts have been undertaken to minimize their concentrations. Very recently, the presence of glycidyl esters in fats and oils has also been proven. Up to now, all these compounds are quantified with indirect analytical methods only providing the sum of all 3-MCPD esters and glycidyl esters, respectively. Thus, the aim of the present study was to develop a direct quantitation method for the single determination of each important 3-MCPD ester as well as glycidyl ester via stable isotope dilution analysis (SIDA). Application of the newly developed assays in combination with the LC-MS technique on different types of edible fats and oils revealed considerable differences in the concentrations of the esters. For example, in three sunflower oil samples glycidyl palmitate (40.2–83.9 µg/kg in 3 samples), glycidyl stearate (31.7 µg/kg in just 1 sample), glycidyl oleate (289–341 µg/kg in 3 samples), and glycidyl linolate (1110–1680 µg/kg in 3 samples) as well as in three rapeseed oil samples glycidyl palmitate (30.2 µg/kg in just 1 sample), glycidyl oleate (108–170 µg/kg in 3 samples), glycidyl linolate (45.5–146 µg/kg in 3 samples), and glycidyl linolenate (44.4–45.9 in 2 samples) were quantified. The results showed a clear influence of the refining process in regard to the formation of glycidyl esters within one type of oil. Further, the amounts of glycidyl esters were in good correlation with the respective fatty acid composition in the samples. In contrast to about 20 different types of refined fats and oils, which were analyzed and which all contained glycidyl esters, in virgin olive oils no esters could have been detected. Thus, with this new method at hand, definite contents of the different esters could be analyzed in a reliable way with a high sensitivity and selectivity as well as low limits of detection using various isotopically labeled standards. In the lecture, the obtained results will be discussed and compared to existing indirect analytical approaches, which are on the basis of a derivatization step after cleavage of the esters and only offer the sum of the respective esters. Furthermore, the lecture will give deeper insights into the formation pathway, which was investigated by labeled precursor substances in model systems.

Keywords: 3-MCPD esters, glycidyl esters, edible fats and oils, stable isotope dilution analysis

L-72*
STUDIES ON THE FORMATION OF IMPORTANT FLAVOUR COMPOUNDS IN WHEAT BEER AS WELL AS OF THE TOXICOLOGICAL RELEVANT STYRENE FROM PHENOLIC ACIDS

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Enzymatic reactions during mashing, thermal reactions while wort boiling, and yeast fermentation are key processing steps in the formation of desired aroma-active compounds on the one hand, but also for the formation of toxicological relevant compounds, the so-called “food-borne toxicants”, on the other. Recently, attention has been drawn to styrene in wheat beer, hence it is evaluated as “possibly carcinogenic to humans” by the International Agency for the Research on Cancer (IARC). Thus, it is a great challenge for the brewing industry to produce wheat beers with good aroma in combination with a reduced styrene concentration. The aims of the present study were therefore, (i) to characterise the key aroma compounds in wheat beer by application of molecular sensory science, (ii) to track the formation of aroma compounds and styrene during the processing steps using stable isotope dilution assays (SIDA), and (iii) to correlate the quantitative data with the concentrations of precursors present in the raw materials as well as in intermediate products during the brewing process. First, the most important aroma-active compounds in wheat beer were characterised by application of the aroma extract dilution analysis (AEDA), followed by identification experiments. Phenylethanol, 4-vinyl-2-methoxyphenol, 3-methyl-1-butanol, methionol, acetic acid, methional, 2-phenylethyl acetate, 2-methoxyphenol, 3-methylbutyl acetate, β-(E)-damascenone, and 4-vinylphenol were found to be the most odour-active compounds in wheat beer. Some of these aroma-active compounds, like 4-vinyl-2-methoxyphenol, 2-methoxyphenol, and 4-vinylphenol are known to be formed as desired aroma compounds by decarboxylation of phenolic acids (ferulic acid, vanillic acid, and p-coumaric acid) during the brewing process. On the other hand, the decarboxylation of cinnamic acid may result in the formation of the toxicologically relevant styrene. To get a deeper insight into the steps of the brewing process responsible for the formation of styrene as well as of the desired odorants, stable isotope dilution assays were newly developed for the quantitation of styrene, the aroma compounds as well as for the respective precursors caffeic, cinnamic, p-coumaric, ferulic, sinapic, and vanillic acid. Starting with the raw material (wheat, barley), followed by malt, subsequently mash, wort, and, finally, some ready-to-drink beers huge differences in the amounts of precursor acids were found. For example, in 10 different wheat beers the concentrations of p-coumaric acid varied between < 0.09 and 3.77 mg/L, while ferulic and vanillic acid varied between < 0.02 and 1.54 mg/L and 2.15 and 4.91 mg/L, respectively. In the lecture, the data will be discussed regarding possibilities to optimise the aroma of wheat beer but reducingthe generation of styrene.

Keywords: wheat beer, phenolic acids, styrene

L-73
NON-TARGET STEP-WISE ANALYTICAL SCREENING OF PAPER FOOD CONTACT MATERIALS TO ASSESS THE SAFETY

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Before a food contact material (FCM) can be used, a safety assessment of all contaminants/migrants that may migrate from these materials is required. Identifying and quantifying all of the hundreds or thousands of substances that a FCM may contain is not always feasible since it is time and money consuming and it is sometimes not practically possible to identify all. A strategy was developed to evaluate the safety of complex matrices in an efficient manner and is demonstrated for paper FCM focusing on non-intentionally added substances (NIAS). Innovative safety assessment In order to increase the efficiency of the safety assessment process of FCM, a stepwise multidisciplinary, exposure-driven strategy was developed. State-of-the-art analytical techniques and toxicology are combined with a pragmatic risk assessment tool known as 'Threshold of Toxicological Concern' or TTC (Rennen et al., 2011, Food Chem. Tox.). The main advantage for the analytical chemist is that much fewer substances need to be identified and quantified compared to conventional approaches. The TTC concept is about to be accepted by the European Food Safety Authorities (EFSA) as risk assessment tool. Paper FCM migration and analysis Migration experiments were performed into tenax, 95% ethanol and isooctane that simulate the intended use of the different paper FCM tested. To enable the application of the TTC principle to paper FCM, a stepwise analytical exclusion approach was developed. In step 1 a non-target forest-of-peaks approach is used to profile (semi-quantitative holistic analysis) all substances that may be present in the FCM. This profiling was performed with; – GC-MS for apolar and medium polar semi-volatiles. – GC-MS silylation (metabolomics approach) for non-volatile and semi-volatile polar and medium polar substances. – headspace GC-MS for volatiles. – LC-UV/NQAD/MS for non-volatiles. Substances that exceed a general health limit of 90 µg exposure per person per day (derived from TTC) were identified and their risk assessed. In contrast to conventional approaches, substances below this general health limit did not require assessment (and thus did not require identification) if excluded that these are not highly potent toxic or genotoxic substances (see step 2 and 3). The main challenge is to quantify substances in FCM with unknown structure. This is done with GC-FID and LC-NQAD and is presented. In step 2 the presence of highly toxic substances such as dioxins, aflatoxins and others were excluded to be present in the FCM. This was performed with target screening methods by GC-MS and LC-MS. Step 3 comprises the exclusion of genotoxic substances using a novel 96-well high-throughput genotoxicity assay (Bluescreen HCTM). After identification of the substances exceeding the 90 µg exposure per person per day, a full risk assessment on the paper FCM was performed. The pragmatic step-wise approach to evaluate NIAS will be presented.

Keywords: food contact materials, non-intentionally added substances, holistic analysis, genotoxicity, TTC

L-74
ANALYSIS OF FOOD PACKAGING CONTAMINANTS BY LC-MS

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Introduction: The new European Plastics Implementing Measure (PIM) and other recent developments in legislation make the analysis of food contaminants and chemical migration from food contact materials more complex. Important changes in the last years were

Expansion with plastics layers in multi materials in the PIM

Separate sets of standard test conditions for overall and for specific migration testing and last but not least a

Non detectable (10 ppb) migration limit for non-evaluated substances

Traditional methods of analysis like GC-FID or HPLC are often hampered by limitations like the achievable detection limit, specific matrix and a restricted number of analytes. GC/MS, LC-MS and other advanced analytical techniques can be used to avoid a number of these problems, but provide other challenges for the analyst. Whereas GC/MS is a relatively easy-to-use instrumentation, LC-MS is much more sophisticated especially for the analysis of unknowns. Source conditions are often specific for individual compounds, and the response is often very varying for detectable compounds.

Examples

Primary aromatic amines

In the past the primary aromatic amines were quantified with a UV/VIS spectrophotometer. In order to provide lower detection limits and more specific information an LC-ESI-MS/MS method has been developed for the analysis of 20 primary aromatic amines in aqueous food simulants[1]. Firstly, the predominating primary aromatic amines in real samples had to be identified and, furthermore, a quantification concept had to be developed.

Perfluorinated compounds

PFOS, PFOA and several other perfluorinated chemicals can be analyzed by HPLC-ESI-MS with detection limits in the sub-ppb area. This is backed up with the presence of isotope labelled standards. There are known problems like interferences such as taurodeoxycholic acid[2] and the compound specific analytical technique (MRM). Volatile fluorinated compounds which pose as precursors for PFOS and other PFC, can be analyzed with GC-EPED. High molecular polyfluorinated coating materials for food packaging, however, are the main source for fluorinated compounds in packaging. HPLC/MS serves as an excellent analytical means for identification, whereas quantification remains a challenging issue due to the lack of labelled and certified standard substances.

Screening for not known non-evaluated substances

A real challenge is the non detectable (10 ppb) migration limit for non-evaluated substances. There are several approaches. GC/MS in combination with GC-FID is a valuable tool. It combines identification (MS) with quantification, but there are many inappropriate compounds for GC. Often LC-MS can be the choice, for analytical screening methods, preferable in combination with methods like HPLC-CAD (Charged Aerosol Detector)

Summary

Recent developments in legislation force the analysts to use LC-MS and other advanced analytical techniques for the analysis of food contaminants and chemical migration from food contact materials. For these techniques there is a need for a suitable quantification method.

[1] Mortensen et al. (2005): Journal of *Chromatography* A, Vol 1091 (2005) 40–50

[2] Gruber et al. (2007): Organohalogen Compounds Vol 69 (2007), 142ff.

L-75*
QUANTITATIVE TRACE ANALYSIS OF EIGHT CHLORAMPHENICOL ISOMERS IN URINE BY CHIRAL LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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During the last decade, findings of chloramphenicol (CAP) residues in food products such as poultry and sheep casings, and feed products have had a major impact on international trade. CAP is banned for use in all food producing animals and a minimum required performance limit (MRPL) of 0.3 µg kg-1 has been established. CAP is a chiral molecule with two chiral centers and occurs in the meta- and in the para-configuration and therefore eight different stereoisomers exists, namely four (RR, SS, RS, SR) meta- and four para-isomers. According to regulations a confirmatory method should be able to unequivocally identify the compound present and thus, in the case of CAP, a method that is able to discriminate the active form from the inactive isomers, is mandatory to prevent false non-compliant results. We found that reversed phase high resolution liquid chromatography methods with mass spectrometric (MS) detection do not discriminate the active isomer from its enantiomer (dextramycin). It is concluded that these methods lack selectivity, resulting in complicated situations during lawsuits. We studied the mass spectrometric fragmentation pattern of the different isomers and found that some of the isomers can be discriminated by selecting the correct combination of product ions in selected reaction monitoring. As expected, this did not result in the discrimination of the enantiomeric pairs and therefore, additionally we developed a sample clean up procedure in combination with a chiral-LC method capable of adequately separating the CAP isomers. Using the combination of chiral-LC and triple quadrupole MS detection we were able to specifically detect all eight isomeric configurations of CAP in urine at trace levels. This is the first method reported that is suitable for selectively detecting the active isomer of CAP and therefore for characterizing samples as being non-compliant.

Keywords: chloramphenicol, chiral liquid chromatography, enantiomers, mass spectrometry

L-76*
ANALYSIS OF ALPHA-DICARBONYL COMPOUNDS IN HIGH FRUCTOSE CORN SYRUP AND CARBONATED SOFT DRINKS

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α-Dicarbonyls (α-DCs) are formed in food mainly by degradation of monosaccharides, which takes place during thermal processing or storage. After absorption, α-DCs may induce dicarbonyl stress and be responsible for protein modifications by advanced glycation end products (AGEs). The latter are discussed as risk factors for diseases connected to chronic inflammation. Thus, α-DCs play an important role for the quality and safety of food products. Until now, however, little is known about the structures and concentrations of α-DCs in food products. In the present study, α-DC profiles of high fructose corn syrup (HFCS) and carbonated soft drinks (CSDs) were investigated. HFCS is a liquid sweetener produced from corn starch by hydrolysis and partial enzymatic conversion of glucose into fructose. Two types of HFCS are of commercial importance: HFCS-42 and HFCS-55, containing approximately 42% fructose or 55% fructose, respectively. CSDs produced in Europe are mostly sweetened with HFCS-42 and sucrose, whereas HFCS-55 alone is used for products for the US market. HFCS has become a common substitute to sucrose, since it is cheaper, easier to handle, and more stable in acidic conditions compared to sucrose. For the analysis of α-DC profiles of various HFCS products (both HFCS-42 and HFCS-55) and assorted CSDs, targeted screening was applied to identify major α-DCs. As a probe, o-phenylendiamine was used, which converts α-DC structures into UV-absorbing quinoxaline derivatives. The latter were then analyzed by ultra-high performance liquid chromatography (UHPLC) with hyphenated diode array - tandem mass spectrometry detection. This method admits unequivocal peak identification and reliable quantification of the major α-DCs in one run. After validation, the method was used to record and quantify α-DC profiles in diverse commercial HFCS products as well as in HFCS-sweetened CSDs, which are available in Germany or the USA. In almost all HFCS and CSD samples, six major α-DCs, namely 3-deoxyglucosone (3-DG), glucosone, 3-deoxygalactosone (3-DGal), methylglyoxal (MGO), and 3,4-dideoxyglucosone-3-ene (3,4-DGE), were identified. Concentration of total α-DCs in HFCS samples ranges from 300 to 1150 µg per ml and from 20 to 115 µg per ml in CSDs. CSDs purchased in Germany contain lower concentrations of α-DCs than US products. German products were sweetened by combination of HFCS and sucrose, whereas HFCS is the only sweetener used for US-products. In all HFCS and soft drink samples, 3-DG was the main sugar degradation product ranging from 200 to 730 µg per ml in HFCS and from 12 to 87 µg per ml in CSDs. Additionally, glucosone is also important and was detected in amounts up to 400 µg per ml in HFCS and 21 µg per ml in CSDs. The remaining α-DCs were detected in lower concentrations with 3-DGal>3,4-DGE>MGO.

Keywords: α-dicarbonyl compounds, high fructose corn syrup, carbonated soft drinks, sugar degradation products, ultra-high performance liquid chromatography

L-77*
RAPID SPE-GC-FID DETERMINATION OF MOSH (MINERAL OIL SATURATED HYDROCARBONS) AND MOAH (MINERAL OIL AROMATIC HYDROCARBONS) IN PRINTING INKS, RECYCLED CARDBOARD AND IN DRIED FOOD AS A CONSEQUENCE OF MIGRATION UNDER ACCELERATED TEST CONDITION

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Mineral oil contamination in foods has been well known for a long time, coming from different sources. More recently the attention was focused on migration from paperboard packaging. The responsible of this contamination are mainly printing inks used in newspapers entering recycled fibres used in the production of packaging and, to a lesser extent, inks used in the printed surface of the packaging. Use of alternative mineral oil free offset printing inks surely represents one of the possible solution to reduce contamination. Since these printing inks consists of both saturated (MOSH) and aromatic hydrocarbons (MOAH) having a different toxicological relevance, it is important to quantify these fractions separately both in cardboard and in food. A rapid off-line SPE-GC-FID method based on the use of silver-silica gel was optimized and used for MOSH and MOAH determination in printing inks, cardboard and dried food packed in direct contact with recycled cardboard. The proposed method allows optimal separation between MOSH and MOAH with minimal sample preparation and solvent consumption and can represent a valid alternative to the on-line HPLC-GC method. Sample preparation involves an extraction step with an organic solvent or a mixture of solvent, followed by a rapid purification/separation step on a glass cartridge filled with 1 g of silica gel treated with silver nitrate. The MOSH fraction elutes (soon after the dead volume of the cartridge) with 2 mL of eluent, while the MOAH fraction elutes, well separated from the MOSH fraction, with 7 mL of eluent. After reconcentration the MOSH and the MOAH fractions are injected separately into the GC-FID. Quantification was performed by using external calibration. Two different injection mode, the first using a conventional on-column injector with the retention gap technique, the second using a multimode injector working as a PTV (with a packed liner), are proposed. The multimode PTV injector gives performance comparable to that of the on column injector with minimal discrimination effect and allows to inject larger sample amounts. The possibility to rapidly heat the GC oven allows to increase sample throughput (about 3-4 samples per hour) and sensitivity. The developed method, which presented good performance characteristics (in terms of repeatability, reproducibility, accuracy, linearity, limit of detection and quantification), was used to characterise different printing inks, to quantify MOSH and MOAH in recycled paperboard, to analyse a selected number of low fat dried foods packed in direct contact with recycled paperboard and to study mineral oil migration into these foods under accelerated test condition.

Keywords: Mineral oil saturated hydrocarbons (MOSH), mineral oil aromatic hydrocarbons (MOAH), migration, recycled cardboard

L-78
ADVANCED ANALYTICAL STRATEGIES FOR MEASURING MIGRANTS AT TRACE LEVELS IN FOOD SAMPLES USING TANDEM OR HIGH RESOLUTION MASS SPECTROMETRY – PARTICULAR CASES OF BISPHENOL A, PHTHALATE DIESTERS AND PERFLUORINATED COMPOUNDS

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Over the last decade, growing attention has been paid to food contact material migrants, such as bisphenol A, phthalate diesters or perfluorinated compounds, evidencing them as a new potential chemical risk for human. In particular, pieces of evidence relying some of them to reproductive or developmental troubles have been established by the scientific community. Together with an increasing and significant media relay, these results largely participate to the current "hot" debate associated with these substances, on either the scientific, regulatory, risk management or consumer's perception points of views. But still, at various national and international levels, accurate management of the associated issue requires additional and extended investigation of the exposure and the related risk. In particular, besides the "traditional" approach consisting in measuring migration of substances from material to food simulants following standardized protocols in order to statute on the conformity of a material, data on effective dietary exposure of the population is also highly valuable for the risk managers. In this way, a standardized method recommended by World Health Organisation and known as the Total Diet Study (TDS) aims at providing contamination data for food prepared as consumed by the population and exposure data, in order to help the risk manager with public health decisions. The TDS consists in 3 major steps: (i) a food sampling, (ii) the analysis of the samples, and (iii) the evaluation of the exposure by combining the contamination data with the national consumption data. However, measuring migrants – which also became environmental contaminants – at trace or ultra-trace levels in complex biological matrices is known to be challenging to the analytical chemist, in terms of sensitivity, specificity, matrix effect or procedural contamination. To reach required performances, one must usually deal with state of the art methodologies, including the most advanced techniques in the fields of sample preparation or mass spectrometry for the detection. In this context, the aim of this work is to illustrate the potential of recent advances in food analysis by describing three innovative analytical strategies dedicated to bisphenol A, phthalate diesters and perfluorinated compounds, using (i) improved sample preparation techniques (liquid/solid or liquid/liquid extraction, reverse phase and weak anion exchange SPE cartridges, molecular imprinted polymers), (ii) associated original detection modes (gas or liquid chromatography coupled to tandem or high resolution mass spectrometry: GC-EI(+)-MS/MS and LC-ESI(-)-HRMS, with derivatisation if required) and (iii) drastic rules for procedural contamination management (from sampling, sample preparation and instrumental origins).

Keywords: phthalates, bisphenol A, perfluorinated compounds, food, mass spectrometry

POSTER SESSIONS

TOPICS

ALLERGENS

(A-1 – A-12)

A-1
A STUDY ON PROPERTIES OF GLIADIN REFERENCE MATERIAL CANDIDATE

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Hypersensitivity reactions (allergy, intolerance) triggered by certain food proteins affect an increase rate of population. At the moment European Union defines 14 foodstuffs responsible for the highest number of these cases. One of the most important groups is wheat and other cereals causing such significant disorders like wheat allergy and coeliac disease. The only effective treatment of these illnesses is the total avoidance of the problematic proteins in the patients' diet. In case of wheat these are the proteins of gluten, mainly gliadins. In order to observe the regulation, the stakeholders (food manufacturers. laboratories, governmental bodies, etc) need different tools like right technological solutions, food safety arrangements validated analytical methods. Today gluten is the only allergenic protein with a regulated threshold level: under gluten concentration of 20 ppm a food can be declared as gluten-free and in the range of 20-100 ppm they can be considered as low gluten level. At present the most commonly used methods in allergen analysis are ELISA and LFD. Development and validation of these immunoanalytical methods have many challenges. The most important ones are lack of reference methods and materials and the insufficient information on the effects of food processing steps on the properties of allergenic proteins. The first goal of our work was to develop a processed food matrix which contains gliadin in defined amount. Two type of matrices were produced, one with gliadin isolate and one with standard wheat flour. We analyzed samples from every step of the production process. The main investigated properties were the homogeneous distribution and recovery of allergenic protein and the stability of our model products. Our results showed that homogeneity and recovery of gliadin were satisfactory. Stability tests are in progress. According to the primary results stability of unprocessed matrices (powder mixture, raw dough) are inadequate. Our results are contributing to improvement of allergen analytical methods, their validation and the related legislation as well.

Keywords: allergy, gliadin, ELISA, reference material

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A-2
QUANTIFICATION OF RESIDUAL MILK ALLERGENS IN CASEINATE-FINED WHITE WINES BY HPLC COUPLED WITH SINGLE-STAGE ORBITRAP MASS SPECTROMETRY

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Milk/dairy products are considered among the most widespread food allergens and thanks to their peculiar characteristics they are frequently used as ingredients in several foods thus representing a threat for allergic individuals. Due to the ability of milk proteins to bind and induce precipitation of phenolic and off-flavour compounds that might impair the organoleptic properties of commercial wine, caseins and caseinates derived from bovine milk are routinely used by wine makers as fining agents for clarification purposes[1]. The resulting complexes between proteins and phenolic compounds are usually removed from wine after filtration and/or decantation steps. However, the presence of casein residues in fined wine cannot be completely excluded. The European Food Safety Agency (EFSA) has issued opinions on this matter stating that a real risk of caseins remaining in some wines might exist[2]; moreover, the lack of good manufacturing practices represents a further issue for consumer's health concern. The most recent directive on allergen issued by the European Commission, the 2007/68/EC[3] required that all milk products intentionally used for food or beverage (wine included) manufacturing had to be declared in the respective label. On the other hand, the deadline for mandatory labelling of egg and milk ingredients used as wine fining agents has been postponed to the end of June 2012[4]. In order to protect sensitive consumers from allergic reactions, sensitive and reliable analytical methods tailored to confirm the presence of milk allergens in white wines are strongly required. A method based on LC-ESI-High Resolution (HR)-MS analysis, using a single stage Orbitrap mass spectrometer, for the quantification of casein allergens present in white wines is here described. The method is based on protein extraction/purification, tryptic digestion followed by detection/quantification of residual caseins by monitoring the response of four representative peptide-markers[5]. Method linearity was assessed on caseinate solutions prepared either in water or in wine matrix. Limits of detection ranged from 0.1 to 0.3 µg/mL in water, and between 0.15 and 0.7 µg/mL in wine matrix, depending on the peptide selected. The method was validated on casein-free white wines fined with caseinate at different concentrations.

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Keywords: Orbitrap-MS, wine, allergens, milk proteins

A-3
PROPOSAL FOR GUIDELINES AND GENERAL CRITERIA TO PRODUCE REFERENCE MATERIALS FOR FOOD ALLERGEN ANALYTICAL METHODS

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Effective allergenic risk assessment and management are important to limit the use of precautionary statements such as “may contain” and to be able to protect allergic consumers. However, such approaches require reliable analytical tools for the detection of allergens in food, in order to inform risk managers about the extent of carry-over of allergenic ingredients on common processing lines, problems of cross-contact from dusts in factory environments and to monitor clean-up procedures. They are also required by those enforcing legislation to monitor food products for the presence of allergens in foods. Very few validation data are available for the comparison of results obtained with different allergen detection methods. The current lack of reference materials suitable for the development of allergen detection methodologies, particularly in different food matrices, must be urgently remedied in order to assess the output of different validation studies as well as to allow comparability between different methods. The establishment of guidelines for producing reference materials has to be defined. It is one of the aims of the subgroup “Reference materials” of the CEN (Centre Européen de Normalisation) TC 275/WG 12 Food allergens. This draft Standard provides guidelines and general criteria for producing references materials in order to assess allergen detection methods. The most important characteristics of a reference material for analytical quality control are homogeneity and minimum sample size, stability during transportation and storage, commutability and a measurement value, if possible with traceability properties and an uncertainty value.

Keywords: Allergens, Incurred Reference Materials, Validation, Analytical methods

A-4
COMPREHENSIVE ANALYSIS OF THE B-VITAMIN COMPLEX IN FOOD AND BEVERAGES BY LC-MS/MS

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Novel Aspect The ability to test all B complex vitamins simultaneously, with significant gains in sensitivity over typical microbial and LC-UV assays Introduction The B-complex vitamins are essential for growth and a variety of bodily functions. Playing a major role in enzyme activity and protein reactions, the B-complex is comprised of eleven water soluble analytes, which vary significantly in chemical structure, yet all are highly polar. Food samples can be challenging and the different matrices are complex; sensitive methods require selective sample clean up. These features make water soluble vitamin analysis a challenge for food manufacturers, who are required to provide accurate labeling on nutritional content of packaged products. To streamline the values reported amongst a variety of food matrices, a simple method to extract and quantitate water soluble vitamins across a variety of matrices was developed. Methods Homogenized samples were subjected to enzymatic digestion to mimic digestion in the body. Samples were digested in triplicate to ensure the precision of the digestion. Filtered samples were analyzed by LC-MS/MS with a seven minute gradient separating the eleven analytes on a polar embedded C18 column. The mass spectrometer was operated in multiple reaction monitoring mode (MRM) to achieve low detection limits and maintain specificity in the complicated matrices. Two MRMs were collected for each analyte of interest. All analytes were monitored in positive electrospray ionization mode. Preliminary Data Analytes showed linearity for 3 orders of magnitude with r> 0.998, with low part-per-billion detection limits for Vitamins B1 (thiamine), B2 (riboflavin), B3 (niacinamide, nicotinic acid), B5 (pantothenic acid), B6 (pyridoxamine, pyridoxal, pyridoxine), B7 (biotin), B9 (folic acid), and cyanocobalamin (B12). Spiked extract samples showed recovery accuracies within 20%, and reproducibility among injections was

Keywords: Vitamins, LC-MS/MS, matrices

A-5
VALIDATION OF A RAPID, ON-SITE TESTING METHOD FOR FOOD ALLERGENS

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Food allergy, an immune response to proteins present in food that the body mistakenly believes are harmful, is an important health problem of increasing concern in developed countries. The major risk for food manufacturers in this context is the potential for cross contamination with food allergens during the production processes. Allergens are the largest single cause of global product recalls. The aim of any food manufacturer's Food Allergen Management program is to minimize this risk. An important tool in any allergen management plan is testing for the presence or, better still, absence of allergens. While there are many laboratory based test systems available, rapid, on-site results are preferred for immediate corrective actions. The aim of this study was to validate the on-site application of the AgraStrip® Allergen Test Kits (Romer Labs®), which are antibody-based, rapid tests in a lateral flow format. The new application allows for the detection of allergens in food samples, as well as for cleaning control using rinse water samples or environmental swab samples. The food sample is extracted and then transferred to an incubation vial that contains specific ready-to-use antibodies. If the sample contains the allergenic food, an antigen-antibody complex will form. This is subsequently detected by the AgraStrip test strip. Swabs are also extracted before being added to the incubation vial. Rinse waters, neutralized to approximately pH 7, can be directly pipetted into the incubation vial with the extraction buffer. Limits of detection for a range of pure allergens (in solution) have been determined to be between 1 and 10 mg/kg in food samples. Results with spiked commodities showed limits of detection ranging from 1 ppm for casein in different commodities, up to 20 ppm with walnut in chocolate. A panel of various commodities was also analyzed to check for possible cross reactivity. The detection limit for allergens in rinse waters was found to be approximately 2 to 5 µg/mL, depending on the allergen. Protein concentrations down to 0.2 µg/mL were detected by surface swabbing of stainless steel and plastic. In conclusion, AgraStrip Allergen Test Kits are easy to use and with this on-site method give results in only 11 minutes. These lateral flow tests can be conducted without the need for further equipment and can be stored at ambient temperatures thus making them well suited for on-site testing directly in the manufacturing facility.

Keywords: food allergen, on-site, rapid, allergen management

A-6
DEVELOPMENT AND VALIDATION OF A REAL-TIME PCR METHOD FOR THE SIMULTANEOUS DETECTION OF BLACK MUSTARD (BRASSICA NIGRA) AND BROWN MUSTARD (BRASSICA JUNCEA)

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Mustard, a member of the Brassicaceae family, is able to induce allergic reactions, including severe anaphylaxis. About 1-7% of all food allergic patients are affected from mustard allergy. French studies demonstrated that mustard is the fourth common allergenic food for children, after eggs, peanuts and cow milk. In the European Union, 14 potentially allergenic ingredients, including mustard and products thereof, have to be labelled according to the Directive 2007/68/EC. Sensitive analytical methods are necessary to verify correct food labelling. Currently, protein based methods (e.g. enzyme linked immunosorbent assays, ELISAs) and DNA based methods (e.g. polymerase chain reaction, PCR) are applied in routine food analysis. So far, two real-time PCR methods have been published for the detection of mustard in foods (Mustorp et al., 2008; Fuchs et al., 2010). Since the developed method presented by Mustorp et al. showed some cross-reactivity with other Brassica species, it is, however, not applicable for the specific detection of mustard in food. The real-time PCR method presented by Fuchs et al. enables the specific detection of white mustard (*Sinapis alba*). The method does not show any cross-reactivity with 67 biological species, including 12 members of the Brassicaceae family. Since commercial food products may not only contain white but also black (Brassica nigra) and/or brown (Brassica juncea) mustard, the aim of the present paper was to develop and validate a real-time PCR method for the simultaneous detection of these two mustard species. The primers and the TaqMan probe were designed for a sequence of the Brassica nigra gene encoding reverse transcriptase from gypsy-like retroelement 13G42-26 (NCBI accession number AJ415649). After optimization of the primer and probe concentrations and the annealing temperature, the specificity of the method was tested by analyzing 73 different biological species, including 11 members of the Brassicaceae family. Low cross-reactivity was obtained with white mustard, cinnamon, cumin, fenugreek and ginger. Cross-reactivity could, however, be neglected when the DNA amount was reduced from 100 ng to 1 ng. The LOD and the amplification efficiency of the real-time PCR method were determined by analyzing serially diluted extracts from black and brown mustard as well as extracts from model sausages spiked with various amounts of black and brown mustard.

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Keywords: black mustard, Brassica nigra, brown mustard, Brassica juncea, real-time PCR

A-7
ASSESSMENT OF HISTAMINE LEVELS IN FISH PRODUCTS: A 3-YEARS CONTROL ACTIVITY OF A EU LABORATORY

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In recent years the demand of consumers for food safety has promoted the investigation for food with harmful compounds. Among these toxic compounds, the histamine in fish has received considerable interest due to its effects in humans health as allergy-like food poisoning, known as scombroid poisoning, which can lead to death in sensitive subjects. Histamine can be readily produced by bacterial decarboxylases in fish with high free histidine levels. Therefore, the reasons for determination of histamine in fish are twofold: first its potential toxicity; second the use of histamine as food quality marker. The Regulation (EC) No 1441/2007 states legal limits for fish and fishery products. According to guidelines issued by the US Food and Drug Administration (FDA), good quality fish should contain less than 10 mg/kg of histamine, whereas a level of 30 mg/kg indicates significant deterioration, and 50 mg/kg is considered to be a conclusive evidence of decomposition. In this work a combination of two analytical methods is used to evaluate histamine occurrence in fish, to produce useful data for preliminary surveillance study. Survey was performed on 305 fish samples (fresh fish, canned fish, processed anchovies) received in our laboratory for official control from September 2009 until August 2011. Fish products, arising from the Puglia region, and imported fish products were analyzed previously by ELISA test screening. The non-compliant samples were subsequently processed by an optimized HPLC/FLD method with post-column derivatization, validated according to the European guidelines. Histamine was detected (C >2.5 mg/kg) in 58% of total samples number with 5% of non- compliance. Among fresh fish samples, 70% had a content of histamine less than 10 mg/kg indicating good quality of products while a percentage of 12% of fresh fish showed histamine level major than 50 mg/kg. Among these samples three of fresh anchovies related to suspect sgombroid poisoning had an histamine content greater than 200 mg/kg. In the case of canned tuna, all the samples had an histamine content below 50 mg/kg, with 81% of samples below 10 mg/kg. Despite for processed anchovies, in 75% of samples a contamination below 50 mg/kg was observed, the highest percentage of “non-compliant” samples (14%) was found. In conclusion, although the most of the fish analyzed was of good quality, occurrence of histamine at high levels was frequently observed, so there is a need for constant monitoring to ensure the safety of fish product and public health.

Keywords: histamine, survey, liquid chromatography, fluorescence detection, food safety

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A-8
COMPARING THE PERFORMANCE OF DIFFERENT ANTIBODIES OF GLUTEN USING ELISA KITS AND LATERAL FLOW DEVICES

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From the 1st of January 2012, new European Allergen legislation will come into force that will apply to pre-packed and food sold loose labelled as gluten free or very low gluten. The levels that will apply will be 20mg/kg or less for gluten free and 100mg/kg or less and which contain cereal ingredients that have been specially processed to reduce the level of gluten for very low gluten. The legislation will not specify the frequency of testing required, but good practice and due diligence will be expected. While Enzyme-linked immunosorbent assay (ELISA) is the common routine method for the analysis of food in the laboratories, the use of lateral flow devices (LFDs) as a qualitative method for rapid allergen analysis for verification of the cleaning regimes is increasing being used in factories. There are currently several ELISA kits and lateral flow devices (LFDs) on the market which use different antibodies (R5 Mendez method, Skerrit & Hill and G12) to detect the levels of gluten (gliadin x2) in food. Each antibody has been raised against specific proteins (α, β and or ω-gliadins) or toxic peptides which potentially can create a disparity between the results when a food product is analysed. Codex alimentarius recommends the use of ELISA based on the R5 Mendez method for the analysis of gluten from wheat, rye and barley in food samples. The AOAC official method is based on the Skerrit & Hill antibody (specific to ω-gliadins) for the analysis of gluten from wheat, rye and barley although an over performance (recovery?) of the concentration of gliadins in rye and an underperformance (under recovery) of the concentration of gliadins in barley has been reported. The G12 antibody is the latest on the market, developed after recent studies in coeliac patients showing reactivity to toxic peptides present in oats. The aim of the study is to analyse cookie dough (rice flour 40%, vegetable oil 25%, glucose syrup or sugar 25% and milk 10%) spiked at 1, 1.5, 5, 10, 20 and 40 mg/kg with wheat, rye, barley and oats using the three different antibodies (R5 Mendez method, Skerrit & Hill and G12) by ELISA and LFDs and to compare the results.

Keywords: Gluten, R5, LFDs, G12, ELISA

A-9
RAPID IDENTIFICATION OF ALLERGENIC COMPOUNDS IN COMPLEX FRAGRANCES USING A HIGH SENSITIVITY GC TIME-OF-FLIGHT MASS SPECTROMETER WITH CHEMOMETRIC DATA ANALYSIS

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The analysis of complex samples such as essential oils and fragrances for target compound identification (eg allergenic compounds) typically combines the techniques of gas chromatography and mass spectroscopy (GCMS). Important parameters associated with this type of analysis are MS data acquisition rates, spectral purity and their relationship to sensitivity, especially so when the chromatography is fast ie within minutes. Conventional "Quad" based MS systems have limited scan rates and sensitivity values and produce spectra which are skewed when operating in a high speed acquisition mode. To combine high sensitivity with full scan (non-skewed) data and fast spectral acquisition requires time-of-flight (TOF) mass spectroscopy. To achieve these performance requirements a new bench top (GC)-TOF system will be described (BenchTOF-dx) and an example application will be demonstrated showing the high speed analysis of a fragrance sample for the identification of up to 24 allergenic compounds per EU Directive 76/768/EEC. The system provides sensitivity levels equivalent to quadrupole SIM analysis but with full spectrum data, and the spectra have a classical format ie directly compatible with NIST. Reducing the GC analysis time has several benefits, ie higher sample throughput, enhanced TIC signal to noise values, and statistical analysis, however it also provides additional challenges for the analyst. One consequence of faster chromatography is the time compression of the TIC profile potentially resulting in co-elution of compounds and the merging of spectral information. Under these circumstances accurate compound identification using conventional library searching techniques will not be possible. To overcome this problem, new post run MS data mining software will be described incorporating a novel chemometric approach for target compound identification. The software contains a sophisticated background noise suppression algorithm to minimise baseline effects (bleed, air/water) and combines spectral deconvolution with principle component analysis (PCA) to identify target compounds within the MS data. The performance characteristics of the software will be demonstrated by the identification of allergens in the fragrance sample using the TOF MS system described above.

Keywords: Allergens, Fast GC, TOF-MS, High sensitivty, Classical spectra

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A-10
DETERMINATION OF BIOGENIC AMINES IN FISH AND FISHERIES PRODUCTS USING IC-MS/MS

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A new method for determination of underivatized biogenic amines based on ion exchange chromatography coupled with mass spectrometric detection has been proposed. The method has been applied to the analysis of 10 biogenic amines (histamine, cadaverine, putrescine, agmatine, 2–phenylethylamine, tyramine, tryptamine, trimethylamine, spermidine and spermidine) in fresh and processed fish products. The amines were extracted from muscle tissue with water without any additional derivative step or sample clean-up. Biogenic amines were separated on Dionex CG17 (4×50 mm) column, using a gradient eluent by mixing formic acid and Milli-Q water. Linearities of response were obtained in the range 0.01–10 mg/L. The detection limits in fish products ranged from 20 ng/g to around 400 ng/g for histamine and putrescine, respectively. Spermidine and spermine showed significantly higher detection limits, therefore we can say that the procedure can be applied for their semi-quantitative determination. This method can be used for determination of biogenic amines in fresh and processed fish products in terms of regulatory and monitoring food safety issues relating to such amines, especially histamine. It is also a useful method for evaluation of other commercial or commonly used methods that are possibly affected by the food matrix due to processing or other drawbacks arising from derivatization process.

Keywords: ion chromatography, MS/MS, biogenic amine, determination

A-11
MULTISCREENING OF SEVEN ALLERGENS WITH MASS SPECTROMETRY AND COMPARISON WITH COMMERCIALLY AVAILABLE ELISA SYSTEMS

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Allergens are recognized as a major health issue with approximately 8% of children and 2% of the adult population affected. Symptoms occur immediately and can affect the skin, the respiratory and the gastrointestinal tract and may lead to systemic anaphylaxis. More than 160 foods have been shown to evoke a reaction, however only eight of them account for more than 90% of all allergic reactions. In the European Union directive 2007/68/EC lists a total of 13 food allergen groups that are mandatory to label if used as an ingredient. Despite this regulation, total avoidance might be difficult for the allergic consumer, as cross-contamination, e.g. due to the manufacturing on the same production line, occurs. Allergen risk management remains an important issue and analytical methods for the detection of undeclared allergens are needed. Two analytical methods are mainly used for allergen detection: antibody based ELISA and PCR. ELISA test have relatively analysis time and easy handling, however they are not capable of multiplexing. When a sample needs to be analyzed for more than one or two allergens, analysis time and cost increase significantly. Another issue is the influence of processing on the allergen. Processing might destroy the epitopes leading to false negative results. PCR methods have the disadvantage that the DNA is detected and not the allergic protein itself. This might not correlate with the amount of allergenic protein. The presentation will focus on a new multiscreening approach based on triple-quadrupole mass spectrometry. It is capable of simultaneously detecting seven allergens (milk, egg, soy, peanut, hazelnut, walnut, and almond). After extraction the allergens are digested with trypsin and separated by HPLC and analyzed in multiple reaction mode. The selection and the validation of the peptide marker are shown. To evaluate the influence of processing on the detection method, spiked flour samples and incurred bread reference material containing the seven allergens have been produced and analyzed. Results were compared with commercially available ELISA test kits. Both methods were capable of detecting peanut, hazelnut, walnut and almond in processed and unprocessed samples. MS could also detect egg in the processed samples. With the exception of one kit, egg could not be detected with ELISA.

Keywords: food allergens, mass spectrometry, multiplexing

A-12
A NOVEL APPROACH TO DETECT ALMOND ALLERGENS BY THE USE OF HIGH RESOLUTION MELTING ANALYSIS

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Almond is responsible for trigging atypical immune responses in allergic individuals, which can range from mild to life-threatening reactions (anaphylactic shocks) [1]. For this reason, almond and other tree nuts were included in a list of 14 groups of potentially allergenic foods with mandatory labelling, regardless of their amount (Directive 2007/68/EC). To ensure labelling compliance, proper analytical methodology is required to verify the adequacy of allergen label statements and to evaluate the risk to food-sensitive consumer. Immunological and DNA-based methods are the assays most widely used for the detection and quantification of allergens in foods [2]. Although immunological assays are preferably used since they evaluate the presence of allergen target directly, the DNA-based methods have also proved to be reliable alternatives for the detection of allergens in foods. However, only a few studies based on polymerase chain reaction (PCR) assays have been described for the detection of allergens in almond [3,4]. The novel approach of high-resolution melting (HRM) analysis emerged with the recent advances in high resolution instrumentation and with the specialised fluorescent DNA-binding dyes [5]. In this work, we propose the application of HRM analysis using the new generation EvaGreen dye to detect the gene AL60SRP encoding for the almond allergen Pru du 5 [6]. For this purpose, reference binary mixtures containing known amounts of almond were prepared ranging from 0.001% until 10%. DNA was extracted with Nucleospin Food kit. To endorse the specificity and sensitivity of the designed primers targeting the gene coding Pru du 5 allergen, real-time PCR using EvaGreen dye was successfully applied with high PCR efficiency (95.3%) and correlation (r^2=0.972) in the range of 10–0.005%. HRM analysis permitted the unambiguous identification of almond in several food products that included chocolates, salami and cookies, among others. The effort of using HRM analysis to increase the specificity of the assay was effective in discriminating almond from other plant foods, being the most pertinent accomplishment the ability to distinguish almond from other Prunus fruits (apricot, peach and nectarine) [7]. Here it was demonstrated for the first time that HRM analysis can provide a useful tool for the identification of trace amounts of allergens in foods.

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Keywords: Food allergens, almond detection, PCR, HRM analysis

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AUTHENTICITY, TRACEABILITY, FRAUD

(B-1 – B-44)

B-1
A COORDINATED RESEARCH PROJECT ON THE IMPLEMENTATION OF NUCLEAR TECHNIQUES TO IMPROVE FOOD TRACEABILITY

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Producing safe and high quality food is a prerequisite to ensure consumer health and successful domestic and international trade, and is critical to the sustainable development of national agricultural resources. Traceability systems play a key role in assuring a safe and reliable food supply. Analytical techniques for the determination of the provenance of food provide an independent means of verifying "paper" traceability systems and can also help to prove authenticity, to combat fraudulent practices, and to control adulteration, which are important issues for economic, religious or cultural reasons. To address some of the challenges that developing countries face in attempting to implement effective food traceability systems, the IAEA, through its Joint FAO/IAEA Division on Nuclear Techniques in Food and Agriculture, has initiated a 5-year coordinated research project involving institutes in 15 developing and developed countries (Austria, Botswana, Chile, China, France, India, Lebanon, Morocco, Portugal, Singapore, Sweden, Thailand, Uganda, UK, USA). The objective is to help in member state laboratories to establish robust analytical techniques and databases, validated to international standards, to determine the provenance of food. Nuclear techniques such as stable isotope and multi-element analysis, along with complementary methods, will be applied for the verification of food traceability systems and claims related to food origin, production, and authenticity. This integrated and multidisciplinary approach to strengthening capacity in food traceability will contribute to the effective implementation of holistic systems for food safety and control. The project focuses mainly on the development of techniques to confirm product authenticity, with several research partners also considering food safety issues. Research topics encompass determination of the geographical origin of a variety of commodities, including seed oils, rice, wine, olive oil, wheat, orange juice, fish, groundnuts, tea, pork, honey and coffee, the adulteration of milk with soy protein, chemical contamination of food products, and inhomogeneity in isotopic ratios in poultry and eggs as a means to determine production history. Analytical techniques include stable isotope ratio measurements ($^{2}D/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$, $^{87}Sr/^{86}Sr$, $^{208}Pb/^{207}Pb/^{206}Pb$), elemental analysis, DNA fingerprinting, fatty acid and other biomolecule profiling, chromatography-mass spectrometry and near infra-red spectroscopy.

Keywords: Traceability, Authenticity, Stable isotopes;

B-2
APPLICATION OF MASS SPECTROMETRY-BASED FINGERPRINTING/PROFILING AND MULTIVARIATE DATA ANALYSIS FOR AUTHENTICITY/TRACEABILITY OF OLIVE OILS

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The authenticity of olive oil as associated with genetic variety, geographical origin, and/or quality grade is an issue of high concern. Unfortunately, economic fraud, such as false claims of geographical origin on product labels, cannot be fully avoided. To protect the market from fraudulent practices and false label claims, a wide range of analytical strategies has been developed to confirm olive oil authenticity. Besides of spectroscopic techniques employing nuclear magnetic resonance (NMR), Raman, or infrared spectra, methods employing gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC) hyphenated to MS with atmospheric pressure chemical ionization (APCI), have been implemented for this purpose. In addition, several procedures such as matrix assisted laser desorption/ionization mass spectrometry (MALDI), direct head-space mass spectrometry (HS-MS), direct infusion MS, and/or ambient MS employing direct analysis in real time (DART) ionization allow reduction of analysis time thanks to elimination of chromatographic separation step. In this study, we have focused on the examination of various fractions of olive oils for the authenticity assessment (geographical origin). For the analysis of triacylglycerols (TAGs) ambient mass spectrometry employing DART–orbitrapMS was employed. In this case, the sample was diluted with toluene and immediately analyzed. For the profiling of polar compounds a rapid extraction with a methanol–water mixture was used before DART– orbitrapMS analysis. In addition, volatile compounds were isolated by an automated head-space solid-phase microextraction (HS-SPME) procedure followed by either gas chromatography–mass spectrometry (GC–MS) or direct desorption into an electron ionization ion source (HS-SPME–EI-MS). Since highly complex data matrices were generated by these fingerprinting and profiling techniques and required to be processed, powerful chemometric tools such as principal component analysis (PCA) and linear discriminant analysis (LDA) were used for data interpretation to fully utilize this comprehensive information.

Keywords: authenticity, traceability, olive oils, mass spectrometry

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B-3
CITRUS LIQUEURS QUALITY CONTROL EMPLOYING HEADSPACE-SOLID PHASE MICROEXTRACTION (HS-SPME) COUPLED TO GAS CHROMATOGRAPHY-COMBUSTION-ISOTOPE RATIO MASS SPECTROMETRY (GC-C-IRMS), ENANTIOSELECTIVE-GAS CHROMATOGRAPHY (ES-GC) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Liqueurs derived from Citrus fruits, generally obtained from maceration of lemon, mandarin and bergamot peel in ethanol, water and sugar, are a category of spirit drinks in which the addition of nature-identical flavouring substances and preparations is not authorized. The traditional production methods and the protection of geographical indications of spirit drinks are governed by the Regulation (EC) No 110/2008 of the European Parliament and of the Council. Authenticity assessment of home-made and commercial Citrus liqueurs was performed using Headspace-Solid Phase Microextraction (HS-SPME) coupled to Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS). Additional analyses were performed on all the samples, by means of enantioselective Gas Chromatography (Es-GC), measuring the enantiomeric distribution of the chiral volatile components, extracted by the same HS-SPME technique. Moreover, Gas Chromatography–Mass Spectrometry (GC-MS) measurements were also conducted employing the HS-SPME technique, in order to obtain information on the qualitative aspects of the samples. The data obtained from the GC-MS technique were also able to reveal the lack of the monoterpene fraction in some commercial samples. The GC-C-IRMS measurements of the liqueurs were compared with the authenticity ranges of the Citrus volatile components carbon isotopic ratio, obtained from genuine cold-pressed lemon, mandarin and bergamot essential olis. In particular, it was seen that the carbon isotope ratio of the volatile compounds of the home-made drinks fell into the correspondent authenticity range of the cold-pressed essential oil. GC-C-IRMS, ES-GC and GC-MS techniques coupled with HS-SPME extraction method have shown to be a complete and rapid tool for the quality control investigation of Citrus liqueurs, and were in good agreement in the revealing of non-natural Citrus aromas in some commercial liqueurs, as well as the assessment of the genuineness of the home-made ones.

Keywords: liqueurs, citrus oils, isotope ratio mass spectrometry, enantioselective GC, GC-MS

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B-4
IDENTIFICATION OF THE VEGETABLE AND ANIMAL FOOD ORIGIN

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Multi-element compare analysis using ICP-MS and gas chromatography are the base of food origin geographical identification. Now for geographical origin and falsification of wines, tea, juice and olive oil identification a “fingerprint” method is used. Success of technique’s reali-zation depends of a suitable elements choice. It is connected with geochemistry of soils. Quantity of elements for this function is limited, it is required the authentic information about their ratios. Method realization becomes complicated with many natural and anthropogenesis factors, such as climate conditions, affinity of industrial productions etc. Using «fingerprint» techniques with mass spectra and chromatograms foodstuff’s extracts allows identifying product origin more reliable. Multi-element analysis by means of ICP-MS and chromatograms comparing are the base of food origin geographical identification. Ĕass-spectrometer with inductively coupled plasma «Elan 9000 DRC II» and gas chromatograph «Clarus-600» (Perkin-Elmer, USA) were used. Data were processed with program «Elan ICP-MS Instrument Control ver. 3.4» (Perkin-Elmer, USA). It is chosen elements which concentrations at the samples differ more than 50 % as geochemical markers for various kinds of raw materials and production (meat, sugar, tea, coffee, oils, juice and fault). For reliability identification increasing through the error minimization macro and micro component ratios is offered: K/Na, Ti/Ge, Mg/Ca, Rb/Sr, Ni/Co, U/Bi, U/Th, Pb/U, Pd/Ag, Cl/P, Mo/Pd, Li/U, U/Ce, Sr/Zr.

Keywords: Multi-element compare analysis, food origin identification

B-5
MULTICOMPONENT ANALYSIS OF SEED OILS BY DIRECT SILYLATION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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In recent years in food analysis there is an aim in developing new direct methods that would determine different chemical compounds in one separation analysis. For the authenticity and composition studies of bio-oils a simple method for the determination of free fatty acids, squalene, free and esterified phytosterols, tocopherols and triglycerides was developed. Oils were derivatized directly by mixture of pyridine and BSTFA/TMS (99:1). Derivatized samples were separated on capillary column coated with 65%-diphenyl-35%-dimethyl polysiloxane copolymer using gas chromatography – mass spectrometry (GC/MS) and TIC/SIM detection mode. The chromatographic profiles of analysed compounds by using selective ions monitoring could be used for authenticity and chemometric studies of bio-oils and their blends. Developed method could also be used for GC/MS profiles analysis of animal fats and as a tool fats fraud protection. The usage of internal standard allows quantitative determination of different classes of compounds.

Keywords: multicomponent analysis, bio-oils, fats, authenticity, GC/MS

B-6
AUTHENTICATION OF PARMIGIANO-REGGIANO GRATED CHEESES BY MEANS OF NMR ANALYSIS

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Parmigiano Reggiano (PR-RE) cheese is a well known Italian hard cheese, long ripened, made from raw and partially skimmed cow's milk. It is included in the list of Italian cheeses bearing the Protected Designation of Origin (PDO, EU regulation 2081/92). This definition includes technological characteristics and geographic restrictions. During the ripening period, the cheese chemical components undergo important chemical, physical and enzymatic modifications: proteolysis and other reactions, such as lipolysis and lactic and propionic acid fermentation, influence the organoleptic properties of the final product. Proteolysis directly contributes to flavour (release of peptides and amino acids) and off-flavours (bitter hydrophobic peptides), also liberating substrates for others reactions. Thus, for the development of an acceptable cheese flavour, a well-balanced breakdown of the protein (i.e., casein) into small peptides and amino acids is necessary. On the other hand, during the last few years, a great interest has been grown for the protection of typical food products, such as PR-RE, from adulteration, sophistication and falsification. In particular, in this work we have been focused on the development of an easy and quick method able to characterize grated PR-RE from other cheese. As analytical technique, we chose 1H NMR, which has been already successfully applied for the analysis and characterization of different food matrices, for example cheese, fruits, tomato and meat. The major advantages of using this technique are the short and easy sample preparation step and duration of experiments, if compared to other kind of analyses. We analyzed 52 samples classified as: a) PR-RE at four different ripening periods, b) PR-RE which has been subjected to sophistication processes; c) three different cheeses which are PR-RE fakes. The samples for NMR analysis were prepared by dissolving grated cheese in deuterate oxide phosphate buffer, and the aqueous fraction was extracted after centrifugation. The extracted phase was directly used for NMR analysis. After setting up instrument operative conditions, all the samples were acquired. The samples analyzed contain mostly amino acids and organic acids. The NMR spectra were processed and the major components were identified by performing monodimensional (1H, 13C) and bidimensional (HSQC, TOCSY) NMR experiments. Then, all the 1d protonic spectra were divided in integral regions (binning method) and a multivariate statistical analysis (PCA) was applied in order to evaluate significant variations between the different groups of grated cheese sample, allowing to discriminate the samples belonging to the different groups. Statistical data elaboration will be presented and discussed.

Keywords: NMR, authenticity, Parmigiano-Reggiano

B-7
DETECTING ADULTERATION OF ANIMAL FEED OILS BY NEAR-INFRARED AND RAMAN SPECTROSCOPIES

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Oils used in the animal feed industry can be adulterated with transformer and mineral oil as a means of illegally increasing profit. In spent form, transformer and mineral oils can contain dioxins/PCBs. A set of basic vegetable blends (BVBs) and Soya oil samples adulterated with transformer oil and mineral oil were characterised using both near infrared spectroscopy (NIRS) and Raman spectroscopy. Applying chemometrics to the NIRS and Raman spectral data, very good calibration and prediction statistics were obtained for the calibration models generated. For NIRS, coefficient of determination values greater than 0.99 were obtained with corresponding values for root mean squared error of calibration and prediction (0.313–0.775 and 0.316–0.739 respectively). For Raman spectroscopy, coefficient of determination values greater than 0.97 were obtained with the root mean squared error of calibration (0.221–4.68) and prediction (0.489–4.68) calculated. The results for the calibration models and validation values depended on the aligritms used to process the spectral data of the adulterated oils. This study demonstrates that both NIRS and Raman technology can be successfully applied as rapid screening techniques for the detection of oil adulteration and fraud in the food and feed industry.

Keywords: Raman, NIRS, Adulteration, Oils

B-8
QUALITY AND AUTHENTICITY OF PLUM JAM

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Plum (*Prunusdomestica L.*) is a nutritionally and technologically important fruit and the harvesting of plum trees and plum based products manufacturing belongs to the Czech tradition. Plum jam is originally Slavic fruit product, which was traditionally boiled for a long period in non-covered pot. Nowadays, the production is based on "lekvar" (plum intermediate product) or dried plums. The plum jam should be tough, glossy, and of specific pure fruit taste. Chemical composition of plum depends on a many factors (variety, degree of maturity, climate, etc.). The most common way of plum jam adulteration is the reduction of the plum content in the jam, which should be according to Czech legislation at least 170 g of plums in 100 g of plum jam. A part of plums is replaced by sugar or more often by other fruits, apples mainly. Frequently also other stone fruits are used instead of plums, and it is very often, when intermediate products (lekvar) of uncertain origin are used. The suitable markers for estimation of authenticity and quality of plum jam were chosen. The followed markers were: dry matter content, phosphorus, ash, organic acids, sugars, phenolic acids, anthocyanins, potassium, calcium, magnesium, floridzin, titratable acidity and formol number. The ranges and regression relationships for fruit content estimation according to the above markers were proposed. The procedure was used for the evaluation of the sets of industrial and commercial samples (plums, plum jams, plum intermediate products).

Keywords: Plum jam, authenticity, quality

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B-9
PROFILING OF HERBAL SUPPLEMENTS USING A NOVEL RAPID VAPORIZATION SYSTEM COMBINED WITH DIRECT ANALYSIS IN REAL TIME (DART) MASS SPECTROMETRY

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Rapid determination of the origin and quality of herbal supplements is facilitated by using a novel sample analysis method to enable faster heating of samples during desorption ionization in Direct Analysis in Real Time (DART). The ID Cube ionization source uses a stainless steel wire mesh onto which various extracts of either the finished supplement or raw materials used in its preparation are deposited as liquids. The wire mesh is positioned between the ID Cube source and the API-inlet of the mass spectrometer just prior to analysis. The mesh is attached to a variable-current power supply that can deliver sufficient current to heat the sample at a rate > 20× faster than the conventional DART cartridge heater. This method facilitates thermal profiling of samples by permitting a more rapid temperature change in the desorption region. Desorption at low, medium and high mesh temperature settings is completed in a quick step-wise fashion. For the analyses pre-cleaned stainless steel mesh are cut into bow-tie shapes 2” in length. Electrical contacts were fixed at opposite sides of the mesh strip and the other ends were attached to a high-current power supply. A small quantity of liquid sample (5 µL) was spotted on the center of the mesh. It was then attached to an electrically isolated clamp and placed between the ID Cube and the API inlet of a Thermo Exactive high resolution accurate mass mass spectrometer. Utilizing Direct Analysis in Real Time (DART) ambient mass spectrometry (MS) as a quick and efficient means of characterization of herbal supplement standards is needed to quickly screen and qualify both national and international herbal products on the market. We are creating a database of DART-MS spectra of fruit and oil dietary supplements in order to be able to routinely screen large numbers of samples that could be detained for inspection at border crossings or sampled as part of a quality inspection. The next generation DART ionization source, the ID Cube will also be evaluated as a low cost, simpler method of screening since the operation of this ionization source has been significantly simplified and miniaturized from the current DART-SVP ion source making it of greater interest as a tool in a mobile lab setting. The herbal supplement standards will be characterized by DART-MS based on their fingerprint spectra and also subjected to accurate mass high resolution mass spectrometry for compound identification. The subject samples will be analyzed against a library of standards and detection limits for gross adulteration will be fine-tuned. Analysis time per sample is approximately 10–12 seconds and this screening technique is evaluated for both gross and residual levels of adulterants.

Keywords: DART, Herbal Supplements, QC, Screening

B-10
HPAE-PAD DETECTION OF UNDECLARED SUGAR ADDITION

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Some producers adulterate fruit juices or honey by undeclared addition of inexpensive sweeteners to increase quantitiesand reduce manufacturing costs. The difficulty of detection of sugar addition depends on the kind of sugar preparation (e.g. inverted syrup, high fructose corn syrup, glucose syrup) and on the amount of added sweeteners to foodstuff. The fingerprint of characteristic oligosaccharides in the preparation, which results from the conditions of hydrolysis of starch syrups, can be used to detect this type of adulteration. In the presented study, the high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) was used to determine the fingerprints of saccharides in various sugar containing materials. Our first goal was to optimize and validate the method for the analysis of oligosaccharides in various sugar materials. We focused on the sample preparation; solid phase extraction was used to remove monosaccharides and small oligosaccharides and to concentrate simultaneously traces of polysaccharides. The optimized procedure was used for the analyses of the set of commercially available syrups. Finally, the possibilities of the detection of addition of these syrups were evaluated in model juices and model honey samples.

Keywords: HPAE-PAD, fingerprints of saccharides, undeclared sugar addition

Acknowledgement: The study was supported by MŠMT 6046137305 and MZe QI91B283.

B-11
ALTERNATIVE PROFILING APPROACHES TO TEA ANALYSIS

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Tea belongs to the most consumed beverages all over the world due to its special characteristics associated with taste and flavour. The authentication of this popular commodity represented by a wide range of products is a complicated task that needs to be addressed to ensure fair conditions for both the consumer and producer. Among many analytical approaches applicable for the authentication, such as spectroscopic techniques (NMR, Raman, IR spectra) or methods employing GC–MS and/or LC–MS, solid phase micro-extraction (SPME)-based sampling procedure coupled to gas chromatography–mass spectrometry (GC–MS) is becoming to be used as a key method for the analysis of tea volatiles. An ambient mass spectrometry employing a direct analysis in real time (DART) ion source in combination with a time-of-flight mass spectrometer presents an alternative approach to tea profiling. The aim of this study was to discriminate among 58 tea samples according to their origin and fermentation degree. For this purpose, solid-phase micro-extraction followed by gas chromatography and time-of-flight mass spectrometry (SPME–GC–TOFMS), as well as direct analysis in real time ionization coupled to a time-of-flight mass spectrometric detector (DART–TOFMS), were introduced. The multivariate data analysis techniques including analysis of variance (ANOVA), principal components analysis (PCA) and linear discriminant analysis (LDA) were used for a statistical evaluation.

Keywords: tea, volatiles, authentication, SPME–GC–MS, DART–MS

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B-12
TRACING THE GEOGRAPHICAL ORIGIN OF CHINESE AND JAPANESE APPLE USING STABLE CARBON AND OXYGEN ISOTOPE ANALYSIS AND TRACE ELEMENT ANALYSIS

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Recently, Japanese food products attract attention all over the world and are exported to a lot of countries. Delicious NIPPON is the program of the Ministry of Agriculture and Forestry (MAFF) for the export promotion of a Japanese ingredient. This program introduces the enchantment of Japan's dietary culture to the world for further promoting export opportunities. Especially, the apple is one of the aggressive export promotion fruits in Japan. Apples grown in Japan are high popularity as the gifts in Asia because they have the finest quality and safety. On the other hand, Japanese fruits are very expensive and have been targeted for the mislabeling. The cultivation area is important factor in determining the market value of apple. A simple analytical method which identifies their cultivation area is required to resolve food authenticity problems. Stable isotope analysis has become increasingly important as a solution tool for food authenticity problems. It has also become increasingly important as a solution tool for food authenticity problems. Trace element analysis has also been used as a rapid tool for discrimination of cultivation areas. In this study, we determined stable isotope ratios and trace element compositions of apples from various cultivated areas in China and Japan to discriminate their geographical origin. Of 188 samples, 98 were from Aomori Pref. (Japan), 42 were from Nagano Pref. (Japan) and 48 were from China. Stable carbon and oxygen isotope ratios were determined by using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS). Eighteen elements (Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Cd, Cs, Ba, Tl, Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS). The ƒÂ13C and ƒÂ18O values of Chinese apples are significantly higher than those of Japanese apples. Using the analytical results of ƒÂ13C and ƒÂ18O values and 18 elements (Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Cd, Cs, Ba, Tl, Pb), apple samples were analyzed by cluster analysis and canonical discriminant analysis to categorize into particular groups. As a result, the apple samples were divided into three groups: China, Aomori Pref. (Japan), and Nagano Pref. (Japan). Thus, stable isotope analysis and trace element analysis would be potentially useful for discriminate geographical origin of Chinese and Japanese apples.

Keywords: stable isotope analysis, trace element analysis, geographical origin, apple

B-13
CHARACTERIZATION OF THE GEOGRAPHICAL ORIGIN OF APULIAN VIRGIN OLIVE OILS BY INSTRUMENTAL AND MULTIVARIATE STATISTICAL ANALYSES

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In this work, free acidity, peroxide and spectrophotometric values, chlorophyll content, sterol, fatty acid and triacylglycerol composition were measured for virgin olive oils coming from three different geographic origins of the Southern Italy (Apulia region). The analytical parameters were studied by applying univariate and multivariate statistical methods, with the aim to find models able to discriminate the geographic origin of the olive oil samples. It was evidenced that univariate statistical techniques could not distinguish the three classes under investigations, while multivariate techniques, such as General Discriminant Analysis (GDA), Partial Least Squares-Discriminant Analysis (PLS-DA) and Soft Independent Modelling of Class Analogy (SIMCA) produced interesting results, finding the best prediction results with GDA (average prediction ability higher than 80%).

Keywords: Virgin olive oil, oil quality parameters, purity parameters, multivariate statistical analysis, geographical origin

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B-14
OFFICIAL FOOD CONTROL IN ITALY DURING THE YEARS 2007–2011 TO DETECT FRAUDULENT TREATMENT OF FISH WITH CARBON MONOXIDE USING A SPECTROPHOTOMETRIC METHOD.

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In the European Union the use of carbon monoxide (CO) in vacuum and modified atmosphere packaging (MAP) is not permitted. Therefore, in Italy fish products are routinely under control to check the presence of carbon monoxide. Two methods should be used for the determination of CO, a gas chromatographic or a spectrophotometric. In our laboratory the latter is usually applied as qualitative and quantitative method. It has been developed and validated as reported by Smulevich et al., [1] and by Droghetti et al., [2]. It is based on the analysis of the Soret region of the electronic absorption spectrum of a meat drip dissolved in phosphate buffer. In fact, CO forms a very stable cherry-coloured complex with myoglobin (Mb) of muscle tissue (Mb–CO). The presence of Mb–CO is evaluated by its intense band at 420 nm and confirmed by its persistence, after addition of sodium dithionite; both spectra, obtained before and after the addition of the reducing agent, are elaborated in normal and second derivative modes. During the period 2007–2011 the majority of the controls have been performed on tuna fish. Recently we have extended the analysis to samples of tilapia. The Limit of detection (LOD) of the method, determined during the single laboratory validation procedure, has been tested in the routine analysis of tuna and tilapia samples. Quantitative results will be shown for CO-treated samples.

[1] Smulevich et al, Food Chem. (2007), 101, 1071
[2] Droghetti et al, Food Chemistry (2011), 128, 1143

Keywords: carbon monoxide, tuna fish, tilapia, electronic absorption

B-15
LINEAR DISCRIMINANT ANALYSIS ON TRIACYLGLYCEROL STEREOSPECIFIC COMPOSITION FOR THE DETECTION OF MILK ADULTERATION

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Dairy products are frequently subjected to adulteration due to the addiction of lower price milk to more expensive one; the quality of milk can be compromise for example by the undeclared addition of cow milk to goat and ewe milks [1]. Species identification has a remarkable importance on account of frequent human adverse reactions [2]. For a long time methods for the quali- and quantitative determination of cow milk in mixtures with other milks have been studied. For this purpose different analytical approaches can be used: lipid and protein analysis or DNA-based methods. The lipid fraction has been used less for milk species identification, in particular the most commonly used methods were based on some fatty acid (FA) ratios or triacylglycerol (TAG) profile, but these do not always allowed detection of milk adulteration. To differentiate milk from different origin multivariate statistical analysis can be applied, in particular linear discriminant analysis (LDA) is useful to examine multivariate differences between groups and to determine which variables are the most helpful for discriminating between groups [3]. This research is part of a more extensive work concerning the study of pure and mixed milks of different animal species. In a previous research [4] it was reported that TAG stereospecific analysis, important analytical method based on chemical-enzymatic-chromatographic procedures, is useful to characterize milk fat of different origin, because of TAG fraction of each lipid matrix has a characteristic FA distribution on the glycerol backbone. The aim of this work was to apply LDA to TAG stereospecific analysis experimental data of pure milks to select the best variables to characterize and distinguish milk samples according to animal species and to identify different milks and their mixtures.

[1] Ramos M. and Juráez M., Bull. Int. Dairy Fed. 1986, 202, 175-190.
[2] Commission Regulation 2001, EC n. 213/2001 of 9 January 2001.
[3] Cossignani L., Blasi F., Bosi A., D'Arco G., Maurelli S., Simonetti M.S., Damiani P., J. Dairy Res. 2011, 78, 335-342.
[4] Blasi F., Montesano M., De Angelis. M., Maurizi A., Ventura F., Cossignani L., Simonetti M. S., Damiani P., J. Food Comp. Anal. 2008, 21, 1-7.

Keywords: milk adulteration, triacylglycerols, stereospecific analysis, linear discriminant analysis

B-16
FT-IR SPECTROSCOPY AND CHEMOMETRICS FOR DETECTION OF CONTAMINATED OR COUNTERFEIT INGREDIENTS

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Glycerol is widely used in food, personal care and pharmaceutical products as a sweetener, humectant, filler or preservative. Glycerol is not harmful, but there have been numerous cases worldwide of counterfeit products using highly toxic diethylene glycol as a substitute for glycerol. Due to the similarity of their physical properties, it is not straightforward to detect this substitution by inspection alone, so there is a need for simple, rapid, sensitive methods of analysis to verify the identity and purity of this ingredient. Fourier transform infrared (FT-IR) spectroscopy is a powerful chemical fingerprinting tool that has long been used as a tool to verify the identity of materials in diverse industries. The most common approach is to determine a correlation coefficient between the sample spectrum and that of a reference material. Where there is little variation between "good" samples, this method can provide a sensitive test for purity as well as identity. However, when there is significant variation, this can mask the contribution of small concentrations of contaminants. This is a significant issue for glycerol, as it is hygroscopic and water concentrations of 1% or more may be allowable. In this submission, we show that the chemometric method of soft independent modelling by class analogies (SIMCA) can be used to develop a simple spectroscopic method to screen glycerol for unknown contaminants, in the presence of a variable amount of water. Impurities below the percent level can be detected, and the method is generally applicable to other situations in which the variability among legitimate samples is significant.

Keywords: FT-IR, Chemometrics, Contamination, Ingredients

B-17
GEOGRAPHICAL AND BOTANICAL CLASSIFICATION OF ITALIAN CHERRIES BY MEANS OF 1H NMR AND ISOTOPIC RATIOS COMBINED WITH CHEMIOMETRICS

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Isotope Ratio Mass Spectrometry (IRMS) and the Nuclear Magnetic Resonance Spectroscopy (NMR) were used in combination with chemometric techniques to assess the geographical (Emilia Romagna and Puglia) and varietal (Bigarreau, Ferrovia, Giorgia) authenticity of Italian cherries. When applying Discriminant Function Analysis (DFA) on NMR and IRMS data to distinguish cherry samples of different geographical origin, prediction abilities equal to 94.3% and 83.0% were obtained, respectively. In addition, applying DFA to the entire dataset (NMR and IRMS data) very good results were obtained in geographical prediction (98.9%), demonstrating the validity of a synergic approach. All these results highlighted the goodness of the models obtained, especially considering that these were constructed from a dataset in which the variability, in addition to their geographical origin, is linked to many other factors such as the degree of ripeness and varietal origin of cherries. Finally, for each of the two growing Italian regions, the NMR and IRMS results have been used for the discrimination of the botanical origin among the three cultivars, obtaining a prediction percentage equal to 100.0% and 98.9% for Emilian and Apulian samples, respectively.

Keywords: IRMS, NMR, cherry, geographical and botanical origin, chemiometrics

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B-18
DEVELOPMENT OF TWO COMPLEMENTARY REAL-TIME PCR METHODS FOR THE QUANTIFICATION OF FISH NUCLEAR DNA

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The development of DNA based methods for the identification and quantification of fish in food and feed samples it is frequently focus on an specific fish species and/or the detection of mitochondrial DNA targets or other highly repetitive sequences of fish origin. However a method for the simultaneous detection and quantification of the most common fish species used by the food and feed industry is needed for official control purposes, and such method should rely on the use of a single-copy nuclear DNA target due to its more stable copy number in different tissues. One of the main difficulties of developing such a method is the choice of an appropriate DNA target. On one side, a high number of different fish species are considered commercial in both the food and feed industry; on the other side public databases do not contain sequences for many of the fish species of interest, in particular from single copy nuclear DNA targets, which makes specially cumbersome to find an appropriate target. We have developed a real-time PCR method for the detection and quantification of fish DNA in food and feed using a single copy nuclear DNA sequence as target. The difficulties to find an appropriate nuclear target for such a various number of species without losing the required specificity, have been overcome with the use of degenerate primers and probe. The method was tested in 22 different commercial fish species and in 24 negative control samples including meat samples and some of the most common ingredients in the feed industry. Positive results were obtained with all fish species of the study, excluding mackerel (Scomber scombrus) and horse mackerel (Trachurus trachurus), which did not give satisfactory results. A complementary method was developed for the specific and simultaneous detection of mackerel and horse mackerel, to use independently or combined with the previous one.

Keywords: real-time PCR, fish, nuclear DNA, allergens, authenticity

B-19
WINE ORIGIN DIFFERENTATION USING UHPLC-QTOF MS AND METABOLOMIC APPROACHES

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Wine is considered as a valuable drink and its quality, and so the price, is related to its origin amongst other variables. Thus, ensuring the authenticity of the wine, although not easy, represent an important tool in order to avoid fraud and to improve the confidence of the costumer. Different approaches have been applied to this purpose in wines and other valuable foods. Non-target methods are commonly performed with reverse phase (RP) liquid chromatography coupled to mass spectrometry as well as NMR. These techniques are also applied to targeted purposes for the determination of interesting compounds such as antioxidants, for example. Alternatively, targeted methodology focused on metals by inductively coupled plasma mass spectrometry (ICP-MS) could be used. In this work, target and non-target procedures have been applied to distinguish wines with different origin from the Valencia Spanish region. A combination of several techniques has been used in order to obtain maximum information about markers. Thus, UHPLC-QTOF MS analysis in a non-target way using RP and HILIC chromatography has been performed. Moreover, metal wine composition have been carried out by ICP-MS in a semi-quantitative approach. Data was then analyzed using multivariate Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) to differentiate the wines. Both approaches succeeded in the separation of the different wines according to their origin. Sampling was carried out considering different varieties of grapefruit and vintage. In the non-target approach, best results were obtained when using HILIC column, achieving complete separation of the samples even for these close areas.

Keywords: UHPLC-(Q)TOF MS, multivariate analysis, wine origin authenticity, ICP-MS

B-20
MOLECULAR TRACKING USING CAVITY RING-DOWN: A NEW, PRACTICAL APPROACH TO FOOD TRACEABILITY USING STABLE ISOTOPES

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Food traceability using stable isotope signatures is in high demand. Mechanical tracking tools such as barcodes and RFID are fine for tracking legitimate materials in known supply chains, but immediately break down when faced with illegitimate shipments. Counterfeit labels and documents accompany every fraudulent shipment – a huge problem with dangerous consequences. Molecular tracking provides a significantly higher degree of food safety and significantly higher barrier to fraud by testing the contents, not the container. Stable isotopes are the tool of choice; scientific studies have proved their validity for decades. Yet, the academic standards, Isotope Ratio Mass Spec (IRMS) and Nuclear Magnetic Resonance (NMR) are unwieldy for deployment throughout the food industry. Cavity Ring-Down Spectroscopy is a bench top, fast, easy technique that can be used in any food lab, worldwide and gives the same or better data quality in a 10 minute test. We will present data to show how various food products and ingredients can be authenticated as coming from their stated origin, including coffee, cocoa, bananas, apples and oranges. In addition, we will show how profiles of synthetic products such as ingredients can be matched to a factory and then compared against know counterfeit products to ensure brand quality.

Keywords: traceability, safety, origin, isotope, fraud

B-21
CHARACTERIZATION OF SPANISH HONEYS WITH PROTECTED DESIGNATION OF ORIGIN "MIEL DE GRANADA" ACCORDING TO THEIR MINERAL CONTENT

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Honey authenticity has become a major concern due to adulteration cases because of different values of honeys from various geographical and botanical origins. Spanish honey is a high quality product consumed locally and also exported to other European countries. To avoid misleading labels and fraud, distinctive signs of authenticity for honey are done by the Spanish authorities and honeys from three geographical regions have been protected by an official Designation of Origin: Granada, Galicia and La Alcarria. In this work, Spanish honeys with Protected Designation of Origin "miel de Granada" and different botanic origins are characterized according to their mineral content. Six major elements (Na, K, Ca, Mg, Zn, Fe) were quantified by Flame Atomic Spectroscopy (FAS). Cluster analysis was used for data analysis.

Keywords: honey, mineral content, FAAS, FAES

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B-22
VERIFICATION OF THE TYPE OF FERTILIZER USED DURING ORGANIC AND CONVENTIONAL CULTIVATION OF LETTUCE BY MULTIVARIATE ANALYSIS OF STABLE ISOTOPE, METABOLITE AND MINERAL COMPOSITION

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In spite of the increasing development of organic agriculture, at present, no analytical controls of the fertilizer inputs are validated and fraudulent application of synthetic fertilizers to organic crops are difficult to detect. Several studies have used the natural abundance of nitrogen stable isotopes ($\delta^{15}N$) as a potential tool to detect fraudulent applications of synthetic nitrogen fertilizers to organic crops but results are not always conclusive. On the other hand, metabolite profiling and mineral content of fruits and vegetables has also been used to differentiate between organic and conventional food. The aim of this research work is to achieve a good classification of organic and conventional lettuce based on N content, $\delta^{15}N$ values, metabolites (sugars, organic acids, total phenolics, chlorophyll, vitamin C and antioxidant activity) and mineral content by using Canonical Discriminant Analysis (CDA). To this end, plants were grown with different solid and liquid organic fertilizers and synthetic fertilizers. Organic treatments involve the application of high rates of organic manure for soil biosolarization and plant fertilization. In addition to organic and conventional treatments, samples fertilized with organic manure plus synthetic fertilizers were included in the model to reflect a more complex and "difficult to classify" situation in which organic and synthetic fertilizers are used simultaneously. Discriminat functions resulted from shoot total N and $\delta^{15}N$ as predictor variables and allowed 80.8% of the original grouped cases and the 78.8% of cross validated cases to be correctly classified. When metabolite profiling and mineral composition were included in the dataset, the model was improved allowing 92.3% of the original grouped cases and the 82.7% of cross validated cases to be correctly classified by one canonical function that used shoot $\delta^{15}N$ and fresh weight, vitamin C, tartaric acid and antioxidant activity of the lipophilic fraction as predictor variables. In conc! lusion, analysis of $\delta^{15}N$ provided evidence about the origin of the N source used for lettuce cultivation. The inclusion of additional variables improved accuracy of the classification but the identification of other predictor markers are needed to allow more reliable discrimination between organic and conventional lettuce.

Keywords: authenticity, natural abundance, ^{15}N, antioxidants.

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B-23
QUANTIFICATION OF THE RED DEER CONTENT BY REAL-TIME PCR TO DETECT FOOD ADULTERATION

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The poster presents a real-time PCR method for the identification and quantification of red deer (Cervus elaphus) in meat samples to detect food adulteration. The primers and the probe were designed for a sequence of the gene encoding the ubiquitin-activating enzyme E1 (accession number EU219371). The PCR method is specific for red deer and does not show any cross-reactivity with roe deer, fallow deer, reindeer, chamois, wild boar, pork, cattle, chicken, turkey, sheep, goat, horse, rabbit, mouflon, ostrich and kangaroo. The limit of detection (LOD) and the amplification efficiency of the PCR method were determined by analysing serially diluted DNA extracts from red deer. The LOD was found to be 2 pg/µL, the amplification efficiency 99.3%. DNA extracts of meat mixtures containing 2%, 5%, 10%, 25%, 38.5% or 50% red deer in pork were analysed to investigate the applicability of the PCR method to detect meat adulteration. The LOD was 0.07% and the LOQ 0.26% red deer in the red deer/pork mixture. In order to determine the concentration of red deer in unknown meat samples the PCR method was calibrated by analysing DNA extracts from a model meat mixture (containing red deer, roe deer, fallow deer, pork and cattle). The percentage of red deer in unknown samples is calculated by relating the concentration of red deer DNA to the total DNA concentration of the sample.

Keywords: red deer, real-time PCR, food adulteration

B-24
DISCRIMINATION OF SLOVAKIAN ORGANIC AND CONVENTIONAL WINES ACCORDING TO ELEMENTAL AND AMINO ACID PROFILES

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The present study was performed to evaluate the elemental and amino acid composition of 27 conventionally and 15 organically produced Slovakian wines of five varieties sourced during the vintage period 2007-2009. All the samples were analyzed for the content of elements, Ag, Ba, Ca, Cd, Cu, Fe, Hg, Mg, K, Na, Pb, Rb, Sr, Zn selected according to their increased variability in soils of Slovakian vineyard regions. Macro-elements Ca, K, Mg, Na, Zn, Fe, Cu, Sr were determined by atomic spectrometry using an air/acetylene flame. Micro-elements Ag, Ba, Cd, Pb, Cr were measured on graphite tube atomizer and for mercury determination the analyzer AMA 254 was used. Twenty free amino acids in organically and conventionally prepared wines were determined by LC/ESI-MS-MS chromatographic method using the Agilent 1200 equipment with Agilent 6410 Triple Quad detector and ESI interface. Quantitatively discernment of organic from conventional wines was most effectively performed by discriminant analysis. Fe, Mg, Ag, Ca, Cu and K were found as the most effective discriminators for canonical discriminant analysis. Classification of wines resulted in 95% of correctly sorted samples according to methods of grape and wine production. In the case of individually discriminated white and red wines 100% success of classification was achieved due to variation of Fe, Zn and Ag markers in white wines and Pb, Rb, Ag and Cu in red wines. The results obtained confirmed that compared wine productions fulfill the safety limits regarding the examined element contents of wines. Preliminary results of LC/ESI-MS-MS method revealed that contents of majority amino acids in conventional wines are higher compared to their organic counterparts. For example the contents of leucine, methionine and phenylalanine amino acids were found twice and p-tyrosine even threefold higher in conventional sample (Cabernet Sauvignon, 2008) than concentrations in relevant organic wine. The achieved results will be reassessed by further investigation.

Keywords: organic wine, conventional wine, macroelements, microelements, amino acids

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B-25
CLASSIFICATION OF OLIVE OILS ACCORDING TO GEOGRAPHICAL ORIGIN BY USING 1H NMR FINGERPRINTING COMBINED WITH MULTIVARIATE ANALYSIS

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1H Nuclear Magnetic Resonance (NMR) fingerprinting combined with multivariate statistical analysis has been applied to the prediction of the geographical origin of olive oils. Authentic extravirgin olive oils from 7 different regions (3 regions of Italy and 4 regions of Greece) have been investigated. For each sample, two 1D-NMR experiments have been acquired, a simple one pulse experiment to detect the dominating lipid signals and an experiment with multiple saturation of the lipid signals in order to detect lower concentrated compounds. The dynamic range of concentrations covered by the two experiments was of the order of 100.000, thus allowing for a more comprehensive NMR assessment of the samples. Monte-Carlo embedded cross-validation was used to demonstrate that a combination of principal component analysis, canonical analysis, and classification via nearest class mean can be used to predict the origin of olive oil samples from 1H-NMR data. Given the rather limited number of samples tested, correct prediction probabilities of 78% were achieved with region specific correct predictions between 53 and 100%.

Keywords: olive oil, geographic origin, multivariate statistical analysis, 1H-NMR, fingerprinting

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B-26
QUALITY VALIDATION OF BRUKER NMR-BASED SCREENING: THE EXAMPLE OF FRUIT JUICE

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1H-Nuclear Magnetic Resonance (1H-NMR) screening is a powerful method for the fast and simultaneous evaluation of numerous parameters linked to quality and authenticity in food products. 1H-NMR is a global, non-targeted approach allowing quantification of multiple relevant compounds, as well as classification and verification of samples within minutes. This allows not only to assess the authenticity of the samples but also to detect unknown frauds that would not be detected by conventional targeted approaches. In order to guaranty the reliability of the quantification and of the classification results, in terms of accuracy as well as reproducibility, the methods are submitted to extended validation evaluations. Long-term reproducibility and inter-lab reproducibility have to be demonstrated. Participation to proficiency testing organized by FAPAS® is regularly undertaken. The accuracy of the quantification results is assessed through the comparison of the NMR results to the results of official methods. The accuracy of the classification prediction is given by the matrix of confusion. As an example, results of the validation evaluation will be presented for the fruit juice screening method SGF Profiling™.

Keywords: long-term reproducibility, validation, accuracy, reliability, NMR

B-27
DIFFERENTIATION OF WINE GRAPE VARIETIES BY MEANS OF 1H-NMR PROFILING

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1H-Nuclear Magnetic Resonance (1H-NMR) screening is an efficient method for both targeted and non-targeted analysis of food products. Applied to the analysis of wine, 1H-NMR profiling allows simultaneous quantification of targeted compounds as well as classification of samples, thus making it a method of choice for authenticity assessment as well as fraud detection. Multiple wine components can be directly quantified from the mixture, in a large dynamic range (order of 4 or 5). Furthermore, application of extended statistical analysis to the spectra allows sample classification. 1H-NMR profiling has been applied successfully to the prediction of the grape variety of German wines. 600 authentic German wines of 10 different grape varieties have been investigated. For each wine, two 1D-NMR experiments have been acquired. A simple one pulse experiment allows to quantify the ethanol content of the wine, whereas an experiment with multiple suppression of water and ethanol signals allows optimal usage of the dynamic range. The mean probability of correct prediction of grape variety of the model is of 96%, excluding the lower represented grape variety. The advantages of the method presented is the simple sample preparation required as well as the fast and fully automated measurement allowing multiparametric data analysis.

Keywords: Wine grape varieties, 1H-NMR profiling, authenticity

B-28
ISOTOPES RATIOS OF LEAD IN BRAZILIAN WINES AND GRAPE JUICES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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In Brazil, the Serra Gaúcha is the main wine producing area and it produces about 90% of wines, juices and other derivatives of a grape. Brazil started the process of recognition and certification of their regions and wines in 1995, but the first indication of origin (IP) only happened in 2002 for the Vale dos Vinhedos region. Wines from four important wine-producing regions, Campanha, Serra Gaúcha, Vale do São Francisco and Vale dos Vinhedos were analysed by inductively coupled plasma mass spectrometry. Lead and its isotope ratios were measured for the first time in 100 Brazilian wines and 20 grape juices. Lead had a medium value of 14.4 $\mu g.L^{-1}$, ranging from 4.36 up to 27.9 $\mu g.L^{-1}$ in Campanha, of 14.2 $\mu g.L^{-1}$, ranging from 9.05 up to 33.5 $\mu g.L^{-1}$ in Vale do São Francisco, of 14.1 $\mu g.L^{-1}$, ranging from 0.568 up to 66.4 $\mu g.L^{-1}$ in Vale dos Vinhedos, of 18.8 $\mu g.L^{-1}$, ranging from 5.77 up to 111 $\mu g.L^{-1}$ in Serra Gaúcha, of 15.7 $\mu g.L^{-1}$, ranging from 9.13 up to 27.1 $\mu g.L^{-1}$ in wines produced in other regions and of 11.1 $\mu g.L^{-1}$, ranging from 1.52 up to 39.8 $\mu g.L^{-1}$ in grape juices. The values of the isotopes ratios were (mean±standard deviation) 0.0564 0.0037 for 204Pb/206Pb, 0.8679±0.0238 for 207Pb/206Pb, 2.115±0.0369 for 208Pb/206Pb; 0.0469±0.0170 for 204Pb/206Pb, 0.8593±0.0058 for 207Pb/206Pb, 2.1012±0.0171 for 208Pb/206Pb; 0.0665±0.0729 for 204Pb/206Pb, 0.8642 0.0179 for 207Pb/206Pb, 2.1081±0.0314 for 208Pb/206Pb; 0.0562±0.0047 for 204Pb/206Pb, 0.8599±0.0154 for 207Pb/206Pb, 2.0995±0.0430 for 208Pb/206Pb; 0.0553±0.0003 for 204Pb/206Pb, 0.8588±0.0107 for 207Pb/206Pb, 2.0955±0.0168 for 208Pb/206Pb; 0.0544±0.0023 for 204Pb/206Pb, 0.8521±0.0139 for 207Pb/206Pb, 2.0768±0.0284 for 208Pb/206Pb, respectively. Based on the isotopic ratio, it was possible to distinguish between the wines produced in Bahia state of wines from Pernambuco state in the same producing region called Vale do São Francisco. The wines produced in other regions could be separated from the ones produced in the Southeast region (São Paulo st ate) and from the ones produced in the South region (Santa Catarina and Paraná states). In the Serra Gaúcha and in the Vale dos Vinhedos two curious facts were observed by isotopic analysis: in the first, extreme were results obtained are related like chaptalized wines or which the concentration of Pb was estimated above 30 $\mu g.L^{-1}$; and in the second these values corresponds like blend wines or are special wines produced by same vineyard. The grape juices were defined. Therefore, the results suggest that the Pb isotope ratio is a promising fingerprint of wine and grape juices origin of Brazil.

Keywords: Wines, Pb isotope ratios, ICP-MS, geographic origin

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B-29
AUTHENTICATION AND TRACEABILITY OF HAZELNUT (CORYLUS AVELLANA L., TONDA GENTILE TRILOBATA CV) EXPLOITING CHEMOTYPING, GENOTYPING AND CHEMOMETRIC ANALYSIS

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The common hazel plant (Corylus avellana L.) is a shrub native to Europe and Asia that belongs to family of Betulaceae, genus Corylus. It grows in temperate climates; Italy is the second worldwide hazelnut's producers, following Turkey. The hazelnuts kernels (predominantly in their roasted form) are largely used by bakery and confectionery industry. Up today, the cultivar identification is primarily and commonly based on morphological analysis of seeds. The use of chemical and/or genomic parameters in order to obtain a successful identification of hazelnuts at cultivar level was already reported in literature. Aim of this work was i) to provide some methods to identify and authenticate the Tonda Gentile Trilobata (TGT), cultivar, covered by Nocciola Piemonte PGI designation, and ii) to differentiate them from other cultivars either from Italy and Turkey, investigating the relationship between chemical and genetic parameters. We have considered in this work some chemotype parameters, like proximate composition; antioxidant activity; polyphenols content and fingerprint; protein patterns by SDS-PAGE electrophoresis and FAMEs by GC-FID. Concerning the genetic fingerprint, we used a PCR-related approach, the RAPD (Random Amplified Polymorphism DNA) markers. This technique resulted efficient in its ability to clearly detect the polymorphisms among different cultivar of hazelnuts. Finally, Principal Component Analysis (PCA) on genomic and chemical data-sets was able to clearly identify/authenticate the TGT cultivar, according to its genotype and chemotype characteristics. The PCA allowed also the clustering of samples according to their geographic area of production, a significant result useful to validate a "food authenticity" protocol. Finally, we confirm and highlight that hazelnut chemotype do not depend only on the genetic bases, but also on the environmental and geographical parameters.

Locatelli, M. et al. Chemotype and genotype chemometrical evaluation applied to authentication of "Tonda Gentile Trilobata" hazelnuts from Piedmont (Italy). Food Chemistry (2011), doi:10.1016/jfoodchem.2011.05.134

Keywords: Hazelnut, traceability, chemotyping, genotyping, PCA

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B-30
COUNTERFEITING: USING LC-MS TO DETECT AND DIFFERENTIATE BETWEEN CARAMELS E150 A, B, C AND D

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Counterfeiting of spirits is reportedly increasing year after year as the worldwide market continues to expand, and poses a serious risk to consumer safety. With exports of Scottish Whisky predicted to be worth Ł3.2 billion in 2011, protecting industry against losses through counterfeiting is key to ensuring brand reputation and job security. The analysis of Whisky using mass spectrometry has traditionally been performed using gas chromatography as the separation technique, and is often targeted at specific additives to identify a genuine product. Here we have used liquid chromatography coupled to time-of-flight mass spectrometer to differentiate Whisky samples containing caramels E150 a to d, with the aim of determining markers indicative of each caramel type to aid in the rapid determination of counterfeiting. This analytical technique is not restricted to explicit target analytes, so also shows promise for detecting adulteration of Whiskies with vanillin and sucrose, as well as a powerful means for research into differences between blends, cask types and ageing.

Keywords: LC-ToF MS, whisky, authenticity, brand, counterfeit

B-31
METHOD VALIDATION FOR ISOTOPIC RATIOS DETERMINATION (18O/16O AND 13C/12C) IN WINE

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This paper reports the validation procedure for measuring $\delta^{13}C$ in wine ethanol and $\delta^{18}O$ of water in wine samples. This was necessary to verify that the performance parameters of the methods used in our laboratory are adequate for assessment the quality and authenticity of wines. Standardized methods used to determinate $\delta^{13}C$ in wine ethanol and $\delta^{18}O$ values of water in wine samples were: EEC No.2676/1990 supplemented with EC No. 440/2003 from 10.03.2003, Annex II for the analysis of $^{13}C/^{12}C$ ethanol from wine, and EEC No.2676/1990 supplemented with EEC No. 822/1997 from 6.05.1997, Article 1, Chapter 43 for the analysis of $^{18}O/^{16}O$ wine water. The isotopic ratios $^{13}C/^{12}C$, $^{18}O/^{16}O$ from wine samples were carried out with an isotopic ratio mass spectrometer (IRMS), type Delta V ADVANTAGE, produced by Thermo Finnigan. The measurements $\delta^{13}C$ and $\delta^{18}O$ were made on CO2. The certified reference materials used were BCR–659 (12% vol. water-ethanol mixture) and BCR–660 (water-ethanol solution 12%). Beside, to determinate the method performance parameters, we used as sample a sweet white wine and two standard working samples with a know $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratio respectively, calibrated against international reference materials. The following performance parameters were determined: range and linearity, precision expressed as repeatability and reproducibility, accuracy express as trueness (bias) and uncertainty of the method. The obtained repetability limit (r) and the reproductibility limit (R) of the method were: r= 0.22, R=0.23 for $\delta^{13}C$ and r=0.23, R = 0.23 for $\delta^{18}O$. These values were compared with r and R values obtained in standardized methods that are: r=0.24, R=0.6 for $\delta_{13}C$, (method EEC No. 440/2003), respectively r=0.24 and R=0.5 for $\delta^{18}O$ (method EEC No. 822/1997). The trueness (bias) was determined by measuring the two standards, BCR-659 and BCR-660, and the obtained values were: bias =0.14% for $\delta^{13}C$, respectively bias=4.34% for $\delta^{18}O$. The trueness for $\delta^{13}C$ measurements is very good, and for $\delta^{18}O$ is good. The possible sources of uncertainty were identified and taken into account, and the uncertainty was calculated. The expanded uncertainty (Ue) was calculated for a normal distribution of values $\delta^{13}C$ and $\delta^{18}O$ with a 95.45% confidence level for k=2. It was Ue=0.18‰ for $\delta^{13}C$.

Keywords: stable isotopes, wine, validation

B-32
STABLE ISOTOPES COMPOSITION OF SOME AUTHENTIC TRANSYLVANIAN FRUIT JUICES

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Authenticity has probably always been a major concern of many consumers and it is still gaining more and more importance. Isotope ratio mass spectrometry is a promising tool for origin assignation of food, thus ^{13}C, ^{18}O and ^{2}H measurements are intensively used in forensic study to prove product authenticity. This application has been particularly useful in food quality control, because it allows the detection of added sugar and water in fruit juices and in tracing the geographical origin of food. One of the greatest limitations to the applications of the technique in origin assignation is the lack of large databases of isotopic abundance in food items. In this work, H, C, O stable isotope ratios of 35 “summer” fruit juices collected between (May – July 2011) from different Transylvanian areas are presented and discussed. We measured $^{2}H/^{1}H$, $^{18}O/^{16}O$ ratios from water juice and $^{13}C/^{12}C$ from pulp and we compared these results with those already reported in literature for single strength juices, in order to see how the geographical, climate conditions of Transylvania and the meteorological peculiarities of year 2011 influenced the isotopic composition of the investigated fruit juices. Our data set may serve in the detection of illegally adulterated fruit juices as references.

Keywords: Stable isotope, IRMS, fruit juices, authenticity, traceability

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B-33
UHPLC- HRMS UNTARGETED METABOLOMICS APPLIED TO THE DISCRIMINATION OF SPANISH WINES

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Metabolomics is a powerful tool that may be very useful to solve problems related to biologically complex systems. Even though initial works in this area were mostly focused on clinical and pharmaceutical fields, recent applications also deal with food classification and characterization issues. Traditionally, wine classification studies have been carried out following a targeted approach, where selected compounds of one or more families are used as descriptors to be related with wine appellation, vintage, grape type, etc. On the other hand, untargeted approaches try to find differences between classes by detecting as many metabolites as possible in a single analysis. In order to obtain the maximum number of ions with enough signal intensity, care must be taken in order to avoid problems arising from ion suppression. Therefore, a typical analysis set-up in metabolomic studies relies on the use of electrospray mass ionization after separation of compounds either with liquid or gas chromatography. After experimental work, typical data preprocessing includes retention time alignment, peak filtration and identification, peak matching across samples and integration. For this step several options, both free and commercial, are available. Finally, discriminant features can be selected by means of different multivariate data analysis methods. This work shows the potential of using metabolomic analyses to discriminate among wines of different Spanish appellations. Data arising from an ultra high performance liquid chromatography – high resolution mass spectrometry (UHPLC-HRMS) method using an Orbitrap analyzer were further analyzed with XCMS (an open source software package for R) as described above and chemometrically analyzed to try finding descriptors and establishing models for characterization and classification of the samples.

Keywords: wine, HRMS, discrimination, chemometrics

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B-34
CHARACTERIZATION OF SERBIAN MONOFLORAL HONEY ACCORDING TO THEIR AMINO ACIDS COMPOSITION

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Honey is produced by honey bees from nectar of plants, as well as from honey dew. Some of the components (carbohydrates, water, traces of organic acids, enzymes, amino acids, pigments, pollen and wax) are due to maturation of the honey, some are added by the bees and some of them are derived from the plants. Amino acids in honey amount for 1% (w/w), and proline is the major contributor. Besides proline, there are 26 amino acids in honey whose relative proportions depending on the honey origin (nectar or honey dew). It has been shown that there is a relationship between the amino acid composition of honey and its origin, most commonly botanical. Honey samples from seven floral sources: acacia, sunflower, linden, basil, rape, buckwheat and giant goldenrod, were collected from six different regions of Serbia, during the harvesting season 2009. The total numbers of 157 honey samples were provided from the Association of the Beekeeper Organizations of Serbia (SPOS). The content of free amino acids was determined by reversed-phase high-performance liquid chromatography. The aim of this study is to characterize main monofloral types of Serbian honey according to their amino acids composition and to establish criteria for their classification by using multivariate statistical analysis of obtained data. A significant difference in content of different amino acids among botanical species could be observed. The major amino acids present in all honey samples were prolin, phenylalanine, alanine, arginine, tryptophan, and serine. It can be seen that the mean phenylalanine content is much higher in samples of basil honey, comparing to sunflower, rape, buckwheat and giant goldenrod, acacia and linden honey with very low percentage concentration. It could be said that the phenylalanine is thus characteristic of this origin. The Principal Component Analysis has been performed on the entire data set, in order to reveal the most important factors influencing the grouping pattern among the several honey species. PCA resulted in four principal component model explaining 93.04 % of the total data variance. Considering mutual projection of PC1 and PC2 score values reveals the six distinctive groups of honeys belonging to different botanical origin, while buckwheat samples are grouping with sunflowers honey. Acknowledgement: The authors are grateful to The Ministry of Education and Science of Serbia for financial support (grant 172017) and to the Association of the Beekeeper Organizations of Serbia for kindly collection of honey samples.

Keywords: Honey, Multivariate data analysis, Amino acid composition, Authenticity

B-35
FISH SPECIES IDENTIFICATION BY RFLP ON THE AGILENT 2100 BIOANALYZER

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The global demand for seafood has grown considerably. New regulations and an increasing number of cases involving substitution and fraud drive the need of stakeholders for a robust, easy to use and well accepted method of species identification. PCR-RFLP authentication of fish species is a method that has a number of advantages over other means of species identification. It is capable of working with mixed samples and works with all but the most heavily processed food samples. This paper describes the development of a fast and userfriendly solution based on industry grade reagent mastermixes, an optimized protocol and an analysis software for pattern matching. Enhancements to the method are shown that allow improved discrimination of sturgeon species from roe.

Keywords: fish species, PCR RFLP, pattern matching software, sturgeon, roe

Acknowledgement: Steve Garrett, Campden BRI (UK) and Pat DeHaan, US Fish and Wildlife Service (USA) for helpful discussions and providing samples

B-36
APPLICATION OF UPLC-MS/MS FOR DETERMINATION OF SYNTHETIC ADULTERANTS IN SLIMMING FOOD SUPPLEMENTS

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A new UPLC-MS/MS method was developed for the confirmatory analysis of 12 adulterants (bisacodyl, caffeine, fenfluramine, N-nitrosofenfluramine, norfenfluramine, orlistat, phenolphthalein, phentermine, N-monodesmethylsibutramine, sibutramine, rimonabant and yohimbine) in slimming dietary supplements. The analytes were extracted with acetonitrile without clean-up and the extracts were subsequently analysed by UPLC-MS/MS operating in positive electrospray ionisation mode. The method was validated according to Commission Decision 2002/657/EC. The following performance studies were carried out: specificity, linearity, recovery, within-laboratory repeatability/reproducibility, decision limit (CCα) and detection capability (CCβ). The method has been extensively evaluated through application for routine examination of authentic adulterated food supplement samples available in Republic of Ireland. Various undeclared drugs were detected and of 63 samples tested, the most frequent adulterations were sibutramine, its analogue N-monodesmethylsibutramine and phenolphthalein. It confirms that slimming food supplements, regardless of the label claim, are often purposely adulterated with synthetic drugs to enhance desired action.

Keywords: synthetic adulterants, slimming food supplements, UPLC-MS/MS

B-37
UTILISING THE INCREASED PEAK CAPACITY OF UPLC ION MOBILITY TOF MS AND MSE TO OVERCOME SAMPLE COMPLEXITY

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Several Passiflora (Passifloraceae) species are utilized as phytomedicines (sedative / tranquillising). Medicinal Passiflora species contain flavonoids, mainly C-glycosylflavones (apigenin and luteolin derivatives; frequently occurring as isomers). Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties. Such attributes mean many flavonoid-containing plant species may be used as functional foods or phytomedicines. LC-MS techniques such as CID (collision-induced dissociation) combined with accurate mass measurement may be an important tool for unequivocal identification of flavonoid isomers in complex mixtures such as phytomedicines. High definition mass spectrometry has been utilised to profile the hydroethanolic extracts of P. incarnata, P. alata, P. edulis and P. caerulea, all of them grown in Brazil. This technique offers some unique advantages to profiling complex mixtures. It is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility (IM) mass spectrometry is a rapid orthogonal gas separation phase technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape and charge, as well as mass. The study undertaken investigates the use of UPLC-IMS-CID-MSE using a Synapt MS platform. HDMS can provide a route to specific and unambiguous identification, enabling the unequivocal distinction of flavonoid isomers. The results obtained clearly show the benefits of using HDMS and that it is possible to separate co-eluting analytes, giving increased peak capacity. This enables single component accurate mass spectra of chromatographic co-eluting components to be obtained. These were used to generate elemental composition information. The enhanced peak capacity enables more information to be extracted from fragmentation studies and the individual MSE fragmentation spectra have been obtained for flavonoid isomers which are co-eluting, from which structural elucidation has been performed. Characteristic assignment for 6-C and 8-C flavonoid glycosides isomers (vitexin and isovitexin) (orientin and isoorientin) has been possible using accurate mass measurement and elemental composition calculation for precursor and fragment ions produced.

Keywords: Ion Mobility, TOF-MS, Peak, Capacity, UPLC

B-38
PROFILING AND QUANTITATION OF C-GLYCOSIDIC MARKER FLAVONOIDS IN NATURAL PRODUCTS USING UPLC TIME OF FLIGHT MASS SPECTROMETRY

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In the profiling study performed it possible to illustrate the advantages UPLC in combination with advances in TOF technology to enable full spectra acquisition profiling and quantitation to be performed using a Synapt based platform. This is an alternative to the traditional selective approach taken using quadrupole LC-MS and LC-MS/MS systems. Utilising the functionality of TOF low level analyte detection can be achieved when acquiring data over a wide mass range with accurate mass measurement. This approach has been used to routinely provide specific identification and quantification of flavonoid marker isomers. Four Passiflora species, P.incarnata, P.edulis and P.caerulea and P.alata were profiled. They are utilised as phytomedicines in Brazil due to the sedative properties that are related to the presence of flavonoids in leaves. As a result of the importance of flavonoids and their glycosides to these species, the identification and/or structural determination of such compounds occurring in leaves play an important role. Using isoorientin. orientin, vitexin and isovitexin as the target flavonoid of interest, it has been possible to illustrate four orders of dynamic range and quantify the level of these marker flavonoids in the plant extracts analysed. As a result of the increase in sensitivity produced by incorporating new technology in the Synapt MS platform, combined with peak capacity of UPLC, it has been possible to resolve, detect and quantify all four marker flavonoids in all four species, which has not been achieved in previous studies. The resultant profile 6-C and 8-C flavonoid glycoside isomers further allows for this approach to be utilised to achieve the specific identification of the species from which the flavonoids have been extracted.

Keywords: Profiling, Quantitation, TOF-MS, UPLC, Flavonoids

B-39
THE DETERMINATION OF FRUIT JUICE AUTHENTICITY USING HIGH RESOLUTION CHROMATOGRAPHY, UV, TIME OF FLIGHT MS AND MULTIVARIATE ANALYSIS

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The verification of food sources and authenticity is an activity that has increased in importance over the last decade. The adulteration of food & beverages has emerged as a growing problem that can pose potential threats to the health of consumers and to the integrity of the industry. There are different types of adulteration that can occur and whilst some can be harmful to health (e.g. melamine) & others can be very misleading to the consumer - especially if they are purchasing the product to support a healthy lifestyle. Food laboratories require reliable analytical methods in order to correctly characterise product quality & integrity. High resolution chromatography and mass spectrometry is a robust platform for authenticity studies providing extremely informative separation and identification information. Whilst the resulting data is complex and comprehensive (consisting of retention time, exact mass and intensity data for each component in each sample), multivariate analysis can be used to simplify data visualization & interpretation of these highly complex data sets. Pomegranate juice samples - known to be either authentic or adulterated were analysed by LC/UV/QTof MS. Several marker compounds indicating adulteration were proposed & using the QTof MS data it was possible to confirm the structure, compound identity, & identify the type of adulteration that had occurred.

Keywords: Authenticity, Juice, QTof MS, UPLC

B-40
APPLICATION OF UPLC-MS/MS FOR DETERMINATION OF SYNTHETIC ADULTERANTS IN SLIMMING FOOD SUPPLEMENTS

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A new UPLC-MS/MS method was developed for the confirmatory analysis of 12 adulterants (bisacodyl, caffeine, fenfluramine, N-nitrosofenfluramine, norfenfluramine, orlistat, phenolphthalein, phentermine, N-monodesmethylsibutramine, sibutramine, rimonabant and yohimbine) in slimming dietary supplements. The analytes were extracted with acetonitrile without clean-up and the extracts were subsequently analysed by UPLC-MS/MS operating in positive electrospray ionisation mode. The method was validated according to Commission Decision 2002/657/EC. The following performance studies were carried out: specificity, linearity, recovery, within-laboratory repeatability/reproducibility, decision limit (CCα) and detection capability (CCβ). The method has been extensively evaluated through application for routine examination of authentic adulterated food supplement samples available in Republic of Ireland. Various undeclared drugs were detected and of 63 samples tested, the most frequent adulterations were sibutramine, its analogue N-monodesmethylsibutramine and phenolphthalein. It confirms that slimming food supplements, regardless of the label claim, are often purposely adulterated with synthetic drugs to enhance desired action.

Keywords: synthetic adulterants, slimming food supplements, UPLC-MS/MS

B-41
GEOGRAPHICAL INDICATIONS FOR HONEY: A PHYSICO-CHEMICAL PROFILE OF ACACIA HONEY PRODUCED IN ROMANIA

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Geographical Indications (GI) are a form of certification which has not been used in Romania, although the legal framework has existed since 2004 (order no 285/2004). In view of the positive impact of Geographical Indication, Romanian honey industry representatives have expressed interest in labeling the honey as GI product. In this context through the study of acacia honey quality, we intend to evaluate how interesting and feasible the GI denomination, in order to contribute to the development of the registration and protection procedures. The analysis of the main physico-chemical parameters has been regarded as a very promising way of studying honey quality. We studied the following physico-chemical propreties: electrical conductivity, content of water, free acidity, lactone acidity, total acidity. In addition, sugar, phenolic and volatile content was determined. Most of the parameters obtained showed good compliance with national an international requirements, as well as values typical for acacia honey from other European countries. Nevertheless, volatile profile seems to be different in Romanian acacia honey then in honey produced in anothers countries. This suggests that the physico-chemical profile of acacia honey can be considered for a future Geographical Indication of Romanian acacia honey, but also other chemical markers could be analyzed by fingerprinting techniques.

Keywords: Geographical indications, honey, physico-chemical profile

B-42
THE OLIVE OIL CHARACTERIZATION OF SOME NATIVE AND FOREING OLIVE CULTIVARS FROM ALBANIA

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The fond of olive cultivars in Albania is increased during second part of 20th century by a number of foreign olive cultivars. The trends of increasing the production on olive oil and the table olives is focused in two main efforts: increase of the olive tree numbers by plantation, and application of the Good Agriculture Practices. Actually is important to be highlighted the lack of data on chemical composition of olive oils extracted by the autochthon cultivars as well as to foreign cultivars. Fatty acid profiles, total phenol of three native cultivars (Kalinjoti, Mixan, Ulliri i Zi and Kushani), and two other foreign cultivars (Frantoio and Leccino) indicate the importance of genetic factor on chemical characteristics of the studied cultivars. Kalinjoti cultivar actually is most abundant, by 50% of total olive trees. While Frantoio, despite its classification as foreign cultivars is well acclimatized, hence it represents over 8% of total number olive trees. The oleic acid content vary from 70.826% (Ulliri i zi) to 77.138% (Kushan), while oleic acid in two foreign cultivars Frantoio (71.646%) and Leccino (75.125%). The content of Palmitic acid is relatively low to Kushan (9.121%) and relatively high to Frantoio (14.259%). The levels of Linoleic acid is considered relatively low to Leccino (4.683%) and Kushan (6.952%). Total Phenol contents in studied olive cultivars vary 70.28 mg GA/kg olive oil (Kushan) to 245.45 mg GA/kg olive oil (Frantoio). The stability of olive oils evaluated by the Oleic/Linoleic acid ratio results in acceptable values except the Ulliri i zi olive cultivar (6.57). The nutritional value of n-6/n-3 show very interesting values to Leccino cultivar by 9.00.

Keywords: Kalinjoti, Mixan, Frantoio, Leccino, Fatty Acids

Acknowledgement: Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Porto, Portugal

B-43
TRACEABILITY AND AUTHENTICITY OF FEED MATERIALS – REPORT ON QSAFFE WORK PACKAGE 2 ACTIVITIES

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The increasing complexity of food and feed production systems, globalisation of feed trade, new feed and food processing technologies and production of feeds from new sources will probably lead to new and unforeseen risks for animal and human health. Particularly if a risk has been associated with a product linked to certain areas of origin, analytical strategies for identification of affected products have to be developed. Thus 'place of origin' and its proof will be increasingly linked to the quality of feed material in a globalized market and will become increasingly important. Furthermore, traceability of products in a globalized market is not always available or reliable by trade documents. Therefore, Work Package 2 of the EU research project QSAFFE (Quality and Safety of Feeds and Food in Europe) will focus on strategies to determine the botanical and geographical origin of feed materials. Major tasks in the project will be the improvement of traceability and the development of analytical authentication approaches suited to 'proof of origin' of feed materials. Different partners from 5 EU countries and China will investigate the potential of different analytical techniques (FT-IR, NIR, FT-NIR microscopy, Raman-spectroscopy, IR-MS, DART-MS, PTR-MS and high-resolution mass spectrometry). QSAFFE Work Package 2 is primarily concerned with analysis of new feed materials. One example are co-products from the distillation process of fuel-ethanol production, the so-called Distillers Dried Grains and Solubles (DDGS). As a result of rapid upgrowth of fuel-ethanol industry on the one hand and the high nutrient content of DDGS (proteins and fat) on the other hand, DDGS play an increasing role in the world feed market. Determination of geographical origin of these DDGS is of particular interest. During their production the main focus is located on the ethanol and practices – e.g. for increasing the fermentation yield – could be locally applied, which possibly implement risks to the food chain (use of antibiotics or fermentation supplements). The poster presents principal objectives of the QSAFFE Work Package 2 and describes the main focus of analysis on DDGS, which will be analyzed by participating laboratories from Europe and China.

Keywords: traceability, authenticity, feed, DDGS, QSAFFE

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B-44
APPLICATION OF METABOLOMIC FINGERPRINTING/PROFILING FOR HONEY AUTHENTICITY

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Honey, with its high world production rate (approx. 1.4 million tons/year), is popular not only as a source of energy but also for its potentially health-promoting properties. The price of honey is usually dictated by its botanical origin (unifloral honeys) and/or by production in a specific region (protected denomination of origin, PDO). Recently, an increased number of alerts concerning the safety and adulteration of honey have been posted. For honey characterization various parameters such as pollen analysis, moisture content, 5-(hydroxymethyl)furan-2-carbaldehyde concentration, sugar composition, proline content, invertase and diastase activity are typically considered. In addition to these traditional approaches, the examination of the profiles of volatiles and phenolics might be considered as a strategy enabling honey authentication. In this work, solid-phase microextraction–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (SPME–GC×GC–TOFMS) and direct analysis in real time–time-of-flight mass spectrometry (DART–TOFMS) were used as the tools for metabolomic fingerprinting/profiling (analysis of volatiles, phenolics, and other compounds) with the aim of distinguishing the botanical and/or geographical origin of honeys. Advanced chemometric strategies were employed for the interpretation of acquired data sets.

Keywords: Honey, Authenticity, Metabolomics, Mass spectrometry

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BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

(C-1 – C-30)

C-1
DEVELOPMENT AND VALIDATION OF A NOVEL MICRO-ASSAY FOR THE DETERMINATION OF THE ANTIOXIDANT CAPACITY OF LIPOPHILIC COMPOUNDS

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Peroxyl radicals ($ROO^{\bullet}$) are oxidant agents related to food degradation and to the development of chronic degenerative diseases. The oxidative damage can be prevented by antioxidant compounds, such as carotenoids, which have the ability to scavenge $ROO^{\bullet}$. Carotenoids are lipophilic compounds which absorb light in the visible region and these characteristics make it difficult to determine their antioxidant capacity by the most currently used methods. The antioxidant capacity was determined by monitoring the fluorescence decay of C_{11}-BODIPY$^{581/591}$ oxidized by $ROO^{\bullet}$ generated from the thermodecomposition of azobisisobutyronitrile (AIBN) at 42°C, using a microplate reader. The solvent choice is critical since three basic characteristics are required: allow the complete dissolution of the reagents and test compounds, do not react with the microplate material (polystyrene) and do not evaporate under the temperature for radical generation. Five solvents were tested: octane:butyronitrile (9:1), methanol, ethanol, methanol:ethanol (1:1) and dimethyl sulfoxide:methyl terc butyl ether (DMSO:MTBE) (10:1). The only appropriate solvent was DMSO:MTBE (10:1) which presented all the desired characteristics, since octane:butyronitrile (9:1) reacted with the microplate and the alcohols evaporated during analysis. The AIBN concentration of 175 mM was chosen in order to achieve 0.5% of the initial fluorescence signal in about 60 min in the control assay (without antioxidant). The reaction mixtures in the wells contained the following reagents at the indicated final concentrations (final volume of 225 µL): C_{11}-BODIPY$^{581/591}$ in DMSO (178 nM), AIBN in DMSO:MTBE (10:1) (175 mM) and test compounds in DMSO:MTBE (10:1). The fluorescence signal was monitored every 2 min with excitation wavelength at 540 nm and emission at 600 nm. α-Tocopherol was used as standard to validate the method and the following parameters were evaluated: probe stability, linearity, limits of detection (LOD) and quantification (LOQ), repeatability and accuracy. The probe was thermo and photo-stable during 120 min at 42 ºC in the absence of AIBN. The regression analysis showed a linear relation (r^2 = 0.99, p<0.01) between α-tocopherol concentration and the net area under the curve (net area) in the range from 24 to 119 µM of α-tocopherol. The net area was obtained by the difference between the area under the curves of α-tocopherol and the control assay. The LOD and LOQ were 9 and 26 µM of α-tocopherol, respectively. Repeatability and accuracy were calculated from 4 independent experiments using 3 levels of α-tocopherol (35, 70 and 105 µM). The repeatability, expressed as relative standard deviation, varied from 8 to 14% and recoveries varied from 97 to 99%. This assay was successfully applied to β-carotene, β-cryptoxanthin, lutein, astaxanthin, quercetin, rutin, gallic acid, both lipophilic and hydrophilic extracts from Amazonian fruits.

Keywords: micro-assay, antioxidant capacity, lipophilic compounds

Acknowledgement: CNPq and FAPESP

C-2
METABOLOMICS: A NEW STRATEGY FOR THE EVALUATION OF MICROALGAE AS A SOURCE OF BIOLOGICALLY ACTIVE SUBSTANCES

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Microalgae represent an interesting source of biologically active substances used in food, feed and pharmaceutical industries. Not every microalga has suitable properties for these purposes were therefore selected two species of microalgae (Trachydiscus minutus and Monodus subterraneus) on the basis of available information about their composition. For obtain the best of amount of microalgae is needed select a suitable conditions of cultivation microalgae. Thus for optimization of bioreactor operation is necessary to have comprehensive data on the composition of biomass. In particular case, content of lipids and their composition was of concern since preliminary data showed very favourable features of these cellular components including high content of polyunsaturated fatty acids (PUFA). The common approach to determine fatty acids composition is their release from the ester bonds with glycerol and then converted into methylesters amenable to GC-FID (gas chromatography with flame ionization detector) analysis. In this way, however, the information about the distribution of fatty acids in various triacylglycerols (TAGs) lost. To eliminate time consuming sample preparation and obtain comprehensive information TAGs pattern ambient mass spectrometry was employed for sample extracts examination. For the analysis of TAGs the sample was diluted with hexane and immediately analysed and for the profiling of polar compounds was used a rapid extraction with a methanol–water mixture (80:20, v/v) before analysis. Direct Analysis in Real Time (DART) ion source coupled with orbitrap mass spectrometer was used for analysis of microalgae grown under various conditions. The correlation of data generated by “classic” GC-FID and those obtained by DART-orbitrapMS will be presented.

Keywords: microalgae, biologically active substances, mass spectrometry

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C-3
DETERMINATION OF ROS AND RNS SCAVENGING CAPACITY IN LIPOSOMES USING C11-BODIPY AS PROBE: DEVELOPMENT OF SEMI-AUTOMATED MICRO-ASSAYS USING MICROPLATE READER IN 96-WELL FORMAT

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Reactive oxygen (ROS) and nitrogen species (RNS) are products of normal cellular metabolism which also act as oxidant agents in food and cosmetics, contributing to the degradation of these products. Liposomes have been used as model membrane systems to evaluate antioxidant protection due to the bilayer structure similar to the lipid fractions of cell membranes. This system allows the evaluation of the antioxidant properties both from lipophilic and hydrophilic compounds. Several probes are currently used associated to liposomes; however, just a few are sensitive to a large range of reactive species. C_{11}-BODIPY$^{582/591}$ is a lipophilic fluorescent probe for evaluation of lipid peroxidation in model membrane systems and living cells with good spectral separation of the non-oxidized (595 nm) and oxidized (520 nm) forms, good photo-stability and very few fluorescent artifacts. This probe is not sensitive to superoxide, nitric oxide radical, transition metal ions and hydroperoxides per se and its sensitivity to oxidation is comparable to that of endogenous fatty acids. The methods were developed for determination of the capacity to scavenge peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), hypochlorous acid (HOCl) and anion peroxynitrite ($ONOO^{-}$) based on the loss of the fluorescence of C_{11}-BODIPY$^{582/591}$ at 595 nm due to its oxidation. The liposomes were prepared by extrusion, passing 21 times through a 100 nm polycarbonate membrane. The final concentrations in the liposomes were 5.0 mM phosphatidylcholine, 5.0 µM C_{11}-BODIPY$^{582/591}$ probe and 26 mM saccharose, with or without antioxidants in different concentrations (added before extrusion). Liposomes average diameters were measured by laser light scattering and ranged from 110 to 140 nm. Zeta potential was measured by electrophoretic mobility and the surface charge was near zero. The assays were carried out in a microplate reader with temperature set at 37 °C. The $ROO^{\bullet}$ was generated by the thermodecomposition of AAPH, $OH^{\bullet}$ by a Fenton system ($FeCl_2$-EDTA-H_2O_2). The HOCl was prepared by adjusting the pH value of a NaOCl solution to 6.2. The $ONOO^{-}$ was synthesized mixing $NaNO_2$ with an acidic solution of H_2O_2. The concentrations of the reactive species in the reaction medium were optimized for each species using 100 µL of liposomes without antioxidant in a final volume of 200 µL. Loss of fluorescence was monitored until 10% of its initial signal. The positive controls used to develop the methods were trolox for $ROO^{\bullet}$ and $OH^{\bullet}$, cysteine for HOCl and ascorbic acid for $ONOO^{-}$. No interactions between the probe and the positive controls were observed until 240 min and the loss of fluorescence due to probe photo-bleaching was less than 10%. The developed methods presented good linearity ($R^2 \geq 0.99$) and repeatability (RSD≤10%). All the methods were successfully applied for the determination of the antioxidant capacity of lipophilic food constituents or ingredients, such as carotenoids.

Keywords: antioxidant, liposomes, reactive oxygen species, nitrogen reactive species

Acknowledgement: CNPq and FAPESP

C-4
COMPARISON OF CAROTENOIDS CONTENT IN BIO-OILS OBTAINED BY MEANS OF COLD PRESSING AND SUPERCRITICAL FLUID EXTRACTION

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Due to the increasing consumer awareness of the diet, that is applied in lifestyle and 21st century diseases like obesity, diabetes, heart or cardiovascular diseases, the production and consumption of cold pressed oils increased in Poland as well in Europe. Cold pressed oils are a rich source of polyunsaturated fatty acids and other minor bioactive compounds like sterols, tocopherols, carotenoids and polyphenols that have a positive influence on human health. Carotenoids are natural pigments that exhibit provitamin A activity and antioxidant activity. The most known is beta-carotene but there are other carotenoids like lutein or lycopene that have great benefits for human health. The main objective of this study was to characterize and compare carotenoids content of selected bio-oils that were obtained by two different methods. Oils were extracted from seeds (Flax, Camelina, Pumpkin [five different varieties], Borage, Evening primrose) using cold pressing method and new alternative method – supercritical fluid extraction with carbon dioxide (SFE-CO_2). Carotenoids contents were quantified by means of a HPLC (high-performance liquid chromatography) technique coupled with diode array detector (DAD). ß-carotene was the predominate carotenoid in cold pressed oils and SFE-CO_2 oils. Both pumpkin seed oils, cold pressed and extracted by supercritical CO_2 had the highest carotenoids content.

Keywords: carotenoids, seed oil, cold pressing, SFE, HPLC

C-5
CHARACTERIZATION AND ANALYSIS OF THE ANTIOXIDANT CAPACITY OF FUNCTIONAL PHENOLICS

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Scytalidium thermophilum is a thermophilic fungus found in mushroom compost, where it triggers the production of the edible mushroom Agaricus bisporus. Since S. thermophilum produces immense amounts of melanin, phenol oxidase production was analyzed. As a result, the extracellular phenol oxidase of S. thermophilum was determined as a bifunctional catalase phenol oxidase (CATPO). CATPO is produced constitutively and in a growth-associated manner. Nonetheless, phenolics could affect the growth and enzyme production. Catechol, hydroquinone, myricetin, kaempferol and coumaric acid have influenced the growth adversely in an increasing concentration manner, on the other hand under the influence of caffeic acid, chlorogenic acid, quercetin, catechin, epicatechin, recorcinol, vanillic acid and resveratrol the growth was not adversely affected, in fact, upto 50% increase in biomass was detected. Most phenolics that positively affected growth either did not change CATPO production or, more or less, repressed it, in a dose-dependent manner. Under toxic conditions CATPO production was adversely affected, however, hydroquinone and myricetin, which showed antifungal acitivity, enhanced CATPO, in a dose-dependent manner. It is suggested that phenolics that affect growth positively show antioxidant activity. Among these, especially the phenolic acids and flavonoids bearing ortho-diphenols in their structures were oxidized by CATPO and did not inhibit growth. However, most phenolics acting in an antifungal/toxic manner were not oxidized by CATPO. The oxidation of 14 phenolic substances was analyzed by using both the supernatant and pure enzyme. The oxidation products of 5 of these compounds were further characterized. According to the findings; CATPO could oxidize catechol, hydroquinone, catechin, quercetin, chlorogenic acid and caffeic acid. The products of these phenolic substance reactions were analyzed by FTIR. The results indicate that the polymerization mainly takes place through C-C and C-O-C bon! ds. Dime r, trimer and tetramer structures were determined in another study by HPLC. The presence of multiple products and the progression of polymerization by time were also observed. The ongoing studies will reveal the mystery behind the antioxidant capacity difference between the substrates and final products.

Keywords: antioxidant capacity, bifunctional enzymes, FTIR

C-6
UTILIZATION OF DART-MS FOR CHARACTERIZATION OF ROSE HIP PRODUCTS

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Rose hip fruits are known as a rich source of many nutritionally valuable substances. The most important of these is ascorbic acid (vitamin C), with content around 1%. Besides the high content of ascorbic acid, rose hips have a significant content of other antioxidants, e.g., carotenoids (β-carotene and lycopene), phenolic compounds, folates, tocopherols or triterpenic acids. Furthermore, the triterpenic acids show antitumor activity. Rose hip fruits have been traditionally used for the preparation of tea, infusions and jams and the high antioxidant potential of rose hip is used in food supplements, where the addition of rose hips enhances the effect of synthetic vitamin C. The aim of this study was a quality assessment of various rose hip products purchased from the Czech market. For the rapid screening of rose hip product quality, metabolomic profiling employing a Direct Analysis in Real Time (DART) ion source coupled with a High Resolution Time-of-Flight Mass Spectrometer (HR-TOFMS) was used. The High-Resolution TOF-MS detector provides accurate mass spectra, which can be used for the calculation of elemental composition of ions and subsequent identification of compounds occurring in rose hip. A large number of both primary and secondary metabolites, e.g. ascorbic acid, carotenoids, flavonoids or triterpenic acids, were identified. The DART-TOFMS data were compared with results obtained by conventional methods such as liquid chromatography coupled with UV/VIS or MS/MS detector.

Keywords: Rose hip, metabolomic profiling, DART-TOFMS

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C-7
STABILITY OF PREBIOTIC INULIN IN FRUIT BABY FOODS

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Prebiotics are indigestible food ingredients that stimulate the growth of bacteria in the digestive system in ways claimed to be beneficial to health.The enrichment of baby foods with prebiotics is a common practise and inulin, mixture of oligomers with different numbers of molecules of fructose, is frequently used for this purpose. The aim of the study was to verify the dosing requirements and stability of inulin added to the fruit baby foods. We verified and validated modified AOAC method (997.08) for the determination of inulin from the difference of concentrations of individual monosaccharides before and after enzymatic hydrolysis of inulin. The technological experiments were focused to clarify the phenomenon of decrease in inulin content under the simulated conditions of production and storage. analyses of real commercial samples of fruit baby food with added inulin showed it´s decrease from 20 to 80% six months after the production. Similar results were obtained from model experiments done under the condition, which simulated the production of apple based baby food (the same composition, pH, temperature, time, initial concentration of inulin 3 and 7%).

Keywords: Inulin, fruit baby food, prebiotic activity, stability

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C-8
THE DETECTION OF TRENBOLONE AND MELENGESTROL IN MEAT SAMPLES BY LCMSMS

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Trenbolone and Melengestrol are steroids used by veterinarians on livestock to increase muscle growth and appetite. Melengestrol is approved for use as a growth promoter in livestock, including beef cattle, in the United States but both are not approved in the European Union and were prohibited in 1988. These steroids normally exist at low levels in meat imported into the EU and therefore low limits of detection are required. Although both Trenbolone and Melengestrol are normally administered to cattle in the ester form e.g. acetates, they are quickly metabolised to the native steroids. This work shows where LC/MS/MS can be used to detect Trenbolone and Melengestrol at low levels in real samples. To improve the sensitivity of the assay both compounds are acetylated before samples were extracted. The acetate derivatives were then analysed by reverse phase high performance liquid chromatography with electrospray mass spectrometry. Both MRM and MS3 methods were developed by infusion of the acetate standards and the sensitivity of both methods were compared by analysing meat extracts. MRM quantitation has shown that both steroids can be detected at low part per trillion levels with some of the interference peaks removed by the use of MS3 instead on MRM methods.

Keywords: MS3 quantitation of steroids in meat extracts

C-9
DIRECT ANALYSIS OF CAFFEINE IN VARIOUS TYPES OF COFFEE

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Caffeine is the world's most widely consumed psychoactive stimulant. This xanthine alkaloid can be found in different plant parts such as tea leaves, coffee and cocoa beans or guarana berries. Among various types of beverages containing caffeine, coffee represents a traditional source of caffeine for adult population. High performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection represents a commonly used method for the analysis of caffeine in beverages. The limiting step of this procedure is time demanding chromatographic separation. A new method employing a direct analysis in real time (DART) ion source coupled with a mass spectrometer (MS) enables straightforward examination of caffeine in tens of samples per hour without a necessity of elaborate extraction and separation. The aim of this study was to optimize method for the determination of caffeine in ground coffee, brewed coffee (espresso type, filtered) and instant coffee. For target analyte quantification isotopically labeled caffeine was used. We have managed to introduce a quick and simple procedure to quantify caffeine in solid coffee and coffee infusions with results comparable to those achievable by a conventional HPLC–UV method.

Keywords: Coffee, caffeine, DART-MS

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C-10
DEVELOPMENT OF LC/MS/MS METHODS FOR THE SIMULTANEOUS DETERMINATION OF TOTAL VITAMIN B, CHOLINE AND CARNITINE IN INFANT FORMULA, PET-FOOD AND HEALTHCARE PRODUCTS

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In living cells Carnitine is required for the transport of fatty acids and for the generation of metabolic energy and together with Choline is deemed a water-soluble essential nutrient and often used as a food additive. Building on an old in-house method for the determination of Choline, a method for simultaneous determination of total Choline and Carnitine in infant formula, pet-food and healthcare products has been developed. Samples are subjected to an alkaline saponifikation at elevated temperature thereby converting acetylcarnitine and phosphocholine to free carnitine and choline, the extract is neutralized and diluted to a concentration contained within the calibration curve. Analysis is performed on a LCMSMS system consisting of an Agilent 1200 HPLC coupled to a 3200 Q-TRAP LC/MS/MS system from AB Sciex. The goal is to have the method accredited according to ISO 17025 at the end of this year. In this presentation we would like to talk about the development of this method and due to the fact that Choline can be grouped within the B-complex vitamins we would also like to discuss the further expansion of the scope of the LC/MS/MS method to include a Vitamin B screen. We will also present initial data from this expanded Vitamin B screen.

Keywords: LC/MS/MS, Vitamins

C-11
DETERMINATION OF CARBOHYDRATE COMPOSITION OF YACON (SMALLANTHUSSONCHIFOLIUS) TUBERS

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Yacon *[Smallanthussonchifolius (Poepp. etEndl.*) Robinson, H] is a plant native to the Andes, where belongs to traditionally grown plants. Yacon tubers contain inulin as amajor storage polysaccharide. Inulin and is a chain of fructose linked by β-(2→1)-glycosidic bonds with a terminal glucose unit. (GFn, G=glukose, F=fruktose, n=degree of polymerization).Inulin as a water-soluble fiberwith a positive effect on the colon microflora and for its prebiotic properties it is often added to various food products. The research has evaluated the content and composition of carbohydrates in various regional varieties of yaconoriginated from different parts of the world. Yacontubers for analysis were grown in experimental fields of the Czech Agricultural University Prague. The optimised extraction procedure of inulin was validated. For characterization of the carbohydrate composition of tubers HPAE-PAD method was chosen. The method was optimized andresponse factors of eachdegree of carbohydrate polymerization were determined. The carbohydrate profile for 27 yacon tubers was summarised and the average degree of polymerization of inulin was calculated. The large differences in the content of inulin in various cultivars were found. The total carbohydrate content in tubers ranged from 8.1 to 22.5 g/kg of fresh tubers. Contents of inulin (> GF2) was determined in the range from 4.5 to 17.8 g / kg of fresh tubers. The inulin content was the highest in cultivar PER/75.

Keywords: Carbohydrate composition, inulin, Yacon, HPAE-PAD

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C-12
VARIETAL DIFFERENCES AMONG THE PHENOLIC PROFILES OF TOMATOES AND DIFFERENCES BETWEEN TWO TOMATO JUICE RECIPES

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The health-promoting properties of tomato and tomato products seem to reside in the high amount of phytochemical constituents such as carotenoids, vitamin C, vitamin E and the wide variety of phenolic components ranging from flavonoids to phenolic acids. Despite increasing knowledge of the effect of industrial food processing on carotenoid and vitamin bioavailability, there is a lack of information regarding the extent of changes in the polyphenol/flavonoid content of tomato after mechanical and/or thermal processing treatments, and there is even less available data in the literature on the effects of food matrix on bioavailability. Furthermore, very little is known about the impact of tomato and tomato-olive oil combination on the phenolic bioavailability and bioefficacy in humans. Previous work on phenolic characterization of tomato fruits has been performed using different techniques including nuclear magnetic resonance, gas chromatography and capillary electrophoresis, but the most common is high-performance liquid chromatography (HPLC) coupled to a photodiode detector (UV/Vis) or mass spectrometry (MS). Among the methods used to determine phenolic compounds, liquid chromatography coupled to mass spectrometry with electrospray ionization (ESI) is one of the most powerful tools for the analysis of non-volatile and thermally labile classes of compounds. High performance liquid chromatography has recently been improved by the introduction of ultra performance liquid chromatography (UPLC), which increases the signal-to-noise ratio (S/N), enhances peak resolution, and reduces both analysis time and costs. In this work, we present the study of two tomato juices with and without refined olive oil in order to verify how the oil combination affects the phenolic bioavailability. Before the study, we analysed the phenolic profile of different Spanish tomatoes in order to get the juice with the highest phenolic content. The phenolic content of the raw tomatoes and tomato juices were characterized by UHPLC analysis by triple quadrupole mass spectrometer, with negative ion detection mode. The identification of individual phenolic compounds was carried out on the basis of their mass spectrum in full scan mode and confirmed by MS/MS experiments (PIS, NL). Multiple reaction monitoring experiments were used for quantification purposes. Three tomato varieties (“rama”, pear and “liso”) were studied. The results showed a higher phenolic content in pear tomato. Also, it was noticed that the process for preparing tomato juice and the addition of a lipid matrix during juice preparation change the phenolic composition of the product, probably due to changes in the bioaccessibility of phenolics from the food matrix.

Keywords: tomato, bioavailability, UHPLC, mass spectrometry

C-13
CISTUS EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY AGAINST STREPTOCOCCUS MUTANS

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The plants of the Cistus genus that grow widespread over the Mediterranean area are commonly used in the folk medicine for the treatment of several diseases due to their reported anti-inflammatory, antiulcerogenic, wound-healing, antimicrobial, cytotoxic and antioxidant properties [1,2]. For the watery extracts of Cistus incanus L., Hannig et al. recently observed a reduction of the initial bacterial adhesion in the oral cavity, an effect, which may protect against caries [3]. The numerous Cistus species, however, differ in their composition of the phenolic compounds [4]. Therefore, in the study presented here, 32 Cistus samples belonging to 21 different species, subspecies, and hybrids were analyzed by HPLC-DAD/ESI-MS/MS, and numerous polyphenols were identified and quantified. As the various Cistus samples differed in their HPLC profiles, their watery leaf extracts also differed in their antimicrobial effect as was tested with a special oral cavity bacteria – Streptococcus mutans – using the LIVE/DEAD® BacLightTM Bacterial Viability Kit.

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Keywords: Cistus, polyphenols, caries, Streptococcus mutans

C-14
INVESTIGATION OF TOPINAMBUR (*HELIANTHUS TUBEROSUS L.*) AS A RAW MATERIAL FOR PRODUCING MULTIFUNCTIONAL BIOLOGICALLY ACTIVE FOOD ADDITIVE

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Nowadays, throughout the world, the interest towards the natural food additives enriched with biologically active substances have been significantly raised due to unbalanced nourishment, stresses, high radiation, and other harmful factors. Effective application of plant preparations and use of specific diet in order to prevent diseases and rehabilitate are of great importance. Therefore, the study of inulin-containing plants is very interesting. Inulin has been found to be desirable as a food or a food additive. It may offer a great number of health benefits and has excellent nutritional and functional characteristics. The objective of this work to investigate chemical composition and antioxidant activity of leaves and tubers of inulin-containing plant topinambur (*Helianthus tuberosus L.*) introduced into Georgia, in order to obtain a multifunctional biologically active food additive in the form of dry extract. Chemical compositions of the tubers and leaves of topinambur were determined by using common methods of chemical analysis and their antioxidant activity was estimated by ferric ion reducing antioxidant power (FRAP) assay. Tubers of topinambur was shown to contain high amount of extractive substances (92%) among them inulin was abundant (19%). In the leaves of topinambur, the content of extractive substances was about 50%, but the content of inulin was slight. The leaves of this plant was found to contain high amount of polyphenolic compounds (12%) and pectic substances (9%). The content of polyphenols in the tubers was found to be rather low (0.5%), while in the leaves it was more than 20%. Both tubers and leaves of topinambur contained sugars, amino acids, organic acids, proteins and minerals. Antioxidant activity of topinabur leaves was three times higher than that of tubers. This fact was certainly caused by the high content of polyphenolic compounds. Thus, both tubers (with high content of inulin) and leaves (with high content of polyphenolic compounds and accordingly with high antioxidant activity) of topinambur are expedient to use for producing a multifunctional biologically active food additive.

Keywords: topinambur, inulin, tubers, leaves, antioxidant activity

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C-15
STUDY ON CANDIDA RUGOSA LIPASE SELECTIVITY TOWARDS T,C- AND C,T- CLA ISOMERS

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It is known that conjugated linoleic acid (CLA) isomers have different beneficial health effects such as anti-inflammatory, anti-atherogenic, anti-carcinogenic and anti-diabetic/obesity properties [1]. Furthermore CLA isomers, for their antioxidant characteristics, were topically applied to treat skin damage, such as skin inflammation and atopic dermatitis [2]. For these reasons, CLA could be used as nutriceutical or supplement in food and also in pharmaceutical and cosmetic products. Since CLA effects are isomer-specific, it is important to obtain isomer separation and enrichment. Recently various enzymatic methods for the fractionation of CLA isomers were studied. Lipases are enzyme widely used to catalyze high specific reactions for the separation of the two most representative isomers, 9Z,11E- and 10E,12Z-CLA. Initially CLA isomers were successfully isolated by selective esterification of CLA with lauryl alcohol by Candida rugosa lipase. The disadvantage of this method is that lauryl alcohol cannot be used for the production of food supplements. Therefore it has been developed the selective esterification of CLA with L-menthol, catalyzed by C. rugosa lipase [3], to obtain purified CLA isomers available as food supplements. Furthermore, since L-menthol is a refreshing flavor, it can be used to mask unpleasant characteristics of fatty acid such as smelly [4]. This research concerns the selective esterification of four CLA isomers (11Z,13E-, 10E,12Z-, 9Z,11E- and 8E,10Z-CLA) with L-menthol using C. rugosa lipase. The degree of enzymatic esterification was evaluated by HPLC equipped with light scattering detector, while the isomer profile was analyzed by Ag+-HPLC after the determination of CLA isomer elution order. Since the 8E,10Z- and 11Z,13E-CLA isomers are not commercially available, they were obtained by sigmatropic rearrangement respectively of 9Z,11E- and 10E,12Z-CLA.

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Keywords: conjugated linoleic acid, Candida rugosa lipase, selectivity, L-menthol

C-16
PLANT COMPOSITE WITH HIGH ANTIOXIDANT ACTIVITY

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Plant-based foods contain significant amounts of bioactive compounds, which provide desirable health benefits. Recently, special attention has been paid to edible plants, especially those that are rich in secondary metabolites (often called phytochemicals) and there is an increasing interest in the antioxidant activity of such phytochemicals present in diet. Phenolic compounds are a large group of phytochemicals widespread in the plant kingdom. Tea plant, Camellia sinensis L. is rich in polyphenolics. Another rich source of the polyphenols are grapes. The objective of this work was to investigate chemical composition of green tea extract and red wine lees and to create a compositĺ on the base of them as well as to test antioxidant activity of the composite in order to use it as a biologically active natural food additive. The composite was prepared by mixing liquid Georgian red wine ("Saperavi") lees and dry powder of green tea (instant tea) with the subsequent spray-drying up to the powder form. Chemical composition of the composite was determined by using common methods of chemical analysis. Antioxidant activity of the plant composite was estimated by ferric ion reducing antioxidant power (FRAP) assay. Green tea extract contained large amounts of polyphenols, sugars, amino acids and pectic substances (23.5, 27.2, 14.3 and 9.5% respectively). Abundant polyphenols and pectic substances were found in red wine lees as well (16.4 and 12.8% respectively). Red wine lees also contained high amount of other bioactive compounds, such as sugars and amino acids (14.0 and 19.0 % respectively). The composite from green tea and red wine lees was found to be rich in polyphenols 18.2%, pectic substances (10.5%) and sugars-26.0%. The composite revealed high antioxidant activity. Antioxidant potential of the composite was only 3 times less than antioxidant potential of L-ascorbic and was equal to 4.1 [Fe^{2+}] mmol.l^{-1}. The composite from green tea and red wine lees with high antioxidant activity may be used as a prophylactic bioactive composite and/or natural food additive.

Keywords: green tea, polyphenols, red wine lees, antioxidant activity

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C-17
FATTY ACID COMPOSITION OF OYSTER MUSHROOMS – PLEUROTUS OSTREATUS

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Oyster mushrooms (Pleurotus ostreatus) contains a low percentage of fat, but it is still very important because it is composed of essential fatty acids that have high nutritional and medicinal value. Fatty acid composition was analyzed by gas-liquid chromatography with a flame-ionization detector (GC-FID). Oyster mushroom was analyzed in fresh and in dried form. This study has shown that the oyster mushroom contain important percentage of linoleic acid, an essential omega-6 acids and a high percentage of other essential fatty acids. Nutritional value and these percentages of nutritionally important fatty acids are higher in the dried form of mushrooms.

Keywords: oyster mushroom, Pleurotus ostreatus, fatty acid composition, GC-FID

C-18
SOLID-PHASE SPECTROPHOTOMETRIC DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY WITH FE(III)-FERROZINE METHOD

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Ferrozine is a highly ferrous-stabilizing ligand such that ferric ion in the presence of ferrozine easily oxidizes antioxidants and is itself reduced to Fe(II)-FZ, yielding a very high molar absorptivity and thus enhanced sensitivity for most antioxidants. The Fe(III)-FZ assay was applied to synthetic antioxidant mixtures to yield additive absorbance values, which is a prerequisite for precise determination of antioxidant capacity of complex mixtures. The advantages of ferrozine over other iron-based TAC assays are higher molar absorptivity, relatively lower interference from foreign ions, wide pH tolerance, complex stability constant as high as β_3= 3.4 x 10^{15}, water solubility, and low viscosity.
Solid-phase spectrophotometry (SPS) in the visible region was used for the determination of antioxidants based on their reducing effect on iron(III), followed by formation of the iron(II)-ferrozine chelate. In this work, a sensitive and selective SPS method for determination of total antioxidant capacity is developed. The anionic Fe(II)-FZ complex can be quantitatively sorbed on Sephadex QAE A-25 resin showing an absorption peak at 562 nm. The fixation of the coloured complex on the transparent white anion-exchange resin results in a noticeable increase in sensitivity because of the concentrating capability of the resin. The apparent molar absorptivity, linear concentration range and TEAC (trolox equivalent antioxidant capacity) values of certain antioxidants were found in the proposed assay. The SPS method can be potentially used for flow-through renewable surface optosensing and allows the determination of antioxidants at the ng ml^{-1} level with reproducible results.

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Keywords: Antioxidants, Solid-phase spectrophotometry, Ferrozine, TEAC

C-19
ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF FERMENTED PRODUCTS FROM SEVERAL VARIETIES BEAN BY USING ASPERGILLUS ORYZAE

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Diabetes mellitus is one of the most serious and chronic disease whose incidence rates are increasing all over the world. In 2000, the amount of diabetic patients was 177 million, an estimated 300 million people worldwide have diabetes in 2025 (according to WHO). Globally, type II diabetes (non insulin-dependent diabetes mellitus) accounts for greater than 90% of the cases. Alpha-glucosidase inhibitor is usually used to prevent or medically treat type II diabetes. Although powerful synthetic α-glucosidase inhibitors (i.e. voglibose, acarbose) are available, but they usually can cause hepatic disorders and other negative gastrointestinal symptoms. Hence, natural alpha-glucosidase inhibitors from food sources have become an attractive therapeutic approach for treating post-prandial hyperglycemia. alpha-glucosidase (EC 3.2.1.20, alpha-glucoside glucohydrolase) is an exoenzyme hydrolyzing carbohydrate and release alpha-glucose from non- reducing ends of substrates. In this study, we have investigated the alpha-glucosidase inhibitory activity of fermented soy bean, black bean and red bean prepared by using mold Aspergillus oryzae. The alpha-glucosidase inhibitory activity of hydrolyzed 2 days-molded products from red bean was reached the highest value- 82%, from black bean –value reached 77%, from soybean –value reached only 49.5%. The inhibition activity of fermented beans against alpha-glucosidase from mammalian and microbial source of were also studied.

Keywords: alpha-glucosidase inhibitor, Aspergillus oryzae, beans, fermentation, antidiabetic

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C-20
PLANAR CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY: IDENTIFICATION OF FLAVONOIDS AND PHENOLIC COMPOUNDS IN PROPOLIS

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Propolis is a complex product of bees, which has been used in folk medicine for hundreds of years. As flavonoids and phenolic compounds are of primary interest due to their biological activity and positive action on human health, their pattern was investigated in the propolis samples. For the screening and identification of marker compounds in the still unknown chemical profile of German propolis sorts, high-performance thin-layer chromatography (HPTLC) was used. The differentiation between different types of propolis and the assignment of the origin of the propolis samples was performed. The simultaneous separation of up to 20 samples using HPTLC lasted less than 30 min. Hyphenated techniques, including post-chromatographic derivatization and different couplings of planar chromatography with mass spectrometry, were used for the identification of the components from the characteristic zones of marker compounds on the plate. HPTLC profiles of propolis extracts were compared with the profiles of the reference standard mixtures, and the marker compounds were initially assigned from this comparison, when possible. After that, the mass spectra obtained by DART-MS and ESI-MS were registered in online mode directly from the HPTLC plate. Alternatively, when necessary, also the offline coupling with different mass spectrometric techniques was used by means of collecting of the extracts from HPTLC zones and further analysis of these zones using different mass spectrometric approaches. The capabilities of using different ionization techniques (DART or ESI) and mass analysers (single quadrupole or Orbitrap) for the identification of flavonoids and phenolic compounds in propolis will be discussed.

Keywords: hyphenated HPTLC, DART-MS, propolis, flavonoid, phenolic

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C-21
CAFFEINE AND CHLOROGENIC ACIDS LEVELS IN COFFEE BREW: INFLUENCE OF ROASTING, CULTIVAR AND BREWING PROCEDURE

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Coffee is one of the most important Brazilian crops and the country is the world's largest coffee exporter. Among ground roasted coffee commercially available in the market, some are produced exclusively with Coffea arabica and others with a blend of Coffea arabica and Coffea canephora. Coffee roasting process is responsible for the products characteristics and final quality. In this process, several substances such as chlorogenic acids (CGA) and caffeine are formed or eliminated, providing flavour, acidity and body. Two coffee cultivars were used in this study: Coffea arabica cv. Catuaí Amarelo IAC-62 and Coffea canephora cv. Apoatã IAC-2258. Beans were roasted in order to obtain 3 roasting degrees (light, medium and dark) that were determined by the Agtron/SCAA Roast Color Classification System. After grinding, two different procedures were used to prepare coffee brew: filtered (hot water was poured onto ground coffee held in a paper filter and left to drip), and boiled (ground coffee was mixed with water, the mixture was boiled and filtered in a paper filter). A total of 32 coffee brew samples were analysed for the presence of caffeine and three of the main chlorogenic acids isomers: 3-caffeoylquinic acid, (3-CQA); 4-caffeoylquinic acid (4-CQA) and 5-caffeoylquinic acids (5-CQA). The compounds were determined simultaneously. Analytical method involved dilution in methanol:water, clean-up with Carrez I and II, and analysis by HPLC with diode array detector (detection at 272 nm for caffeine and 324 nm for CQAs). Recoveries ranged from 95.3 to 96% and from 79.5 to 93.4% for caffeine and CQAs, respectively; with RSDs up to 1.2% (caffeine) and 7.5% (CQAs). Coffee brews prepared with C. canephora presented higher caffeine levels than the ones prepared from C. arabica, with mean levels, for filtered coffee, ranging from 87.3 to 99 mg/100 mL, and 123.3 to 165.5 mg/100mL, respectively. The levels of summed CQAs isomers varied according to the degree of roast, with light coffees presenting hig! her leve ls than dark coffees: means of 274.7 mg/100mL and 33.7 mg/100mL, for filtered brew, respectively. For all compounds, despite the roasting degree, boiled coffees presented levels 8% to 43% higher than the corresponding filtered samples. Results indicate that the coffee cultivars studied, roasting degree, and brewing procedure may influence the levels of the studied compounds in coffee brew.

Keywords: Coffee, caffeine, chlorogenic acid, roasting, brewing

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C-22
RAPID DETERMINATION OF ANTHOCYANINS IN BILBERRY BASED NUTRITIONAL SUPPLEMENTS

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Anthocyanins are a subclass of flavanoid polyphenols responsible for the brilliant red, orange, and blue colors of most fruits, vegetables, and flowers. The chemical structures of anthocyanins are naturally electron deficient, which makes them very reactive towards free radicals and consequently powerful natural antioxidants. Major sources of anthocyanins include bilberries, strawberries, black currants, purple grapes, and pomegranates. Consumption of bilberry based nutritional supplements has increased globally due to its reported health benefits. This paper describes a sensitive, fast, and accurate UHPLC method to determine 15 anthocyanins and 5 anthocyanidins (anthocyanin aglycones) in bilberry-based nutritional supplements. The method demonstrates good sensitivity, enabling the detection of a wide variety of anthocyanins in bilberry supplements with concentrations ranging from 0.25–24.3 µg/mL with a total run time of less than 30 min. The reported LODs using the method ranged from 0.20–1.56 µg/mL and LOQs ranged from 0.78–6.25 µg/mL. Each bilberry based nutritional supplement was spiked with known amounts of seven anthocyanins and recoveries ranged from 78–107%, suggesting good method accuracy.

Keywords: Polyphenols, anthocyanins, antioxidants, Biberry, supplements

C-23
METHODS TO QUANTIFY THE PHYTOCHEMICAL RICHNESS OF A DIET

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Numerous nutritional studies have linked the health benefits of fresh fruits and vegetables, whole grains, legumes and plant-based foods such as olive oil and wine to their high levels of phytochemicals. Phytochemicals have been associated with reductions in oxidative stress, cholesterol levels, cardiovascular diseases, inflammation, a lower incidence of obesity etc. [1]. A high intake of these micro-nutrients is important for optimal health and prevention of disease, but quantification of the compounds is costly and time consuming. For that reason Vincent et al. proposed a simple method to express the total phytochemicals content of a diet, the so-called 'phytochemical index' (PI). It is defined as the ratio of the energy from high-nutrient phytochemical-rich foods to overall daily energy consumed [kJ phytochemical rich foods/total kJ consumed]) [2]. In this contribution we discuss methods to express the diversity of the range of phytochemicals present in a diet. This to distinguish diets with a high level of one phytochemical from diets that contain a wide range of different species, each present at lower levels. Three routes for expressing the diversity are considered:

1. Detection of specific compounds from a previously determined list
2. Fingerprinting analysis
3. Effect-quantification

The principles of the above routes will be discussed. Special emphasis will be on the selection of the specific compounds for inclusion in the list for route 1. Methods for fingerprinting will be shown as well. Fingerprints obtained with various forms of chromatography and mass spectrometry will be shown including data from comprehensive GC×GC, LC-ion mobility MS and LC-accurate mass MS. Quantification strategies to convert these complex fingerprints to numbers that are indicators for the phytochemical diversity are discussed.

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Keywords: Phytochemicals, food richness, fingerprinting, chromatography, mass spectrometry.

C-24
ANALYSIS OF COENZYME Q10 IN MEATS FROM DIFFERENT ANIMAL SPECIES

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Coenzyme Q10 is an endogenous hydroxybenzoquinone liposoluble compound which plays an important role as electron carrier in the mitochondrial respiratory chain. Coenzyme Q10 can have a relevant role as antioxidant in meat by protecting cellular membranes and lipoproteins against oxidative damage. The purpose of this work was to analyse coenzyme Q10 in meats from different animal species (pork, beef, lamb, rabbit and chicken) and muscles of different metabolic oxidative patterns for each species. Sample preparation included the homogeneization of meat with a solution containing 0.5 M NaCl + 0.1 M SDS. The coenzyme Q10 in the homogenate (1 mL) was then extracted with a mixture of ethanol (2 mL) and hexane (5 mL) and, after appropriate mixing and centrifugation, the hexane fraction was evaporated under nitrogen and resuspended in 0.5 mL of isopropanol that was injected into the HPLC. The separation was performed with a Ultrabase reverse phase column (100×4 mm, 2.5 µm). Lactate and malate dehydrogenases activity and myoglobin contents were also determined for a better characterisation of each muscle metabolism. The results showed a strong dependence of coenzyme Q10 with the type of muscle metabolism which was much more relevant than the effect of animal species. A clear trend towards higher contents of coenzyme Q10 in oxidative muscles was observed. For instance, 18.4 µg/g pork meat in oxidative pattern Masseter vs 5.3 µg/g pork meat in glycolytic pattern Longissiums dorsi. This indicates a better antioxidant protecting effect in muscles with higher oxidative pattern.

Keywords: coenzyme Q10, meat, nutrients, antioxidant, HPLC

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C-25
DEVELOPMENT OF SPECIFIC ANTIBODIES TO DETECT RECOMBINANT BOVINE GROWTH HORMONE IN MILK

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Recombinant bovine somatotropin (rBST), or bovine growth hormone, has been widely used to increase milk production since the commercial product became available in 1994. However use of this synthetic hormone in the European Union was banned in 1999 due to concerns on animal welfare, food safety and public health risks associated with it's use. Despite the ban, the illegal use of rBST within the EU and importation of dairy products from animals treated with rBST cannot be prevented without methods that allow the unequivocal identification of rBST-treated animal products. An antibody that specifically recognises rBST could be utilised in a variety of immunoassay formats to detect the illegal hormone. The major difficulty of this approach is that the banned rBST is virtually identical to the native circulating form of the hormone. The aim of this project is to generate monoclonal antibodies that will detect rBST in milk with a high degree of sensitivity and specificity. We generated these specific antibodies using a procedure based on the phenomenon of high zone tolerance. We rendered the animals immunotolerant to the native pituitary somatotropin (pBST) prior to immunization of mice for production of anti-rBST antibody. Screening of the hybridomas generated by this method for reactivity to both forms of BST revealed a subset of clones that produce antibodies specifically recognizing the rBST. These antibodies are currently being used to develop immunoassays for rapid detection of rBST in milk.

Keywords: bovine somatotrophin, monoclonal antibody production, immunotolerance, biosensor

Acknowledgement: The project is financially supported by the uropean Comission RP7 Unique-Check (contract no. 230667).

C-26
CHROMATOGRAPHIC METHODS FOR TOTAL CHOLESTEROL IN FOOD OF ANIMAL ORIGIN DETERMINATION

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Dietary intake of exogenous cholesterol together with macronutrients composition and fatty acids profile of the diet are main factors often discussed in context with elevated plasma LDL-cholesterol. The sensitivity of organism to dietary cholesterol is individual. The sources of exogenous cholesterol are eggs and egg products, meat and meat products, milk and dairy products. The latest reported results state that content of cholesterol in this matrix may be influenced by changes in intra-vital and genetic factors caused by changes of conditions in animals breeding. Considering these facts, we used and adapted several methods for cholesterol determination with regard to the particularity of the matrix in our study. The total cholesterol content was determined by chromatographic methods, namely by RP HPLC with PDA and ELSD detection. Parallel analyses by capillary GC were performed. Method for confirmation of target analytes was LC-MS with APCI detection. Prior to the final chromatographic analyses the saponification step with methanolic potassium hydroxide solution was used, followed by the extraction of the unsaponificable residue into non polar solvent. Parameters of RP HPLC method were compared with parameters of GC determination. The parameters of both methods were practically identical. The detection limits (LOD) determined on the bases of blank samples analysis were 5.2 $mg.kg^{-1}$ for cholesterol, 4.8 $mg.kg^{-1}$ for stigmasterol. Stigmasterol was used as an internal standard. Recovery ranged between 80–92%, repeatability expressed as RSD of 12 parallel samples measurements was 4.2–6.8%. Accuracy tested on the SRM 1845 Whole Egg Powder (NIST) was 95.7%

Keywords: cholesterol, HPLC, GC, milk, meat, eggs

Acknowledgement: The study was supported by project MSM 6215712402 "Veterinary Aspects of Food Safety and Quality.

C-27
STUDIES ON THE ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACTS FROM IN VITRO CULTURES OF SALVIA OFFICINALIS L., BY THREE DIFFERENT ANALYTICAL ASSAYS AND THEIR ROLE ON LIPID AUTOXIDATION OF FRESH CHEESE

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This study is designed to examine the chemical composition and antioxidant activity of the ethanolic extracts from shoots and hairy roots as well as from undifferentiated (cell and callus) cultures of Salvia Officinalis L. Antioxidant activities of the samples were determined by three different test systems namely DPPH, β-carotene/linoleic acid and reducing power assay. The concentrations of rosmarinic acid, diterpenoids (carnosic acid and carnosol) and total phenolic compounds in each extract were determined. The ethanolic hairy root and root regenerated plant extracts possessed the strongest effects on reducing Mo and DPPH radical scavenging. On the other hand the best protective effect against linoleic acid oxidation was observed for ethanolic extracts of shoots obtained from in vitro culture followed by the extracts of shoots of intact plants grown in the field, without statistically significant differences between them Antioxidant activity of the polar sub-fraction of ethanolic extract was superior to the all samples tested with an EC50 value of 157.26±1.12 μg ml−1. In the second case, the inhibition capacity (%) of the polar sub-fraction of ethanolic extract (97.39%±0.84) was found the strongest one, which is almost equal to the inhibition capacity of positive control BHT (97.44%± 0.74). In the case of reducing power assay, a similar activity pattern was observed as given in the first two systems. Polar sub-fraction was the strongest radical reducer when compared with the non-polar one, with an EC50 value of 625.63±1.02 μg ml^{-1}. The amount of the total phenolics was highest in polar sub-fraction (25.60±0.74 μg/mg). A positive correlation was observed between the antioxidant activity potential and total phenolic level of the extracts. On the other hand, the in vitro sage extracts were homogenized, each one, in a fat fresh cheese until a pH of 5.6 was reached. Cheese produced from 2.38 g extract/100 g remained stable over 12 days of storage at 4°C. The cheese curd was found to retain between 67 to 86% of diterpenoids, and over 91% of the phenolic compounds from the extract after separation of the whey. The level of conjugated dienes and the peroxide value in the final product were significantly lower ($P < 0.05$) as compared to the untreated control fresh cheese, after two weeks of storage at 4°C. This research shows for the first time that the ethanolic extracts of in vitro cultures of Salvia Officinalis L., rich in bioactives can act as useful preservation agents for fresh dairy products.

Keywords: Antioxidant activity; DPPH; β-Carotene/linoleic acid test, reducing power assay, cheese

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C-28
OPTIMIZATION OF THE METHOD FOR PROFILING OF FATTY ACIDS IN ALGAE

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C18 and C20 polyunsaturated fatty acids are essential for nutrition of many animals and humans since they are used for the biosynthesis of eicosanoid hormones. In addition, there is a growing interest for these compounds in biotechnology and, more recently, in cosmetic industry. An interesting source of polyunsaturated fatty acids represents algae. Therefore, a selection of algae producing a maximum amount of polyunsaturated fatty acids is challenging. However, determination of composition/profile of fatty acids of this matrix is complicated due to the low content of fat (maximum 15%) and typically low sample quantity (0.5–1 g) available for the analysis. Classical methods for the determination of profile of fatty acids in high-fat matrices are based on the Soxhlet extraction followed by hydrolysis of the parent compounds (triacylglycerols) with subsequent methylation catalyzed by BF3. This approach requires somewhat high quantity of fat (approx. 250 mg) for the analysis. Taking into account both low content of fats in algae samples and low amount of those samples available, we have developed a novel approach for the determination of profiles of fatty acids in this type of matrix. This method involves extraction of triacylglycerols with a mixture of dichlormethane–methanol from the dry algae material. During this step, co-extracted pigments are removed by the addition of active charcoal. The methylation procedure takes place in a reaction cup with small amounts of the reaction agents. Subsequently, gas chromatographic separation of fatty acids employing a polar column SP-2560 (100 m × 0.25 mm; 0.20 μm) follows with detection using a flame-ionization detector (FID).

Keywords: Profiling, Algae, Polyunsaturated fatty acids, Sample preparation, Gas chromatography

Acknowledgement: The financial support by the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6046137305) is gratefully acknowledged.

C-29
DEVELOPMENT OF IMMUNOANALYTICAL METHODS FOR THE DETECTION OF RECOMBINANT BOVINE SOMATOTROPIN: THE CHALLENGE OF IMMUNODISCRIMINATION BETWEEN NATIVE AND RECOMBINANT ISOFORMS

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Bovine somatotropin (bST) is a peptidic growth hormone of 191 amino acids, which exogenous administration increases milk production by a 15% on average in dairy cows. The use of recombinant bST (rbST) and the commercialization of dairy products obtained from treated animals are banned in the EU, Japan, Australia, New Zealand and Canada however, its use is authorized for example in USA, Brazil, South Africa, Mexico and Korea and imports from these countries to EU is permitted. Therefore, it is necessary to monitor rbST in dairy products. Current screening methods consist in the analysis of biomarkers which expression is increased upon rbST administration (e.g. Insulin-like Growth Factor-1, IGF-1), and the detection of anti-rbST antibodies in the plasma of treated cows. Recently, the application of instrumental techniques based on HPLC-MS/MS has also been reported for confirmatory purposes. The development of fast, simple, cost-effective, portable and high-throughput screening immunoanalytical methods would be highly desirable. The closed structural similarity displayed by the recombinant and the native bST, with only one different amino acid at the N-terminus constitute the main limiting factor for the production of antibodies able to immunodiscriminate rbST. Several attempts have been conducted to develop immunoassays for rbST, but no specific antibodies have been obtained to date. The Marie Curie FP7 Unique-Check project involving four partners, aims at the unequivocal determination of the presence of rbST in dairy products, by the development of immunoanalytical tools (SPR-based biosensor, ELISA and Lateral Flow devices), as well as confirmatory instrumental methods (LC-MS/MS based). As a first attempt, the production of polyclonal antibodies (pAbs) of different animal origins (rabbit, lama and hen) was accomplished by using a battery of immunogens. Four different strategies were conducted consisting in the immunization with (1) whole rST (equine and bovine); (2) synthetic peptides representing the N-terminus of rbST (between 12 and 18 amino acids); (3) a hapten representing the first two amino acids of the rBST N-terminus; (4) an arachnid-mimicking hapten displaying several units of the first two amino acids of the rbST N-terminus. The preliminary characterization of the generated pAbs showed that several of the used immunogens elicited a positive immune response. Some of the pAbs obtained by the immunization with the peptides showed promising results, displaying a higher recognition to the rbST. The production of monoclonal antibodies by the application of the hybridoma technology employing this immunizing strategy is under work.

Keywords: recombinant bovine somatotropin, dairy products, immunodiscrimination, polyclonal antibodies

Acknowledgement: Unique-Check Project has been financed by the European Community's Seventh Framework Programme (FP7/2007-2013; Grant Agreement N° 230667).

C-30
CHEMICAL CHANGES IN COFFEE ACCORDING TO THE PREPARATION PROCEDURES. PART B: QUALITATIVE PARAMETERS OF COFFEE (ANTIOXIDANT ACTIVITY, PHENOLIC ACIDS, CAFFEINE AND VOLATILE PROFILES)

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Coffee, together with tea, is the most consumed hot beverage all over the world, due to its specific aroma, taste and refreshing effect on human organism. During the roasting of green coffee beans, aside from formation of processing contaminants (see Part A), the changes can occur in the content of compounds with protecting character (mainly polyphenolic compounds and organic acids) that make coffee an important source of antioxidants. Coffee is also the major source of caffeine in human diet. The aim of this part of our study was to characterize the differences in: (i) total antioxidant activity, (ii) chlorogenic acid content, (iii) caffeine content and (iv) volatile profiles of brews prepared from roasted ground coffee as espresso, filtered coffee and so called "mud" coffee. The same parameters in instant coffee, capsuled coffee and roasted ground coffee sold for espresso preparation were also examined. Head-space solid-phase microextraction coupled to gas chromatography - mass spectrometry (HS-SPME/GC-MS) was used for description of volatile profiles. The antioxidant activity was determined as the total ability to inactivate free DPPH radicals using a spectrophotometric method, while HPLC/DAD was used for chlorogenic acid determination. For caffeine determination, a method using direct analysis in real time (DART-MS) was introduced. The antioxidation activity and the content of chlorogenic acid of the brews examined ranged from 0.6 to 7.2 mg $AA.ml^{-1}$ (expressed as the equivalent of ascorbic acid, AA) and from 112 to 2045 $\mu g.ml^{-1}$, respectively. The brewing method together with the type of coffee (roasted beans, instant coffee) had an impact on the examined parameters. On the other hand, no significant changes in these parameters or in the caffeine content were observed depending on the dwell time after preparation of brews.

Keywords: Coffee, SPME/GC-MS, DART-MS, antioxidation activity, caffeine, volatiles

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BIOTECHNOLOGY BASED METHODS

(D-1 – D-3)

D-1
SIMPLE AND RAPID DETECTION OF LISTERIA MONOCYTOGENES IN FERMENTED SAUSAGES USING CULTURE ENRICHMENT COMBINED WITH REAL–TIME PCR

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Listeria monocytogenes is a psychrotrophic, gram-positive, non-spore-forming, facultative anaerobe bacterium, that has become an important cause of human foodborne infections worldwide. Conventional microbiological, immunoenzymatic, and modern molecular approaches are currently used for the isolation and detection of L. monocytogenes in food samples. Advances in molecular technologies, have allowed more reliable microbial identification and surveillance. The aim of this work was to optimize the experimental conditions for Taq Man Real - Time PCR detection of L. monocytogenes in food samples and analyze an influence of enrichment incubation time on the sensitivity of method. For that purpose fermented sausage samples were artificially inoculated with serial dilution of L. monocytogenes strain ATCC 19111. Direct Real–Time PCR detection, without incubation, was compared with detection after different enrichment incubation times (2, 6, 24 h). The method was based on the amplification of the hlyA gene using hlyQF/R primers and hlyQP probe. The signals produced (threshold cycle, Ct) by the serial dilutions of L. monocytogenes were plotted against the Log10 CFU and the standard curves were constructed. In this study we could not quantify less than 104 CFU/g. Also, correlation coefficient (R2) significantly increases over time. The highest correlation coefficient (R^2 > 0.930) and the best sensitivity of Real - Time PCR method was achieved after 24 h incubation, detecting 1 CFU/g. The Real – Time PCR procedure described has proved to be a simple and rapid method suitable for the routine analysis of food samples. This rapid detection and automatization method for L. monocytogenes will be important for verifying food safety and preventing listeriosis outbreaks.

Keywords: Listeria monocytogenes, fermented sausages, Real - Time PCR, detection

Acknowledgement: This work has been supported by Institute of Meat Hygiene and Technology, Belgrade.

D-2
RAPID DETECTION OF TOXICOGENIC E.COLI O157:H7 BY USE OF PCR

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Introduction: E.coli isolation rate by culture method will be reduced if animals take antibiotics before sampling. This study aimed to setup, optimize and introduce a sensitive and specific PCR detection method for identification of E.coli DNA in clinical samples.
Materials and Methods: stx2A gene was selected as a specific target sequence. This primer pair amplifies 556 bp of this target gene. E.coli O157H7 was used as a standard organism for optimization experiments. Phenol – Chloroform method was used for DNA extraction. Amplified product was detected by 1% gel agarose electrophoresis, stained by ethidiome bromide.
Results: Provided data confirmed amplification of expected product. Specificity test proved no cross reaction with tested organisms. Sensitivity test detected 500 fg E.coli DNA as a final detection limit.
Conclusion: Optimized experiment confirming applied PCR protocols is quite fast with high sensitivity and specificity performing in less than three hours. Application of this test to the clinical laboratories can help to rapid diagnosis of the E.coli in samples.

Keywords: E.coli, PCR, stx2A gene

D-3
MICROBIAL PRODUCTION AND DOWNSTREAM PROCESSING OF 2, 3 BUTANEDIOL

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2,3- Butanediol is characterized by interesting properties and wide range of application. This compound could be a valuable component of various polymers which are probably easily biodegradable.This type of production will, however, have to be a microbiological process as the current known chemical processes are not economically competitive. Major achievements of the work entitled "MICROBIAL PRODUCTION AND DOWNSTREAM PROCESSING OF 2,3-BUTANEDIOL" are summarized below. Physical and chemical parameters to achieve high AMC+BD production using E.cloacae ATCC 27613 and K.oxytoca DSM 3539 have been standardized. For initial studies glucose and sucrose were used as carbon sources and basic media for the two organisms was standardized and its composition is given below. A pH of 5.8 and a temperature of 300±0.50°C was found optimum for AMC+BD production. Aeration at a rate of IVVM (at a constant agitation of 180 rpm) was found optimum for AMC+BD yield (% on sugar basis) obtained using a fore mentioned standardized media and conditions was Medium E.cloacae AMC+BD yield(g/100g sugar uti.) K.oxytoca AMC+BD yield(g/100g sugar uti) GYEP 52.3 46.2 SYEP 45.7 43.3 Although AMC+BD yields obtained using these standardized media and conditions approached the theoretically possible yields (50% on sugar basis), effort was made to find some compounds, which could lead to further enhancements of AMC+BD production. Various organic acids and their salts, which influence activities of various enzymes involved in butanediol biosynthetic pathway, or could themselves act as tricarboxalic acid cycle intermediates were found to have a considerable stimulatory effects on AMC+BD production. Acetic acid, lactic acid,succinic acid or their respective sodium salts when added to GYEP or SYEP media were found to resulting considerable enhancement of AMC + BD yields, giving yields which were even higher than the theoretically possible yields. AMC + BD yields (%age on sugar basis) obtained using supplemented media is given hereafter: E.cloacae K.oxytoca AMC+BD Yield (on Sugar basis) Supplemented GYEP 60.2 53.6 Unsupplemented SYEP 52.3 46.2 AMC+BD yields obtained in this study were higher than the maximum yields reported to date which is 52% (on sugar basis).When AMC+BD production was carried out using immobilized cells of E. cloacae and K. oxytoc.

Keywords: 2, 3 butanediol, scale up procedure, biotechnology for 2,3 butandiol

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FLAVOURS AND ODOURS

(E-1 – E-26)

E-1
VOLATILE ORGANIC COMPOUNDS EMITTED BY QUARANTINE POTATO PATHOGENS: NEW PERSPECTIVES FOR BACTERIAL BROWN ROT AND RING ROT DIAGNOSIS

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It is well known that both microorganisms and plants emit volatile compounds, some of which may have odours that are characteristic of the species. The scent of flowers or the bad smell of an Escherichia coli culture describe the large spectrum of the odours that our nose perceives and uses to recognize the environment. Concerning microorganisms, several studies have reported that the type of culture media and growth time, as well as the microbial species, may influence the amount and pattern of volatile compounds that are produced. When these patterns are characteristic for pathogens, they can be used as disease biomarkers. Brown rot and ring rot, caused by the bacteria Ralstonia solanacearum (Rs) and Clavibacter michiganensis subsp. sepedonicus (Cms) respectively, are the most damaging potato diseases worldwide; the former has been reported in more than 30 countries from sub-tropical to cold temperate areas, while the latter occurs in Northern America, North Eastern Europe and Asia. Both bacteria are included in the A2 list of quarantine pathogens in Europe and are subjected to EU directives. In this study, volatile compounds emitted from bacterial cultures of Rs and Cms grown on different nutrient media (TZ-, LPG-, and PD-agar) have been analyzed with GC-MS by sampling of the headspace using SPME: PD (potato dextrose)-agar was chosen to simulate a semplified potato substrate. Dimethyldisulphide (DMDS) was the main volatile compound metabolized by Rs on TZA. A mixture of sulfides (DMDS and trimethyltrisulfide) was produced by Rs on LPGA along with 2-propanone, and methyl ester of 2-methylbutanoic acid. Propanoic acid, 2-butanone, DMDS, 2-methyl propanoic acid, 3-methylbutanoic acid and methyl ester of 3-methyl-2-butenoic acid were detected in the headspace of Rs grown on PDA. Owing to the slow growth of Cms with respect to Rs, only few molecules were identified as markers of pathogen presence: trymethyltrisulfide was observed on LPGA while 2-propanol and 3-hydroxy-2-butanone could be considered molecules characteristic of Cms metabolism on PDA. Cms did not grow on TZA. Preliminary SPME-GC-MS analysis have been performed on potatoes experimentally infected by Rs and Cms. According to the volatile compounds detected in the headspace both of Rs and Cms grown on PDA, for Rs infected tubers, significant variations of relative abundance of 3-methylbutanoic acid, and a mixture of short chain hydrocarbons were found comparing GC-MS spectra of control and infected tubers. Two specific markers of ring rot disease were identified analyzing Cms diseased tubers: 2-propanol and 3-methyl-3-buten-2-one. It seems that the metabolism involved in the Rs and Cms growth on PDA is similar to the metabolism that occurs in potato tubers. These results are encouraging to develop a new non-distruptive method for diagnosis of potato brown rot and ring rot diseases based on the different odours emitted from infected tubers.

Keywords: Brown rot, ring rot, potato, VOCs

Acknowledgement: This work is part of the Q-Detect European project (FP7-KBBE) which is supported by the European Commission through the Seventh Framework Programme for Research and Technological Development.

E-2
ANALYSIS OF THE ODOUR PROFILE OF FOOD PRODUCTS USING A MICRO CHAMBER THERMAL EXTRACTION SYSTEM AND THERMAL DESORPTION (TD) GC–TOF (MS) DETECTION

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The ability to identify the odour profile from food products is commercially important for several reasons. These include product quality/consistency, consumer attraction and off-odour analysis as an indicator of decay or contamination. To study this profile, a micro chamber thermal extraction system (μ-CTE) is described which enables the volatile and semi volatile organic chemicals (VOC/SVOC's) to be monitored from a variety of food products. The work will show the ability of a multi hyphenated technique (μ-CTE-thermal desorption-GC-TOFMS) to analyse the odour profile of pork meat and cheese for both fresh and aged samples. The μ-CTE can accomodate bulk (grms) samples which are placed into a series of chambers, heated (optional) and purged with an inert gas. The effluent from each chamber obtained from dynamic headspace extraction is subsequently trapped by a thermal desorption sample tube containing selective sorbents allowing the whole VOC/SVOC profile to be monitored, including Sulphur compounds. Analysis is performed using a thermal desorption system connected to a high performance GC time-of-flight mass spectrometer. The ability of the TD system to quantitatively recollect a (split) proportion of the sample after desorption from the TD tube and/or cold trap is described which enables re-analysis of the same sample. This provides a technique to look at both the high concentration components (high split), followed by a low split method for trace level analysis. The inherent sensitivity and classical EI spectra derived from the TOF-MS enables trace level compounds to be detected and identified using commercial MS libraries eg NIST.

Keywords: Odour-profile, Micro chamber, Thermal desoprtion, TOF-MS

Acknowledgement: Dr Daniel Cooper, Dr Paul Morris, Markes International Ltd.

E-3
COMPREHENSIVE PTR-MS/TRIBOLOGIC STUDY ON AROMA RELEASE FROM DAIRY-EMULSIONS: THE INFLUENCE OF FRICTION AND FAT LEVEL

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Flavor perception of foods is a key factor determining consumer acceptance. During oral processing flavors (i.e. volatile compounds) are released from the food and transported to the olfactory receptors in the nose. This process is governed by different factors such as the physico-chemical properties of the flavor compounds, the nature of the food matrix, and human physiology. In this context, the fat level plays an important role. With fat acting as a "sink" for hydrophobic compounds, the fat level determines the amount and rate of aroma release from the food matrix. Moreover, fat plays an important role on oral food processing: During and after food consumption, humans tend to rub their tongue across the palate, spreading the food in the oral cavity and generating friction forces. We hypothesized that, with fat acting as an in-mouth lubricant, a change in fat level will alter in-mouth friction and thus alter spreadability of the food in the mouth. This, in turn, may alter the food's surface area and phase mixing of volatiles within the food thereby modulating overall flavor release from the food into the oral cavity. In the present study, we investigated the relationship between fat level, friction coefficient and flavor release using dairy model emulsions (n=15; fat level varying between 0–40%). This was realized by coupling PTR-MS (monitoring real-time flavor release) with a custom-made Tribometer. The Tribometer was used (1) to induce friction by shearing the emulsions between a fixed and a sliding PDMS probe that was operated at different shearing speeds (0, 45 and 90 mm/s) and (2) to measure the change in the resulting friction coefficient with fat level and shearing speed. The emulsions were spiked with 7 volatile compounds that differed in hydrophobicity (logP) and volatility. Flavor release from emulsions upon shearing was monitored and compared to in-vivo flavor release obtained from 6 panelists that were asked to consume the emulsions using a standardized drinking prot! ocol. Fl avor release (intensity in ppbv) changed with the friction coefficient which in turn was determined by the fat level and the shearing speed. The size of the effect depended on the volatile compound (i.e. logP and volatility). In-vivo flavor release changed with fat level and revealed considerable inter-subject differences. We suggest that the observed change in flavor release with fat level is not only determined by hydrophobic fat-volatile interactions. The concomitant change in (oral) friction with fat level also plays an important role. Implications of oral friction on in-mouth flavor release and how this relates to the observed inter-subject differences in in-vivo flavor release are discussed.

Keywords: PTR-MS, aroma release, tribology, friction

E-4
COMPARISON OF THE KEY AROMA COMPOUNDS IN BARTLETT (WILLIAMS CHRIST) PEAR BRANDIES

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The name Bartlett (Williams Christ) Pear Brandy is trademarked and is only used for brandies manufactured from Bartlett (Williams Christ) pears. The first step in brandy production is a fermentation of a pear mash using yeast. After fermentation, the mash is distilled and on the basis of boiling points, separated into first-, middle-, and back running. Only the middle running is stored in special clay jugs for a minimum of two years to finally obtain the pear brandy. Thus, three factors mainly influence the overall aroma of the final product: the type of pear, the fermentation/distillation process, and the years of ageing. Although a few studies have been performed on the volatile composition of pear brandy, data on the key aroma compounds contributing to the overall aroma are scarcely available. Because aroma is one of the most important factors determining the quality of pear brandy, two commercial brandies, significantly differing in their overall aroma profile, were chosen for analysis by the molecular sensory science concept. This concept starts with the localization of the key aroma compounds by applying an aroma extraction dilution analysis (AEDA), followed by identification experiments, and finally a quantification of the most odor-active compounds by means of stable isotope dilution assays (SIDA). Forty-four aroma-active compounds could be identified of which more than twenty-five were rated to be "keys", based on their Odor Activity Value. Besides to the well-known "Bartlett flavor compound" ethyl (E,Z)-2,4-decadienoate, also (S)-ethyl 2-methylbutanoate, (E)-beta-damascenone, ethyl hexanoate, 4-ethyl-2-methoxyphenol, and diacetyl were established as most important odorants. A comparison of the concentrations of the key aroma compounds in the two different Bartlett Brandies revealed clear differences, e.g., in gamma-nonalactone and 4-ethyl-2-methoxyphenol The results will be discussed with special emphasis on their formation during processing.

Keywords: bartlett pear brandy, flavour, AEDA, SIDA

E-5
ANALYSIS OF 4-METHYLIMIDAZOLE: CREAMY CARAMEL COLORS, COLA AND CANCER?

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4-methylimidazole (4MI or 4-MEI) is a by-product formed during the production of caramel colorings. These colorings are added to many foods including beverages (cola), coffee, beer, whisky, baked goods, soy and Worcestershire sauces and meats. To date, studies have not provided a clear indication as to the toxicity of 4MI but the National Cancer Institute has nominated 4MI as a candidate for toxicity and carcinogenicity studies. In addition, the EFSA is currently considering changing the allowable 4MI levels in food products. The chromatographic analysis of 4MI has traditionally been accomplished by GC/MS with derivatization, RP-HPLC with ion pairing, and now HILIC. None of which are simple or easily reproducible. In this presentation we utilize our Ultra PFPP column to analyze 4MI using typical LC/MS mobile phases, water and methanol with formic acid, and isocratic conditions in ESI+. We also explore the retention mechanism of the PFPP phase that allows the retention of small polar compounds such as 4MI.

Keywords: 4-methylimidazole, caramel, coloring, HPLC, mass spectrometry

E-6
LATEST DEVELOPMENTS IN PROTON-TRANSFER-REACTION MASS SPECTROMETRY (PTR-MS) TO IMPROVE FOOD AND FLAVOR ANALYSIS IN REAL-TIME

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Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) has become an approved technique in food and flavour science already shortly after it had been invented at the University of Innsbruck in the 1990s and commercialized by the spin-off company Ionicon in 1998 (see [1] for a recent PTR-MS review and [2] for an example utilizing PTR-MS in the analysis of coffee). In the present work we want to report on the latest instrumental developments that are highly beneficial for food research, namely (i) the improvement of the detection limit that now allows measuring trace gas compounds in a concentration range from several ppmv down to the ppqv (parts-per-quadrillion) region with a typical response time well below 100 ms, (ii) a novel heated Nosespace Air Sampling Extension (N.A.S.E.) for the analysis of flavor compounds in human breath (iii) the coupling of our sophisticated PTR source to two different types of time-of-flight (TOF) mass analyzers and (iv) the possibility to switch between H_3O+, NO+ and O_2+ as reagent ions. Furthermore, our very recently developed Direct Aqueous Injection (DAI) inlet system allows us for dealing with “hot topics” like detecting minute traces of so-called "rape drugs", i.e. γ-butyrolactone and 1,4-butanediol, in liquids (red and white wine, tea, etc.). Amongst the very exciting results obtained with the above-mentioned developments we present data from an IONICON High-Sensitivity PTR-MS coupled to the recently developed N.A.S.E. It demonstrates the on-line detection capability of "sticky" aroma compounds in human breath like e.g. vanillin. Such substances are on the one hand very common in flavour and food science but also very difficult to analyse as they tend to condensate in the inlet lines of trace gas analyzers which reduces the overall sensitivity, extends the response time and leads to memory effects. Furthermore we present very recent studies on potentially dangerous substances that are predominantly (and often unknowingly) consumed highly diluted in liquids, e.g. γ-butyrolactone and 1,4-butanediol traces mixed in different concentrations into plain water, tea, and red and white wine. Both substances are metabolized in the human body to γ-hydroxybutyric acid ("liquid ecstasy") and are therefore frequently abused as recreational drugs (in lower doses) or so-called "date rape or knockout drugs" (in higher doses). Utilizing complementary simple headspace analysis above the liquids' surface and our recently introduced Direct Aqueous Injection (DAI) system coupled to a PTR-MS, we were able to detect both substances in all above-mentioned liquids with great linearity down to concentration levels far below the activation threshold for effects in human beings.

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Keywords: PTR-MS, PTR-TOFMS, real-time analysis, SRI, flavour analysis

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E-7
ODOUR-IMPACT COMPOUNDS OF AN ODOUR REPRESENTATIVE HS-SPME-EXTRACT OF A RED BERRIES YOGHURT DRINK: A D-GC-O AND GC-MS/FID-O STUDY

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Headspace-solid-phase microextraction (HS-SPME) sampling followed by gas chromatography (GC) separation is widely used for the analysis of odorant compounds in dairy products (1, 2). However, extracts with varying odorant properties might result depending on the fibre coatings. Therefore, when studying a product's odour and prior to GC-Olfactometry (GC-O) studies, aiming at obtaining a representative extract is recommended. Direct-GC-O (D-GC-O) evaluates an extract's global odour at the sniffing port without chromatographical separation and a comparison to the original sample's odour is made. Hence, this technique is suitable for HS-extracts where no physical extracts are obtained (3−7).
Here we report on the odour-impact compounds of an odour representative red berries yoghurt drink HS-SPME-extract. Firstly, the odour representativeness of yoghurt drink extracts obtained with four different fibre coatings was evaluated by D-GC-O. The product's odour was best represented by the DVB/CAR/PDMS 50/30 µm fibre, followed by the PDMS 100 µm and the CAR/PDMS 85 µm fibre (7). Based on these results, the volatile chemical and odorant profile of the extracts was studied by GC-MS/FID-O. Detailed analyses were conducted with the DVB/CAR/PDMS 50/30 µm fibre.
The two predominant constituents were methyl hexanoate (41–54% of the total FID peak area) and ethyl 2-methylbutanoate (24–32%) for all fibres. GC-O analyses revealed seven reliably perceived odour-active zones (detection frequency (DF) ≥ 50%; panel n=4) with the DVB/CAR/PDMS 50/30 µm, five with the PDMS 100 µm, and eleven with the CAR/PDMS 85 µm fibre. Fruity odour zones were dominant. Four green-grassy/fruity-chemical odour zones were perceived with the CAR/PDMS 85 µm fibre only. These additional odour zones and the fact that the overall D-GC/FID signal intensity was lower for this fibre might explain why it resulted in a less representative extract. Detailed GC-O-analyses (panel n=8; odour impact evaluation by multiplying the DF with the mean intensity) were conducted with the DVB/CAR/PDMS 50/30 µm fibre on two different stationary phases. Main odour-impact compounds were ethyl 2-methylpropanoate (fruity, red berries, pineapple; present at ~1%), ethyl 2-methylbutanoate (fruity, strawberry, pineapple; ~24%), ethyl butanoate (fruity, banana, pineapple, soapy; ~3%), and (Z)-hex-3-enol (grassy, fruity; ~2%). Methyl hexanoate and the internal standard methyl octanoate were not perceived by the panel

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Keywords: GC-Olfactometry, direct-GC-O, solid-phase microextraction, yoghurt, detection frequency

E-8
CHARACTERISATION OF LIGHT INDUCED OFF-FLAVOUR COMPOUNDS IN BEER WITH EMPHASIS ON 3-METHYL-2-BUTENTHIOL FORMATION

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When exposed to sunlight, i.e. if bottled in green or white glass, beer quickly develops an off-odour usually referred to as "sunstruck-flavour". Since about 50 years, 3-methyl-2-butenthiol (MBT) is suggested as the main reason for the sunstruck-flavour, especially due to its very low odour threshold of 7 ng/L. Several studies have suggested that the thiol is formed from isohumulone in presence of light, riboflavin and cysteine. To prevent the formation of MBT hops are hydrogenated, but, due to the purity law the use of hydrogenated hops is not permitted in Germany. Thus, the dosage of hops has to be significantly reduced when filled into white bottle, which, however, leads to a lower nutriceutical value of beer. Because recently, additional odorants have been made responsible for light induced off-flavour in beer, the aim of the present study was (i) to compare the key odorants in hopped and unhopped beer by means of the molecular sensory science concept and (ii) to get deeper insights into the formation pathway of these aroma-active compounds by comprehensive model studies. The result of the application of an aroma extract dilution analysis revealed MBT (skunky odour), 3-(methylthio)propanal (cooked potatoe-like) and methane thiol (sulphury) as key contributors to the sunstruck-flavour on the basis of odour activity values and sensory experiments. Interestingly, also in unhopped, but illuminated beer 3-(methylthio)propanal and methane thiol were generated, also causing an off-flavour. Using single hop constituents, in depth studies were performed to clarify the formation pathway of MBT. The results will be discussed and reaction pathways will be presented.

Keywords: beer, lightstruck flavour, MBT, SIVA, AEDA

E-9
RELEASE OF CARVACROL AND THYMOL FROM POLYPROPYLENE ACTIVE FILMS FOR BREAD AND STRAWBERRIES PACKAGING BASED ON HS-SPME-GC-MS ANALYSIS

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Active packaging systems interact with foodstuff by positively modifying its sensorial, nutritional and microbiological properties (1). It has long been recognized that some essential oils have antimicrobial properties (2). Oregano essential oil is one of the most widely used and shows high antimicrobial activity (3), being carvacrol and thymol its major components (4). This work focuses on the release study of carvacrol and thymol from polypropylene (PP) antimicrobial films by HS-SPME-GC-MS analysis for food packaging. The polymer used in this study was PP ECOLEN HZ10K (Hellenic Petroleum, provided by Ashland Chemical Hispania). Carvacrol 98% and thymol 99.5% were used as active additives (Sigma-Aldrich). Two active formulations were prepared: PP containing 8 wt% of carvacrol and PP with 8wt% of thymol. PP without any active compound was also obtained as control. The different mixtures were processed by blending the additives with the polymer in a Haake mixer at 190°C for 6 min and a rotation speed of 50 rpm. 200 µm (average thickness) films were obtained by compression-molding at 190°C. The release of carvacrol and thymol was studied from bread stored at room temperature and refrigerated strawberries at 4°C during 15 days; and from strawberries during storage at room temperature for 10 days. The aromatic profile of food samples was also obtained (0 days). Foodstuffs were placed in suitable food containers, with a silicone septum, and the active film in contact with them. A DVB/CAR/PDMS fibre was used for HS-SPME analysis of the additives. The fibre was exposed for 30 min and immediately desorbed for 10 min at 270°C into the injection port of the GC-MS. An increase in the amount of carvacrol and thymol released from the PP films was observed with time for all analyzed food samples. For bread, carvacrol inhibits the release of 2,4-dimethylheptane and 4-methyloctane, while thymol inhibits the release of 2-pentanone. On the other hand, the release of thymol and carvacrol in strawberries samples avoid the presence of 2-methylbutanol and 3-ethylhexane, producing a decrease in the formation of 2,3,4-trimethylhexane. In conclusion, the release of thymol and carvacrol from the active PP films has shown to be effective in maintaining the quality of strawberries and bread during different storage conditions.

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Keywords: polypropylene, carvacrol, thymol, active packaging, HS-SPME-GC-MS

E-10
VOLATILE COMPOSITION OF FONTINA PDO CHEESE RIPENED IN DIFFERENT CAVES

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Fontina is an Italian semi-hard and semi-cooked PDO cheese. It is tipically produced in Aosta Valley, it is made from raw cow's milk and it is ripened for at least 3 months in natural caves. During this period, the cheese develops a unique flavour. The complex reactions triggered during the cheese-making, and principally due to enzymes from milk, rennet and microorganisms play an important role on the development of unique flavours in cheeses. Furthermore, the aromatic profile represents an important tool for quality evaluation purpose. The objective of this work was to study the aroma profile as for Fontina PDO cheese, ripened in different caves, by means of thermal desorption / gas chromatografy / mass spectrometry. Fontina cheese samples (12) came from 6 different cheese wheels, aged 56 and 84 days, produced on the same factory, on the same day, starting from the same batch of milk to reduce the variability which can be usually found in Fontina cheese. Then, cheese wheels were ripened for 3 month in 3 different caves. Cheese samples were stored at – 80°C until analysis. Fontina samples were submitted in triple to a dinamic headspace extraction and the volatile components were concentrated on a Tenax TA® trap. Afterwards, volatiles were automatically thermally desorbed, separated and detected by a TD-GC/MS system. Determination of pH, water activity, humidity, sodium chloride, total protein content, proteolysis index, fat, free fatty acids (C2–C18:1) and peptide characterization were also carried out to obtain a more complete description of the properties of this PDO Italian cheese. In the volatile composition of Fontina PDO cheese, 46 components were identified and classified as alcohols, esters, ketones, aldeydes, terpenes, organic acids and sulphur compounds. From a quantitative point of view, differences were found in the 3 caves for most of the volatile compounds which are present in the aromatic profile: 2-Butanol, 3-methyl-1-butanol, hexanal and 2-butanone were the most abundant compounds which were found particularly in one of the 3 caves. Volatile flavour compounds, in the layer immediately under the surface, were less abundant than the same found in the centre of the product in each 3 caves, in agreement with literature data. This work provides a characterization of the volatile composition of Fontina PDO cheese in different maturing caves and shows a probable influence of the process conditions during ripening on the aroma compounds concentration. Discussion of the other analysis results will allows us to complete the studies of this effect.

Keywords: Fontina cheese, volatile compounds, dinamic headspace tecnique, GC-MS

E-11
CLASSIFICATION OF TURKISH EXTRA VIRGIN OLIVE OILS BASED ON THEIR VOLATILE PROFILES USING SPME-GC-MS IN COMBINATION WITH CHEMOMETRICS DURING STORAGE

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The fragrant and unique aroma of extra virgin olive oils (EVOOs) form the basis of the organoleptic quality and stability of oils. The unsaponifiable fraction of EVOOs includes minor constituents that vary with vegetal species, climatic conditions, extraction, refining procedures, and storage conditions. Lipid oxidation is the main cause of EVOO quality deterioration and its reaction rate determines the shelf-life of oils. Volatile compounds are mainly responsible of the pleasant flavor and change in off-flavors during storage. Therefore, it is important to monitor changes in the volatile profile during storage. Solid phase-micro extraction (SPME) is a simple and fast, solvent-free sample preparation technique for the extraction of volatile compounds. In this study, SPME with gas chromatography-mass spectrometry (GC-MS) method has been applied to qualiatively monitor changes in the volatile profile of EVOOs samples produced from two different olive varieties, Edremit and Memecik, which were harvested from north and south Aegean regions of Turkey, respectively, during 18 months of storage. Partial least squares- discriminant analysis was performed to classify EVOO samples according to the olive varieties and storage times. The results showed that the EVOO samples obtained from south region were successfully discriminated from the samples obtained from north region based on their volatile profiles. This indicated that EVOOs produced from Edremit and Memecik olive varieties had different volatile profiles. Additionally, olive oils stored for 0 and 6 months were classified together and separated from the EVOO samples with 12 and 18 months of storage.

Keywords: Extra virgin olive oil, SPME, GC-MS, Chemometrics

E-12
NOVEL APPLICATION FOR THERMAL DESORPTION GC-MS SYSTEM ANALYSIS OF AGING COMPOUNDS IN BEER

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The profile of smells and odours in beer arise of interrelations between different chemical compounds such as alcohol, ester groups, carbonyl groups, organic acids, phenolic agents and sulphuric compounds [1]. A matter of particular interest is the concentration of volatile carbonyl compounds in beer like 2–furaldehyde and (E)-2-nonenal. These aging compounds have a wide affect on the beer quality. Already low quantities might be detected sensory which reduces the value of the product [2]. Therefore it is necessary for quality control to choose a method for analysis, whose limit of detection is well below the threshold of odours and smells. Although Headspace GC–MS and SAFE (solvent assisted flavour evaporation) – method had been used for this type of analysis for many years [3], the technique lacks the sensitivity of Thermal Desorption GC-MS (TD GC–MS). By TD GC–MS the volatile flavour compounds are adsorbed directly onto Tenax tubes by using the stripping method. Afterward the volatile flavour compounds are desorbed from the Tenax tubes, concentrated on a cryotrap, before being flash desorbed into a GC–MS system. In comparison to the SAFE – method the loss of very volatile compounds will be prevented, due to a less complex sample preparation process. Furthermore the new method establishes fingerprints and allows a good food quality control by identifying unknown or undesired byproducts.

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Keywords: aging compounds, GC-MS, beer, VOC

E-13
MULTIVARIATE MODELLING OF THE FRESHNESS OF COOKED HAM

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Cooked ham is among the most consumed meat products in Europe, made out of pork, turkey or chicken. The occurring flora of microorganisms in the fresh product is strongly dependent on the hygienic circumstances present in the producing company. The common spoilage process of fine precooked meat products is predominated by different homo- and heterofermentative Lactobactariaceae, which are able to suppress other pathogenic spoilage bacteria [1,2]. The deterioration of meat can be followed in many ways. A well-established way to declare the freshness of a product is the microbiological determination of total plate counts. Unfortunately there are no strict regulation values given, but only indicative ones. If the microbiological contamination reaches critical values the decision on the tradability of the product is handed over to human sensory. Human sensory is a very useful tool in quality assessment, but needs a considerable, continuously and well trained panel - requirements which often cannot be fulfilled by small enterprises. The aim of this work is to render the decision of tradability more objective by the implementation of various GC, HPLC- and NIR techniques, with their data being used together with NIR (Near Infrared Spectroscopy), sensory and microbiological results in multivariate modelling of the freshness of the product. During the growth of Lactobacteriacae they produce considerable amounts of aroma active compounds which will be first extracted in the headspace by SPME (solid phase microextraction) and analysed by gaschromatographical separation combined with different detection modes (MS, FID, FPD, olfactometry). The main volatile compounds are alcohols (ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutanol, pentanol and 3-methyl-1-butanol), aldehydes and ketones (3-methyl-1-butanal and 2-methyl-1-butanal, 2-butanone, 3-hydroxy-2-butanone and 2, 3-butandione. Acetic acid is also detected by HS-SPME, whereas other acids like the predominating L-(+)-lactic acid and other metabolites of the citric cycle can only be measured by HPLC. During a cooked ham's deterioration process of 10 days, data of all those techniques mentioned above will be collected and used in the establishment of a robust multivariate model, which also points out the parameters with the highest and low correlation to the freshness of the product. The Unscrambler®, software from CAMO (Norway) will be used for qualitative clustering and the elaboration of quantitative prediction models.

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Keywords: Meat spoilage, multivariate modelling, aroma compounds, GCO

E-14
IDENTIFICATION OF IMPORTANT VOLATILES IN CARROT VARIETIES USING METABOLIC PROFILING AND OLFACTORY DETECTION

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In food science, multivariate analysis of metabolites is an important tool, providing comprehensive information on chemicals effectively. Although, the loading data significantly determines target compounds able to use as biological markers, thresholds of determined compounds representing sensory quality need to be considered in the analysis of volatiles. We identified the important metabolites in roots of twelve carrot cultivars using metabolic profiling using gas chromatography-mass spectrometry (GC–MS) and the GC–olfactory detection system. Fifty-eight compounds were identified and quantified repeatedly by GC–MS analysis. Principal component analysis (PCA) of these compounds determined quantitative changes in among cultivars. Volatiles such as toluen, alpha-bisabolol, gamma-bisabolene, beta-myrcene detected in higher loading in the first factor. Beta-ocimene adjacent to beta-myrcene on the biosynthetic pathways of mono terpene, also revealed higher loading value. On the other hand, 2-nonenal(E), beta-myrcene, terpinolene, 2-sec-butyl-3-methoxypirazin detected intensively by the GC–O system. These compounds seemed to have the lowest threshold among carrot volatiles. These results suggested that beta-myrcene was the important volatile quantitatively and qualitatively explaining the varietal difference in carrot.

Keywords: Metabolic profiling, GC–O, carrot, volatiles,

E-15
DOEHLERT MATRIX OPTIMIZATION OF A HS-SPME-GC×GC-QMS METHOD DETERMINATION OF BOAR TAINT COMPOUNDS ON PORK FAT

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One of the main problems related to pork meat quality is the off-flavor known as boar taint: an offensive odor present especially on meat from non-castrated male pigs. The compounds responsible are skatole and androstenone. Since these compounds are lipophilic, they accumulate on adipose tissues. Their determination on pork fat is an important tool both for quality control; however, it is also a formidable analytical problem. Although the levels present are not low (μg g^{-1}), their affinity with the fatty matrix renders their isolation difficult. Most of the methods available are extremely time- and labor-demanding and unreliable. In this paper we describe a method combining Headspace Solid Phase Microextraction (HS-SPME) and Comprehensive Bidimensional Gas Chromatography with Fast Quadrupole Mass Spectrometric Detection (GC×GC-qMS) to detect and quantify these compounds on pig fat. The GC×GC-qMS system was based on a Shimadzu QP2010+ GC-MS fitted with a lab-made modulator; for the extraction, two SPME fibers were tested (60 μm PDMS/DVB and 50+30 μm DVB/Carboxen/PDMS). For the general procedure 150 mg of finely ground pork fat was transferred to a 5 mL septum-sealed glass vial containing 500 μL of CBase Mol L^{-1} aqueous KOH. The suspension was stirred for tSAP min at TEXT °C to hydrolyze the lipid fraction of the matrix and improve fiber / sample distribution coefficients. Following saponification, 500 μL of aqueous HCl was added to neutralize the excess base and a SPME fiber exposed to the headspace of the solution for tEXT min. After extraction the fiber was exposed to the injector of a GC×GC-qMS. Detection was performed on multi-SIM mode monitoring fragments with m/z = 130, 131, 77 and 104 up to 9.0 min run (skatole) and m/z = 272 e 239 afterwards for androstenone. The sample preparation variables - CBase, tSAP, TEXT and tEXT – were optimized by multivariate experiments according to a Doehlert matrix arrangement. Two series of tries were conducted: one using PDMS/DVB fiber, followed by! a set o f experiments using DVB/CAR/PDMS fiber but optimizing only TEXT and tEXT (since CBase and tSAP are related to the saponification of the matrix and their values should be the same regardless of the fiber); the maximum extraction efficiency achieved with both series of tests was compared to select the appropriated fiber. The conditions that provide the maximum extraction efficiency were CBase = 5.0 Mol L^{-1}, tSAP = 15 min, TEXT = 30 min and tEXT = 80°C, using DVB/CAR/PDMS fiber. Using these conditions, the resulting method proved to be capable to detect and quantify skatole and androstenone in concentrations compatible with those expected on pig fat samples, being the resulting procedure simpler, faster and more dependable than the traditional alternatives.

Keywords: Boar taint, pig fat, SPME, GC×GC-qMS, Multivariate Optimization

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E-16
REAL-TIME MONITORING OF VOLATILE ORGANIC COMPOUNDS FROM FOODS AND BEVERAGES BY HYBRID LINEAR ION TRAP – TRIPLE QUADRUPOLE MS SYSTEM

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Formation of unpleasant odour from processed foods and beverages may indicate the growth of bacteria and fungi, oxidation or other chemical transformations that make these goods unsafe or unpalatable to consume. It will be a great benefit to the food and beverage industry, if one can closely monitor the process on a real-time basis so that a corrective action can be taken in a timely fashion, rather than using a time - consuming sampling – extraction - sample clean-up - determination by GC or spectroscopic methods which may take several hours. We have designed new air sampling inlets for a hybrid linear ion trap–triple quadrupole mass spectrometer and applied to several foods and beverages. We designed and fabricated heat-traced Pyrex glass inlets, one for 10–1000 mL/min air flow and another covering 60–120 L/min. A coaxial glass nozzle was placed at the center of this tube so that a liquid compound of interest can be mixed at a pre-determined speed to generate a desired concentration. By adjusting infusion rate, one can prepare a calibration curve and obtain detection limits. The sample air is introduced to an APCI source of a hybrid linear ion trap–triple quadrupole mass spectrometer. Ions are introduced into mass spectrometer and spectra are obtained in trap modes with good S/N ratio. Quantification is done by multiple reaction monitoring or MS3 when many mass peaks are observed. Several foods and beverages such as milk, fruit juices, cheeses, French fries, cookies, chocolate bars, tortilla and potato chips are placed in a clean Pyrex container, and swept with clean “zero” air or nitrogen. The air sample carrying VOC emanating from these foods was continuously introduced into the APCI source of a hybrid linear ion trap – triple quadrupole (LIT-QQQ) MS system. Precursor ion spectra were obtained; then, information-dependent product ion scans were automatically triggered in both positive and negative ion modes. Product ion spectra of typical VOC such as ketones, low molecular weight fatty acids, aldehydes, ethers, epoxides, esters and amines were compared against our library. A dedicated principal component analysis procedure was applied to differentiate between safe and “rejected” food products. Results can be obtained within a few minutes so that a suitable correction can be taken in a reasonable time frame. Advantages and disadvantages of this approach will be discussed in comparison to traditional monitoring methods being used by the industry.

Keywords: Direct Ionization: Applications, Food Safety, Informatics: Profile Analysis; Informatics: Small Molecule Identification and Characterization

E-17
EFFECT OF STARTER CULTURES ON VOLATILE AROMATIC PROFILE IN GOATS' AND EWES' CHEESES - FOLLOWING THE CONSUMER'S TASTE

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Analysis of volatile compounds (VC) employing solid phase micro-extraction (SPME) in combination with gas chromatography – mass spectrometry (GC-MS) is recently one of the major methods for determining quality of dairy products. VC in cheese and consequently its aroma are affected by many factors including: animal diet, cheese ripening, technological characteristics, indigenous microbiota of raw milk, type of animal (ewe, cow, goat), rennet, environment, season, cheese making technology and finally also the type of starter culture (SC) used. Acceptance of cheese for the consumer depends on its sensorial properties. Consumers sometimes seek typical cow's cheese aromatic properties in cheeses originating from goats' or ewes' milk. As a consequence, cheese producers are forced to use SC in order to mask the “unpleasant” or “typical” goat or ewe odor. Our task was to investigate the effect of adding SC on aromatic profile in cheeses. Raw milk cheeses from 6 farms (2 goats’ and 4 ewes’) in western part of Slovenia were analyzed in their 2 months ripening period. On all farms, animal diet based strictly on hay. For goats' cheeses we compared 2 different SC such as Lyofast 0.31(L31) and Lyofast 0.46 (L46). For ewes' cheese we compared 3 different SC such as Lyofast 0.82 (L82), Lyofast 0.62 (L62), and a mixture of SC for Montassio cheese (M) (2/3) and L82 (1/3). We also compared ewes' cheeses with and without added SC. VC were extracted on 50/30 μm fiber made of carboxen / polydimethylsiloxane / divinylbenzene. The fiber was exposed to VC in closed vial for 24 h at controlled room temperature (25°C) and afterwards it was analysed by GC-MS. In ewes' cheeses made with mixture of SC: M (2/3) and L82 (1/3), the content of some ketones increased (2-nonanone, 2-pentanone, 2-octanone) in comparison with cheeses where L82 and L62 were added, respectively. Only in cheeses with M and L82, a large content of 2-heptanone (fruity, banana-like flavour) was found. Comparison of cheeses made with L62 and L82, revealed the increase of some acids (butanoic, hexanoic and isovaleric) in favor of L62. In goats' cheese, L31 had a major effect on some VC (especially on volatile acids), whereas the addition of L46 resulted in lower content of octanoic acid (goaty note of flavour). The effect of added SC on VC in cheeses is difficult to precisely determine due to the complexity of cheese system but certain differences in the content and presence of some VC due to use of different SC were observed. For more reliable results it would be better to sample cheeses on the same farm, using different SC to minimize the effect of endogenous microbiota. To confirm the effects of SC on VC and to confirm that SC could mask the “unpleasant” or “typical” odor of goats and ewes, more analyses and sampling of cheeses in more different batches must be done.

Keywords: goats', ewes' cheese, volatile compounds

E-18
ESTABLISHMENT OF GAS CHROMATOGRAPHICALLY UNIVERSAL PLATFORM METHOD FOR IDENTIFICATION OF AROMA-KEY COMPOUNDS IN HERBS/SPICES AS QUALITY INDEX

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Herbs and spices (H&S) are valued for their unique flavour and aromas, however they can be lost during production and/or storage. The study of key aroma compounds of H&S is still of interest. Due to their naturally complex matrix for volatiles content, to obtain an accurate identification of aroma-impact compounds which directly leads to a quality index is still troublesome. This study aims to establish a systematic experimental procedure based on gas chromatography (GC) to identify the aroma-impact compounds using ground fennel seeds as a model. The contrasting sample storage times of 6 months (0.5Y) and 5 years (5Y) are subjected to assessment of a comparative aroma quality index. Solid-phase microextraction (SPME) is used to extract the volatile compounds from the headspace of a sample vial which represents the undifferentiated compound suite which is perceived by nose, and is related to olfactory perception of the consumer. Aroma-impact compounds are identified by GC-olfactometry (GC-O) based on the detection frequency approach by 8 assessors. The GC-O system was operated in parallel with GC-FID. By correlation of the aromagram with the chromatogram from FID, it is possible to identify the specific compounds of interest in the GCĂ—GC coupled with time-of-flight mass spectrometry (GCĂ—GC-TOFMS) analysis. Note that correlation of peaks in the GC-O system still results in some uncertainty as to which identified component in GCĂ—GC-TOFMS is responsible for the perceived odour. Additionally, comparing the nasal impact frequency (NIF) from GC-O (i.e. increase or decrease of NIF) analyses can potentially reveal if such compounds affect aroma perception for fennel, leading to the ability to indicate the key aroma compounds as a quality index. To gain more accurate information from GCĂ—GC-TOFMS, chemometrics is used to treat the data with deconvolution in order to efficiently identify the peak of interest and their peak area quantification based on the mass spectrum. The major compounds res ponsible for aroma perception which show a decrease of 30–50%NIF from the 0.5Y to the 5Y, but still have aroma potency are limonene, linalool, terpinene-4-ol, estragole and trans-anethole. Some monoterpenes which may represent for the level of freshness are b-pinene, a-phellandrene, b-myrcene (pungent herbaceous, green smell) by exhibiting aroma-impact for the 0.5Y but completely no smell perceived from 5Y fennel. Meanwhile sesquiterpenes can be used as an aging index by mostly presenting in 5Y fennel, although such compounds do not show strong aroma character for fennel in this study. Interestingly, p-anisaldehyde remains the same aroma perception (sweet creamy, floral flavour) for both samples. This could be the main key compound for overall aroma perception of fennel.

Keywords: comprehensive-two dimensional gas chromatography, gas chromatography-olfactometry, aroma-impact, Solid-phase microextraction, Time-of-Flight mass spectrometry

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E-19
HPLC DETERMINATION OF STEVIOL GLYCOSIDES AND MOGROSIDE V IN SWEETENERS

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Terpene glycoside extracts from plants are gaining popularity as sweeteners and some, such have stevia extracts, have been successfully commercialized as off-the-shelf sugar substitutes. These compounds are low calorie, yet have sweetness greater than 300 times equivalent sucrose solutions making them attractive sugar replacements. Purified individual glycosides and whole fruit or leaf extracts are available on the market as sweeteners, herbal teas, and nutritional supplements. Many glycosides are present in stevia (Stevia reabaudiana (Bertoni)) extracts, with rebaudioside A of primary commercial interest. Another example terpene glycoside sweetener, mogroside V, is extracted from the fruit of the lo-han-guo (Siraitia grosvenorii). Sample analysis can be challenging due to the structural similarity of these compounds. In this work, an HPLC method using a tri-mode column for sweetener analysis is discussed. The method's volatile mobile phase makes it ideal for an aerosolizing detection technique, such as charged aerosol detection (CAD), following UV detection. The tri-mode column separates analytes by one, two, or three mechanisms, reversed-phase, anion-exchange, and cation exchange, depending on the analyte and the mobile phase conditions. This column rapidly separates the analytes of interest with clear differentiation between steviol glycosides and mogroside V. Furthermore, the dual detection method provides greater flexibility in determining the relative glycoside proportions. Two commercial stevia-based sweeteners and a lo-han-guo beverage are analyzed in this work using calibration ranges between 7-280 µg/mL. Retention time and peak area precisions (RSDs) were < 0.1 and < 2.0, respectively, with consistent retention times over several days of analysis. Analyte recoveries from samples ranged from 80–114% by CAD and 78–108% by UV detection, suggesting method accuracy. Additionally, the amount of rebaudioside A found in a commercial sweetener, an average of 4.2 mg/g of sweetener by CAD and 4.1 mg/g of sweetener by UV detection, correlates well with literature target concentrations in tabletop sweeteners. Advantages of employing two detection techniques are illustrated using commercially available samples and these analyses reveal that CAD detection is two- to four-fold more sensitive depending on the analyte.

Keywords: HPLC, CAD, Stevia, Mogroside V

E-20
CHARACTERIZATION OF THE KEY AROMA COMPOUNDS IN RAPE HONEY BY MEANS OF MOLECULAR SENSORY SCIENCE

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Honey has been used as food since thousands of years, and is still today appreciated for its unique aroma by consumers. It is well-known that considerable differences do exist in the overall aromas of different types of honey. However, although honey volatiles have been analyzed in several scientific studies employing SPME-GC-MS and SPME-GC×GC-TOFMS, studies aimed at identifying the aroma-active compounds in honey and their overall importance to honey aroma are rare. Therefore, the aim of this study was to characterize the key aroma compounds of rape honey by application of molecular sensory science. In collaboration with a German bee keeper, rape honey from Bavaria was collected. After characterization of the key aroma compounds by application of an aroma extract dilution analysis (AEDA), followed by identification experiments, the compounds were quantified using stable isotope dilution assays (SIDA). Because honey mainly consists of carbohydrates, the odour thresholds of the key aroma compounds were determined in a fructose-glucose-solution in order to calculate their odour activity values (ratio of concentration to odour threshold). Among the 38 odour-active compounds identified in rape honey, (E)-β-damascenone smelling like cooked apple and the honey-like smelling phenylacetic acid showed the highest odour activity values. The contribution of the odorants to the overall aroma was finally confirmed by an aroma recombination experiment, which was performed in a fructose-glucose-mixture. The results will be discussed with emphasis on their origin.

Keywords: honey, aroma, AEDA, SIDA

E-21
CHARACTERIZATION OF AROMA-ACTIVE COMPOUNDS IN RAPESEED OILS

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The production of rapeseed oil has increased enormously over the past twenty years due to the availability of new rapeseed cultivars (Brassica napus) containing less erucic acid. Because of its high content of essential polyunsaturated fatty acids like linoleic and α-linolenic acid, the oil is appreciated by consumers. However, in particular, refined oils are available on the market. During refining, the rapeseed oil is heated up to 250 °C for 1 – 2 hours (deodorisation) resulting in a huge loss of volatile aroma-active compounds. Recently, more oils labeled as cold-pressed and eliciting a pleasant and nutty aroma are sold. These oils are simply filtered after mechanical pressing without any heat applied. However, up to now, the aroma-active substances in cold-pressed rapeseed oil are yet unknown. Thus, the aim of this study was to elucidate the most aroma-active compounds in cold-pressed rapeseed oil by means of the molecular sensory science concept using an Aroma Extract Dilution Analysis (AEDA) to locate the odour-active volatiles, followed by identification and quantitation experiments. In total, more than thirty odorants were detected in the FD (flavour dilution) factor range of 8 – 4096. The highest FD factors were found for 2-isopropyl-3-methoxypyrazine (green pepper-like), 1-octen-3-one (mushroom-like) and (E,Z)-2,6-nonadienal (cucumber-like). Quantitation of selected aroma compounds by means of Stable Isotope Dilution Assays (SIDA) revealed the methoxypyrazine at a level <10 µg/kg while the highest amount was determined for hexanal (1918 µg/kg). In this study, more than 40 aroma compounds were identified and partly quantified in cold-pressed rapeseed oil for the first time. By contrast, nearly no aroma-active compounds could be found in the volatile fraction of refined rapeseed oil. The results of this work contributed to a collaborative study, in which the effect of aroma compounds on postprandial satiety regulation was investigated, since odorant receptors were recently characterized in the human gastrointestinal tract.

Keywords: rapeseed oil, aroma, AEDA, SIDA, volatiles

E-22
EFFECT OF TEXTURE AND AGING ON THE AVAILABILITY OF IMPORTANT WHEAT BREAD AROMA COMPOUNDS DURING CONSUMPTION (PTR-MS)

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The aroma impression is one of the most important criterion for consumer acceptance of white bread. With respect to orthonasal perception, previous studies have shown that among the over 300 bread volatiles only very few are actually relevant for the perception of the typical aroma of white bread. For example, 2-acetyl-1-pyrroline was identified as key aroma compound in white bread crust, while the typical crumb aroma was mainly caused by 2-phenylethanol, 3-methyl-1-butanol, and fatty-smelling aroma compounds. But, analysing the overall flavour composition of wheat bread does not reflect the retronasal aroma perception, which is also important for the quality of wheat bread. Up to now, the availability and release of bread aroma compounds during the process of eating (retronasal perception), which depends on the texture and aging of bread crumb, are still not clear. Therefore, the aim of this study was to correlate the flavour release during bread consumption with texture characteristics of fresh and stored wheat bread using model breads and an artificial mouth simulating the process of chewing. To change the crumb texture, model breads were prepared with mono glyceride (1%) and ascorbic acid (0.05%). Addition of mono glyceride and ascorbic acid resulted in higher specific volume of breads compared to the specific volume of a control sample. In contrast, crumb firmness was decreased by each addition, but especially for mono glyceride (42% compared to control bread). The flavour release of five selected aroma-active compounds (2-phenylethanol, 3-methy-1-butanol, hexanal, 2-methypropanol, and 3-methybutanal), measured in model breads using Proton Transfer Reaction - Mass Spectrometry (PTR-MS), showed high signal intensities for the control sample bread, which presented a low specific volume and hard crumb. Model bread prepared with mono glyceride and ascorbic acid showed significantly less flavour release. However, breads with each addition presented a high specific volume and soft crumb. Two days stored model breads showed a high flavour release than the fresh breads. In conclusion, the results proved a correlation between crumb texture and flavour release, in which bread with hard crumb showed a higher flavour release.

Keywords: wheat bread, texture properties, flavour release (retronasal), model mouth, Proton Transfer Reaction- Mass Spectrometry (PTR-MS)

E-23
HS–SPME/GC–MS ANALYSIS OF VOC AND MULTIVARIATE TECHNIQUES APPLIED TO THE DISCRIMINATION OF BRAZILIAN VARIETIES OF MANGO

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The high worldwide consumption of mangos is attributed to their pleasant sensory properties, such as aroma, which is a decisive attribute for the good acceptance of any fruit. The present study determined and compared the VOC profiles of three mango varieties, namely Tommy Atkins, Rosa and Espada, using HS-SPME extraction coupled to CG-MS analysis. The fruits were purchased at local markets and once in the laboratory they were washed, peeled and their pulps homogenized in a mixer/blender. After this, sample masses between 0.2 and 1.6 g were put inside 20 mL headspace vials, with 20 mL of deionized water. The vials were finally sealed with Teflon caps and aluminum seals. In order to optimize the extraction and desorption conditions of the VOC, multivariate methodologies by factorial design and response surface methodology were applied. The VOC were extracted with a 75µm Carboxen/polydimethylsiloxane fiber (Supelco), according to the following optimized conditions: extraction time- 4 min; extraction temperature- 40°C; desorption time- 5 min; desorption temperature- 250°C. The analyses were done in a Shimadzu QP-2010 quadrupole GC-MS system and a DB5 capillary column (30m × 0,25mm i.d. × 0,25µm, J&W Scientific) according to the following GC and MS conditions: oven temperature program- 50°C (0 min); 50–100°C at 2°C/min; 100–160°C at 6°C /min and 160–280°C at 15°C/min; injection mode- splitless; ion source temperature- 230°C; Transfer line temperature- 280°C. The extracted VOC were tentatively identified by comparison of their spectra with those contained in the NIST Electronic Library. The data were evaluated using pattern recognition methods, such as principal components analysis (PCA) and hierarchical clustering analysis, in order to visualize grouping tendencies of the volatile compounds. The optimized extraction method was applied to the samples and amongst thirty-seven volatile organic compounds tentatively identified in the three varieties of mango, the most prominent twenty-three, belonging to different chemical classes, such as esters, terpenes and hydrocarbons, were used in the classification step. The multivariate analysis became then possible to visualize the grouping tendencies of the mango samples, according to the presence of their respective volatile substances, and enabled the identification of groups of substances responsible for the discrimination among the three varieties studied.

Keywords: mango fruits, VOC, HS-SPME-GC-MS, multivariate techniques, discrimination analysis

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E-24
EVALUATION AND APPLICATION OF SOLID-PHASE MICROEXTRACTION METHOD FOR ANALYSIS OF ESSENTIAL OILS IN HERBAL TEA INFUSIONS

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The essential oils are complex mixtures of organic compounds occurred in all parts of plants (leaves, flowers, fruits, stems and roots). The main group of compounds belonging to the essential oils are terpenes. These compounds usually don't have the significant influence on the aroma and taste just because these properties are provided mainly by oxygen compounds (e.g. alcohols, aldehydes, ketones, esters, etc.)[1,2]. It is well known the positive influence of various essential oils on the human health. People used to use many kinds of herbs and spices for the treatment of different diseases since antique (e.g. Mentha piperita for digestion, Lavandula angustifolia has an anti-inflammatory and analgetic effect, Melissa officinalis against cough...)[3]. One of the most important nutritional sources of the essential oils components are herbal and fruit beverages including various kinds of tea. For isolation of these compounds from herbal infusion samples the microextraction methods, especially solid-phase microextraction (SPME) and/or single-drop microextraction (SDME) seem to by good tool. With respect to the aromatic properties (related to their volatility) of compounds belonging to the essential oils, it is possible to analyse all obtained extracts by method of gas chromatography equipped with various kind of detectors such as flame ionisation (FID) or mass spectrometry (MS) detectors. The SPME technique is based on the equilibrium establishment of target compound between sample and fibre (direct immersion, DI-SPME) or among the sample, headspace (HS) and extraction phase in the case of HS-SPME. The obtained extract containing entrapped analytes could be easily desorbed inside the injection port of the GC system without any additional equipment.[4,5] The main aims of this study were the evaluation and the application of the method based on the SPME extraction followed by GC/MS for analysis of essential oils in 10 real herbal infusions. In this case 10 compounds were used for the method validation and these compounds were determined. Other compounds were identified using mass spectrometry detection, comparing the MS spectra with libraries, and finally, relative retention indices were evaluated as well. Moreover, sensoric evaluation of selected samples was performed in order to find out some relation between content of target compounds and suitable sensoric properties of analysed beverages.

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Keywords: Solid-phase microextraction, essential oils, herbal tea

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E-25
DIRECT ANALYSIS OF FOOD AND BEVERAGES USING SPME–GC–MS/MS – NO CLEANUP, AUTOMATED AND HIGHLY SPECIFIC

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In the year 2008 the European Parliament and the Council of the European Union released the European Regulation 1334/2008 (1), which lays down rules on flavorings and food ingredients with flavoring properties for use in and on foods. Among other rules it stipulates the flavoring substances, which have the restrictions and regulatory limits for food. There have been developed a lot of methods for determination the individual flavorings in different matrices, however no one was suitable at use for regulatory purposes. The aim of this work was preparing the method, which will be able these needs complete. The presented method was developed using automated headspace solid-phase micro-extraction (HS/SPME) coupled with GC-MS/MS to simultaneously determine the presence of seven biologically active flavoring substances whose levels of use in processed foods is controlled by statutory limits. The method can be applied to identify and quantify the presence of 1,2-benzopyrone (coumarin), β-asarone, 1-allyl-4-methoxybenzene (estragole), menthofuran, 4-allyl-1,2-dimethoxybenzene (methyl eugenol), pulegone and thujone at levels ranging from 0.5 to 3000 mg/kg. The method has been optimized and validated for three different generic food types categorized on the basis of composition and anticipated use levels of flavorings and food ingredients. The food categories are: (1) Alcoholic & non-alcoholic beverages; (2) Semi-solid processed foods (e.g. soups, sauces, confectionary etc.) and (3) Solid foods (muesli, bakery products etc.). The method is simple, inexpensive, rapid, and eliminates the use of flammable and toxic solvents. There is no sample preparation and, using MS/MS, unequivocal confirmation of identification is achieved even in highly complex matrices containing many potential interfering volatiles. The method precision for spiked samples ranged from 2 to 21% with the greater variability associated with solid matrices. The LODs and LOQs were well below 0.1 and 0.5 mg/kg respectively, in all cases for individual substances fulfilling requirements for enforcement purposes. The robustness of the method was demonstrated in a small survey of retail samples of spirits (4), flavored milks (5), energy drinks (3), liqueurs (5), soups (5), sauces (10), herbal teas (5) and breakfast cereals (3). During optimization a developing method were investigated various features of food and beverages, which can have a significant influence on the effectual extraction of target compounds on the SPME fiber. Foremost the different content of ethanol and sugar in alcoholic and non-alcoholic beverages was suspected.

Keywords: SPME, GC-MS/MS, biologically active flavorings, food analysis

E-26
ANALYSIS OF KEY ODORANTS IN GREEN TEA INFUSIONS: COMPARISON OF STEAMED AND PAN-FIRED TEA

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Green tea is one of the most consumed beverages in China and Japan but its popularity has not dethroned black tea much beyond these regions. Behind a common green tea name, a variety of cultivation, harvesting and manufacturing practices are in use and result in brewed green teas with very distinct sensorial characteristics. In a simplified view, typical green tea process involves an enzyme deactivation step on fresh leaves before cutting and drying the leaves. Such deactivation step is traditionally performed via steaming the leaf in Japan and pan-firing (dry heat treatment) in China. To date, about 400 volatile compounds have been reported in green tea composition (1). Lists of odour-active compounds identified in different green teas based on GC-O dilution methodologies have been published (1-5) but the relation between these odour-active compounds and main processes practices has not been well established. To comprehend better compositional differences between steamed and pan-fired green teas a list of 18 odour-active compounds relevant for most green teas was established and was used to compare the aroma composition in 6 Chinese (pan-fired deactivation) and 6 Japanese (steamed deactivation) green teas. A headspace SPME-GC×GC-TOF MS method was used for the comparison of peak intensities, as the improved sensitivity and separation power compared with traditional GC-MS was needed for many key aroma compounds.

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Keywords: odour-active compounds, green tea, GCxGC-TOF MS

FOOD CONTAMINANTS (ENVIRONMENTAL)

(F-1 – F-55)

F-1
DETERMINATION OF PAHS IN HONEY

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This study is focused on monitoring of a contamination level with PAHs in a honey and in a bee glue. Tested kinds of honey were a blossom honey and a honeydew honey. These kinds of honey come from a home-made production and also from a market network in the Czech Republic. Monitored analytes were isolated with a solid – liquid extraction at first, then the extract was cleaned up using a Silica gel column chromatography. Samples were analyzed on a gas chromatography with a mass spectrometer (GC/MS) finally. It was found out that all samples of honey (20) were contaminated with naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene and benzo[k]fluoranthene. In nine samples anthracene was identified, in four samples benzo[b]fluoranthene was identified, in three samples acenaphthylene was identified, in two samples indeno[1,2,3-c,d]pyrene and benzo[a,h]anthracene were identified, and in one sample benzo[a]pyrene and benzo[g,h,i]perylene were identified. Positive samples of honey ranged from hundredths up to units of $\mu g.kg^{-1}$. The bee glue was investigated only in one bee colony and its positive samples ranged from tens up to hundreds of $\mu g.kg^{-1}$. Obtained results show the insignificant honey contamination with PAHs and therefore it is necessary to monitor these analytes during an evaluation of the honey quality.

Keywords: blossom honey, honeydew honey, PAHs, GC/MS

Acknowledgement: This work was supported by MŠMT ČR, grant No. 6215712402.

F-2
NEW SIMPLE AND FAST GC-MS/MS METHOD FOR THE SIMULTANEOUS ANALYSIS OF VARIOUS GROUPS OF ORGANOHALOGEN POLLUTANTS AND PAHS

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Since the number of environmental contaminants which undergo the legislative control or are included in the monitoring programs of the European Food Safety Authority and other international bodies as the U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration, still increases the demand for high throughput, selective, sensitive and non–expensive analytical methods arises as well. In our previous experiments, within the European project CON*ff*IDENCE, a sample preparation method for the simultaneous determination of polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and polycyclic aromatic hydrocarbons (PAHs) in fish muscle tissue based on ethyl acetate extraction followed by silica minicolumn clean–up was developed and validated. Comprehensive two dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC–TOFMS) in electron ionization mode (EI) was used for efficient separation and detection of all desired compounds as well as for potential non–target screening. Within this part of study the previously developed sample preparation method was transfer from the GC×GC–TOFMS which data handling is rather complicated and time consuming to the GC coupled to tandem mass spectrometer with triple quadrupole ion analyzer (GC–MS/MS) in EI that is current standard, widely used in commercial laboratories, and that allows reaching low limits of quantification (LOQs) which are necessary for reliable data assessment conducted within exposition studies. The limits of quantification (LOQs) obtained using this instrumental technique were as follows: PCBs 0.01–0.025 µg/kg, BFRs 0.025–0.1 µg/kg and PAHs 0.01–0.05 µg/kg, which means decrease of LOQs especially in case of higher polybrominated diphenyl ethers (PBDEs). Moreover, further improvement might by possibly achieve by large volume programmable temperature vaporization (LV–PTV) injection technique. With regards to similarities in physico–chemical properties (good thermal stability, hydrophobicity, etc.) of our target analytes and other persistent organic pollutants (POPs) the original list of compounds defined by the CONffIDENCE project was in the following experiments enhanced by other emerging BFRs (e.g, decabromodiphenyle ethane, hexabromobenzene, bis(2,4,6–tribromphenoxy)ethane, etc.), organochlorine pesticides and other congeners of polybrominated diphenyl ethers (PBDEs), PAHs and their methylated analogues, which were not initially included. All target analytes except for four OCPs (dieldrin, endrin, β–endosulfan and endosulfan sufate were irreversibly fasten on the silica minicolumn during the clean–up step) fulfill the key performance characteristics (recovery in the range of 70–120%, repeatability less than 20%) defined in the European SANCO document No. 10684/2009 (originally designed for pesticides residues analysis but commonly applied also for other organic food contaminants) even at the lowest spiking level (1 µg/kg).

Keywords: GC–MS/MS, PCB, PAH, OCP, BFR, fish

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F-3
EPA METHOD 1699: HIGH SELECTIVE MULTIRESIDUE HRGC/HRMS PESTICIDE ANALYSIS APPLIED TO FOOD SAMPLES

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Introduction Among recent official methods introduced by the U.S. Environmental Protection Agency (U.S. EPA) EPA Method 1699 can be found [1]. This method is used for the determination of organochlorine, organophosphorus, triazine and pyrethroid pesticides in environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) using isotope dilution and internal standard quantitation techniques. This EPA method is generally applied to aqueous, solid, tissue and biosolids matrices. Objectives The aim of our study was to extend the scope of applicable matrices for this method to include food samples. Furthermore the compatibility of this method with QuEChERS extracts has been investigated. Material and methods Tea and Rucola salad samples were prepared via the QuEChERS method. Extracts were analyzed according to EPA method 1699 on a Thermo Scientific DFS (high resolution sector field mass spectrometer) coupled to two Thermo Scientific Trace GC Ultra supported by an extra-wide Thermo Scientific Triplus autosampler. Standards according to the EPA method were self prepared and a high resolution MS multi window selected ion monitoring (SIM) method was set up including the usage of suitable reference masses (FC43). A mass spectrometer resolution of 10,000 (10 % valley) was employed for ultimate selectivity. Extracts and standards were injected in splitless mode via a temperature programmable PTV injector on a 30 m DB17ms column (0.25mm ID, 0.25 um film thickness). Results The instrument sensitivity using this EPA method was proven with standard measurements and resulted in the low pg/ul (ppb) range. For a number of components even sub ppb sensitivity can be achieved, which includes pestizides with strong tendency to fragment like Dieldrin, Aldrin, Endosulfans, Endosulfansulfate. The measurements of QuEChERS extracts from different food samples showed very good selectivity for most of the targeted pesticides with sensitivity well in the range requested by food regulations. Conclusions It could be demonstrated that EPA method 1699 is applicable to food samples using QuEChERS extraction methods. Furthermore high resolution MS proved to be an alternative to triple quadropole MS technology being equally or at least partly superior in performance.

Keywords: pesticides, HRGC/HRMS, QuEChERS

F-4
THE ESTIMATION DAILY INTAKE OF PCDD, PCDF AND DL-PCB VIA HUMAN MILK

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The level of PCDD, PCDF and PCB in human milk reflects the body burden and can be used as biomarkers of exposure. The aim of this study was to Estimate Daily Intake (EDI) of PCDD, PCDF and dl-PCB via human milk. We followed WHO selection criteria: mothers should be primiparae, healthy, exclusively breastfeeding one child, and living in the area for at least five years. Milk samples (min. 50 ml) were collected between 2 and 8 week after delivery. Samples were extracted manually with n-hexane, diethyl ether and methanol followed by automatic clean-up carried out on multicolumn PowerPrep4 automated system (FMS, Inc, USA). Samples were fortified with corresponding 13C12-labelled congeners. The measurement was carried out on HRGC/HRMS system – Autospec Ultima NT (Waters) and Agilent Techn. 6890N GC and J&W Scientific DB-5MS GC column (60m × 0.25 mm I.D. and 0.25 µm film thickness). All standards and solvents were of analytic grade of PCB/dioxin that are commercially available. Contents of fat in extracts was determined with gravimetric method. PCDD, PCDF and dioxin-like PCB are reported on the basis of toxic equivalents (TEQ) using the WHO toxic equivalency factors. Estimated Daily Intake (EDI) of PCDD/PCDF and EDI of dl-PCB via human milk were calculated on the basis of levels of In 46 samples the content of PCDD/Fs (17 congeners) and dl-PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) in breast milk and infant ingest data (140 mL/kg b.w./day) and body weight data from the study, assuming that human milk is the only food source for nursing infants (0–6 months old). As the levels of measured compounds in certain samples were below the LOD, <LOD was replaced by ½ LOD when calculating EDI. The average EDI of PCDD/PCDF via human milk was 158.44 pg TEQ/day for nursing infants, with the range of 5.15–343 pg TEQ/ day and the average of EDI of dl-PCB via human milk was 36.25 pg TEQ/day with the range of 0.546–124.11 pg TEQ/ day.
When assuming a 5 kg body weight of 2 months old infant, the mean EDI of PCDD/PCDF and dl-PCB was 31.69 and 7.25 pg TEQ/kg b.w./day, respectively.
The World Health Organization established 4 TEQ pg/kg bw/day as a recommended TDI for dioxins.
The level of PCDD/PCDF and dl-PCB in breast milk collected in urban area in central Poland are 6.027 pg TEQ/g fat, with range of 0.196–13.047 pg TEQ/g fat, and 1.438 pg TEQ/g fat, with the range of 0.022–4.923 pg TEQ/g fat, respectively. Those results are lower than average levels of POPs measured in most of European countries within fourth WHO programme.

Keywords: daily intake, PCDD, PCDF, dl-PCB, human milk

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F-5
THE QUECHERS EXTRACTION APPROACH AND COMPREHENSIVE TWO – DIMENSIONAL GAS CHROMATOGRAPHY OF HALOGENATED PERSISTENT ORGANIC POLLUTANTS IN COW MILK AND HUMAN BREAST MILK

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Persistent organic pollutants (POPs) are a group of chemicals that include halogenated pesticides, brominated diphenyl ethers (BDEs) and polychlorinated biphenyls (PCBs). Due to the lipophilic nature of these components they accumulate in the fatty tissue of animals and bioaccumulate up the food chain. According to the World Health Organization human breast milk is an ideal matrix to monitor the levels of POPs in not only the mother and infant, but also as a key indicator of the levels of these chemicals in the local environment. Current methodology for the analysis of halogenated pesticides, PCBs and BDEs can be expensive, solvent intense and time consuming. The QuEChERS extraction approach coupled to a silica cartridge SPE cleanup may be an attractive sample preparation alternative for biomonitoring efforts for halogenated POPs in milk. Comprehensive two-dimensional gas chromatography (GC×GC) with an electron capture detector (ECD) may also offer a more cost-effective alternative. Method development was done using whole cow milk and later compared to a NIST Standard Reference Material of Human Breast Milk.

Keywords: Milk, GC×GC, PCBs, BFRs, QuEChERS

F-6
BIVALVE MOLLUSCS AS BIOINDICATOR OF HEAVY METALS CONTAMINATION: CASE STUDY AT MANGROVE PARK LOCATED IN THE METROPOLITAN REGION OF RECIFE, PERNAMBUCO, BRAZIL

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The process of industrialization and sprawl of big cities have been of great concern regarding the use of natural resources. The metals introduced by human activities, often exceed the natural inputs, representing potential harms in terms of actual contamination. Living organisms, which are present in aquatic environments, can be used as bioindicators of the contamination degree from a given area. Bivalve molluscs are widely used in environmental monitoring programs, which present the essential features of a biological monitor, such as sedentary habits, reaction to changes in environmental contamination levels, and others. This study aimed to evaluate the concentrations of trace metals in bivalve molluscs (Anomalocardia brasiliana (Gmelin, 1791) and Mytella charruana (Orbigny, 1842), their seasonal variations and the risk to human health. Collections of snails were held in a natural bank, during both the rainy and the dry season, during the months of January and July 2007. The methodology used for the opening of the samples was adapted from AOAC, 2000. For the quantification of the metal an ICP-OES method was used. For purposes of interpreting the results, the values established by Brazilian legislation, EPA and the WHO were used. The results of Anomalocardia brasiliana for zinc ranged from 10.1 $mg.kg^{-1}$ during the dry period and 6.8 $mg.kg^{-1}$ during the rainy season; manganese ranged from 15.6 $mg.kg^{-1}$ and 3.0 $mg.kg^{-1}$ in the dry season, 1.0 $mg.kg^{-1}$ during the rainy season; Copper was not detectable in the dry season and 2.6 $mg.kg^{-1}$ in the rainy season; iron varied between 28.5 $mg.kg^{-1}$ in the dry season and 23.0 $mg.kg^{-1}$ the first period. For Mytella charruana, the content of zinc ranged from 10.4 $mg.kg^{-1}$ in the dry season to 7.7 $mg.kg^{-1}$ during the rainy season. For manganese, the results ranged from 5.2 $mg.kg^{-1}$ during the dry period and 3.3 $mg.kg^{-1}$ during the rainy season; copper ranged from 4.3 $mg.kg^{-1}$ in the dry season and 2.1 $mg.kg^{-1}$ in the rainy season. Iron concentrations ranged from 4.1 $mg.k\ g^{-1}$ in the dry season to 81.0 $mg.kg^{-1}$ during the rainy season. Zinc and copper concentrations in the molluscs studied were within the values allowed by Brazilian legislation and the results presented for iron and manganese concentrations were above the maximum recommended by the international literature, indicating that a long-term consumption of these shellfish can cause damage to health.

Keywords: Bivalve Molluscs, Bioindicators, Trace metals, Environmental Contamination

F-7
SIMULTANEOUS DETERMINATION OF PCB/PBDE IN MILK FAT BY GC-ITMS. EVALUATION OF THE UNCERTAINTY OF MEASUREMENT

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PCBs and PBDEs are man-made industrial chemicals. Due to concerns arising on its wide environmental spread and its impact on human health, levels of those compounds should be monitored both in food and the environment. PCB/PBDEs are regarded as micro-pollutants present in food at low pg level. The method of choice according to the standing European Union regulation 1883/2006 for the purpose of dioxin-like PCB confirmatory analysis is the high resolution mass spectrometry. However the latter regulation permits the use of other analytical techniques (including low resolution mass spectrometry) for the screening purpose. The aim of this study was to develop a screening method for the determination of 6 indicator, 12 dioxin-like polychlorinated biphenyls and 14 PBDE congeners in milk fat with purpose of high resolution gas chromatography and low resolution ion trap mass spectrometry. Statistical parameters of the method were evaluated on the basis of laboratory made spiked samples and milk powder certified reference material. Results uncertainty was also assessed. Recovery of the studied compounds from the spiked samples calculated against the recovery of the $^{13}C_{12}$ labeled internal standards was above 91% and 87% for PCB and PBDE respectively. While recovery of the $^{13}C_{12}$ labeled internal standards was above 60% in most cases. The only exception was the $^{13}C_{12}$ BDE 209 which showed the recovery values in 50–60% range. Limits of quantification obtained with the proposed method were in low pg g^{-1} level (0.07–10.72 pg g^{-1} fat for PCBs, 0.1–4 pg g^{-1} fat for PBDEs). Calculated extended (k=2) uncertainty of the analytical results in the studied concentration range varied from 7–15%.

Keywords: PCB, PBDE, milk fat, ion trap, mass spectrometry

F-8
ASSESSMENT OF SPATIAL AND TEMPORAL DISTRIBUTION OF PCB AND PBDE IN MILK FAT FROM POLAND

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PCBs and PBDEs are dangerous industrial chemicals covered by the Stockholm Convention of Persistent Organic Pollutants (POPs). Both PCBs and PBDEs shares the similar toxicological profile and have the same environmental fate. Cows milk and milk products have shown to be good indicator samples for the contamination of persistent organic pollutants in the food chain. The aim of this study was to assess the levels of 19 PCB (6 indicator + IUPAC 194 and 12 dioxin-like PCBs) congeners and 14 PBDE congeners in milk fat. Seasonal and geographical differences in the concentrations of studied chemical contaminants in milk fat were assessed. Results of this study showed that determined average concentration of the 6 indicator PCBs in the studied material was 1500 pg g^{-1} (fat). Dioxin-like PCBs concentration expressed as a lower-bound dioxin equivalent toxicity was 0.684 pg TEQ g^{-1} (fat). Average summary concentration of 14 investigated PBDE congeners was 105 pg g^{-1} (fat). Statistically significant concentration differences between summer and winter samples were found. Results of this study indicate also a strong geographical differentiation of milk fat contamination as a consequence of regional differences in environmental contamination. Strong seasonal differences found in PBDE profiles evidence transformation of PBDE within the environment. Average daily intake of 6 indicator PCB, dioxin-like PCB and PBDE congeners was calculated as 717 pg kg bw^{-1} day^{-1}, 0,329 pg TEQ kg bw^{-1} day^{-1} and 50 pg kg bw^{-1} day-1, respectively.

Keywords: PCB, PBDE, milk fat, food contaminants

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F-9
SEARCHING FOR THE HOLY GRAIL: SEPARATION OF ALL PRIORITY POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR KNOWN INTERFERENCES BY SERIAL COMBINATION OF DIFFERENT HPLC COLUMNS

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Polyaromatic hydrocarbon analyses are performed regularly throughout the world for both food safety and environmental testing. Many organizations test for PAH residues in food products but the analytes lists vary from organization and country. For example, in the United States, the EPA, FDA, AOAC and NOAA all test for PAHs but do not share the same target compound lists. Internationally, the EU and individual countries will test for different PAH compounds. Chromatographic methods are typically tailored to a specific target compounds and is not transferable when target compounds change. A further complication is the need to separate interfering compounds that are not considered target compounds but that do occur in samples, like triphenylene, perylene and benzo[e]pyrene. In this work, we developed chromatographic solutions that allow the separation of twenty-nine PAH compounds including interfering compounds by both GC and HPLC. Chromatographic resolution of PAH compounds from EPA and EU lists, including isobaric compounds and know interfering compounds was achieved on the Rxi-17Sil MS column. HPLC separation was accomplished by combining two HPLC columns in series; a PAH specific phase and an aromatic bond selective phase. Separate column selectivities were tested and then combine based on a predicted ratio of the column lengths that would results in no coelutions. Temperature was also determined to play a critical role in the elution order and resolution of the PAHs tested. Time programmed fluorescence detection was optimized to yield the best sensitivity possible, ppb range, with closely eluting compounds.

Keywords: Polyaromatic hydrocarbons, HPLC, GC, PAHs, interferences

F-10
MULTIANALYSIS OF CELLULAR BIOMARKERS IN VARIOUS TOX CHIP-FORMATS

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Environmental chemicals, ingredients in food and food additives, especially if encapsulated in nano-scale structures are known to potentially alter the immune response and function. Recent investigations revealed that inflammatory immune response contributes to the development of immune-mediated diseases and the progression of tumors and cancer. Currently, measurements of immunomodulatory effects caused by exposure to complex chemicals include detection of specific key components of adaptive and innate immunity (e.g. transcription factors, chemokines and cytokines) as well as analysis of cell viability and functional responses. Due to the heterogeneity of the immune system there are actually no suitable methods available which allow an integrated assessment of altered variable key factors leading to immune response as well as rapid analysis of many different samples in parallel. These facts are the main drivers for the rapid expansion of assay-development in this field. In order to meet the demand of rapid multiplexing, we have developed a high-throughput assay system for simultaneous measurement of multiple biomarkers on a single platform. To do so, various chip-formats were employed: microwell plates, tubes, and planar chips. Antibodies and antigen-conjugates which are specific for the targeted biomarkers were spotted at the bottom of the wells and tubes, and onto the chip surface. To measure low and high abundant biomarkers at the same time sandwich and inhibition immunoassay formats were combined. Detection was done via a biotin-streptavidin bridge and a fluorescent label (λ_{ex}=635 nm). The assay design is based on the alteration of specific cellular biomarker expression-levels which are basically involved in pro-inflammatory immune responses: IL-1ß, IL-6, and IL-12 are known as essential biomarkers of the innate immune response, while MIP-2 and MIP1-α act as macrophage inflammatory proteins. Pro-inflammatory cytokine MCP-1 plays a crucial role in the recruitment of monocytes; Granulocyte-macrophage colony-stimulating factor (GM-CSF), and the tumor necrosis factor TNF-α. On-chip immunoassays for this selected set of biomarkers were established in all described chip-formats using various standard cell culture media (GIBCO® RPMI Medium 1640, GIBCO® DMEM-Dulbecco`s Modified Eagle Medium, and X-VIVO™15 LONZA Medium) and validated to assess the precision and comparability of measurements. These high-throughput multiplex detection assays allow rapid, simultaneous analysis in a broad range of applications, especially related to food safety and quality control of raw materials.

Keywords: Immunotoxicity, cellular biomarkers, multiplex detection system, protein-chip formats

F-11
VOLATILE COMPOUND METABOLIC SIGNATURES IN POULTRY FAT FOR BACK-TRACING DIETARY EXPOSURE TO HEXABROMOCYCLODODECANE (HBCD)

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The study investigated the feasibility of using volatile compound metabolic signatures of adipose tissues in laying hens to detect previous dietary exposure to hexabromocyclododecane (HBCD). This flame retardant has been increasingly used in recent years as a substitute for other prohibited molecules (PCBs, PBDEs). As a persistent bio accumulative and toxic substance, HBCD is currently under consideration for listing under several international food safety legislations including Stockholm Convention, US EPA, EU REACH. A recent study evidenced that after a dietary exposure to this compound, the main HBCD isomer was rapidly and partly metabolized in some more persistent toxic compounds (1). In order to point out a previous dietary exposure to HBCD, it is then necessary to develop alternative method to the direct HBCD quantification. Based on a previous report showing the relevance of volatile compound metabolic signature in chicken liver for back-tracing a dietary exposure to rapidly metabolized xenobiotics (2), the present study investigates the relevance of this approach to evidence a previous HBCD contamination in laying hens. Two groups of laying hens were fed a similar feed either non contaminated (control group) or contaminated with HBCD. For this second group, layers hens were given a HBCD-contaminated diet for 21 days (contamination period) then a control diet for 18 days (depuration period). Abdominal fat of animals sequentially slaughtered throughout the experiment was collected and analysed by liquid Chromatography-tandem mass spectrometry (LC-MS/MS) for HBCD direct quantification and by solid phase micro-extraction – gas chromatography-mass spectrometry (SPME-GC-MS) for volatile compound metabolic signature. LC-MS/MS quantification evidences that the HBCD content of the adipose tissue increased during the contamination period and rapidly fell down to the basal level found in the control samples confirming the rapid HBCD metabolization. The volatile compound metabolic signature! s eviden ced that changes in laying hen metabolism occurred in response to HBCD dietary contamination. The metabolic signatures enabled to differentiate control and HBCD-contaminated animals, even at the end of the depuration period. The present finding might pave the way to a new generation of monitoring methods which are not based on the measurement of xenobiotic residues or their parent metabolites.

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Keywords: non targeted approach, environmental toxicants, poultry-derived food products, volatile compounds

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F-12
A RAPID AND SENSITIVE MULTIDIMENSIONAL LIQUID-GAS CHROMATOGRAPHY (LC–GC) METHOD FOR THE DETERMINATION OF HYDROCARBON CONTAMINATION IN FOODS

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The complete separation of complex mixtures through single column GC is often hindered by the fact that sample components belong to numerous chemical families and are present in a wide range of amounts. In many situations, it is much more convenient to isolate more simple and homogenous mixtures prior to GC separation. This may be easily attained by exploiting the high selectivity of an LC fractionation process. On-line LC–GC methods are particularly suited to the separation of compounds with similar physico-chemical properties, in samples characterized by a great number of chemical classes. In the present research, a simple, rapid and highly sensitive on-line LC–GC method, for the determination of hydrocarbon contamination in foods, is described. The instrumentation used is capable of either heart-cutting or comprehensive (LC×GC) analysis. The LC separation was carried out under normal-phase isocratic conditions, using a short conventional packed column; GC separations were carried out in a rapid manner using a micro-bore 0.1 mm ID column. High sensitivity was achieved by transferring high solvent volumes into a programmed temperature vaporizing injector. The developed LC-GC method requires a time measurable in minutes. A series of commercially-available products were subjected to LC-GC analysis with often high levels of contamination found.

Keywords: Multidimensional liquid-gas chromatography, LC-GC, Food analysis, Hydrocarbon contamination

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F-13
RAPID GC-MS METHOD FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SEAFOOD: AOAC COLLABORATIVE STUDY

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Within the EU project CONffIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure) an efficient, cheap, rapid and simple multiresidue analytical method for simultaneous determination of PAHs, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood samples was developed. The procedure based on rapid ethyl acetate extraction (partition supported by inorganic salts addition), followed by clean-up on silica SPE mini-column need for sample preparation (including extraction, clean-up and concentration prior to the gas chromatography-mass spectrometry (GC-MS)) about 30 min and multiple samples can be processed at the same time. Identification and quantitation is performed using GC-MS with time-of flight analyser. The overall method was validated for 32 PAHs including both EU and EPA PAHs and also their methylated analogues. This simple and fast procedure was submitted to AOAC INTERNATIONAL which launched the call for submitting rapid analytical methods suitable for quantification of polycyclic aromatic hydrocarbons (PAHs) in the raw edible portions of fish/seafood after the Mexico Gulf oil spill last year and was selected as the most promising candidate from the 30 reviewed submissions. To become an AOAC INTERNATIONAL Official Method for the determination of PAHs in seafood it was necessary to evaluate the method's intra-laboratory and inter-laboratory performance during the Collaborative study. The Collaborative study coordinated by Covance Laboratories (USA) consists of two parts: (i) Laboratory qualification and (ii) Analysis of test materials. In the laboratory qualification phase, the collaborators were conducted seven steps to check their GC-MS and solvent evaporation conditions, selection of appropriate silica SPE column (with low background), reagent blank contamination and familiarize themselves with the method.Within the study altogether 19 analytes (14 US EPA PAHs and 5 methylated homologues) were tested on three seafood matrices (mussels, shrimp, and oysters). Nowadays, the first part of the study is completed and the second part will be finished during autumn.

Keywords: PAHs, seafood, GC-MS, collaborative study

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F-14
DETECTION OF DEHP IN EDIBLE OILS AND ELUCIDATION OF SOURCES

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In past monitoring studies phthalic acid diesters (PAE) were identified in olive oils and olive oil based pesto samples [1, 2]. Reported concentrations were in the range of low ppb for short chain PAE whereas levels of DEHP, the plastiziser with the highest market share during the last decades, exhibited levels above 1000 ppb. Direct sources of these high levels of DEHP were not identified, yet. Our recent study focused on different food oil types distributed by a German bio food supplier. In a first project phase PAE levels were screened by a fast GC-MS analysis in diluted olive samples. Results were well comparable to levels obtained by GC-MS analysis after an extensive GPC clean-up. Besides olive oils, other alternative food oil types (walnut, sesame, peanut, corn, thistle, almond) were shown to contain elevated DEHP levels. Therefore, two production sites were investigated by analyzing oil and pre-products along the production line. In addition, several oil contact materials were sampled at these sides and analyzed for PAE. However, results did not show a significant increase in DEHP levels during production and thus, no DEHP sources were identified, neither for olive oil nor for the alternative edible oils. In a third project phase, input seeds and olives were analyzed for DEHP and detected DEHP levels in the original products were related to the oil yield typical for the respective seeds or olives. Calculated oil-related levels were well comparable to the measured levels in the respective food oil samples. These results indicate that DEHP levels in food oils in the low ppm range may results from DEHP concentrations present in the original seeds and olives and not from migration out of food contact materials. Basing on atmospheric DEHP depositions rates reported earlier [3] and olive oil yields related to square meter surface space of an olive tree we calculated a yearly DEHP flow into olive oil from typical European environmental levels. Results indicate, that these depositions flows may explain the majority of the detected DEHP amounts in the analyzed samples of edible oils.

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Keywords: DEHP, oil, olive, sources, deposition

F-15
ANALYSIS OF PCDD/FS AND DL PCBS IN DIFFERENT SPECIES OF FISH FROM LAKE GARDA-NORTHERN ITALY

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Fish products acquire contaminants and concentrate them in their tissues by uptake from water (bioconcentration) and through dietary routes (bioaccumulation). Aim of this work was to monitor the levels of some chemical pollutants (PCBs, PCDD/Fs) in Lake Garda Lake. It is the largest Italian lake: its surface is about 370 km2 with a maximum depth of about 346 m. Fishing activities and game fishing were particularly intensive along the lake so a monitoring programme was carried out to detect residues of PCDD/Fs and DL PCBs in different fish species caught from the lake. Sampling was undertaken at ten monitoring stations to cover all areas of the lake. From each monitoring station six different species were to be taken: eel (Anguilla anguilla), shad (Alosa fallax lacustris), whitefish (Coregonus lavaretus), pike (Esox lucius), perch (Perca fluviatilis), tench (Tinca tinca). The sampling was performed by a specialist Company which availed itself of the assistance of local fishermen: fishing was carried out with different mesh nets placed at different depths. Eel and shad were considered most at risk for the contamination, so it was decided to collect 4 samples for both species in each monitoring station while a single sample was expected for the other species. Unfortunately the fishing period and the morphological characteristics of the lake didn't allowed fishing for all species in all areas. Totally 112 fishes were analyzed, different by species, age and size (39 eels, 38 shad 11 whitefish, 10 pike, 9 perch and 5 tench). The analysis were performed according to EPA 1613/B 1994 method for PCDD/Fs and EPA 1668/B 2000 method for DL PCBs. Among the different species, eels had the higher concentrations (PCDD/Fs from 0.17 to 1.38 pg TEQ/g w.w. and sum of PCDD/Fs and DL PCBs from 5.43 to 56.25 pg TEQ/g w.w). Many eel samples (15) exceeded the EU limit (Reg. 2006/1881/CE). The eel samples had many differences of weight, age and lipid percentage but no correlation was found between these parameters and DL PCBs concentrations. One probable explanation for the wide divergence in the results was because the eels are in contact with a wide range of inhomogeneously polluted lake sections and its tributaries. This exposes them to different degrees of contamination, most of which they incorporate and accumulate with their food. The accumulation of PCDD/Fs and DL PCBs from eels are quite different from those in the other species. Due to the larger proportion of fatty tissues in eels, PCDD/F and DL PCBs accumulate more readily in this species than the other. The mean value of sum of PCDD/Fs-DL PCBs concentrations for shad was 3.66 pg TEQ/g w.w., while the mean values for whitefish, pike, perch and tench were less than 2 pg TEQ/g w.w. In all species the concentration levels for the DL-PCBs are considerably higher than those for the PCDD/Fs. Amongst the absolute concentrations of the DL PCBs the non-ortho PCBs predominate over the mono-ortho PCB.

Keywords: PCDD/Fs, DL PCBs, Fish,

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F-16
CONTENT OF MERCURY IN CANED FISH PRODUCTS AVAILABLE ON SERBIAN MARKET

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Fish contains high-quality protein and other essential nutrients, is low in saturated fat, and contain omega-3 fatty acids. However, increased fish consumption may simultaneously increase the contaminants intake to the levels of toxicological concern, especially mercury. Total mercury concentrations were measured in canned tuna and canned sardines originating from five countries imported to Serbia in 2010 and first half of 2011. These countries account for the largest part of total canned fish products import and these product types are of great importance in the diet of the Serbian population. Samples were prepared by microwave digestion (Milestone) with nitric acid and hydrogen peroxide. Analyses were carried out on atomic absorption spectrometer Varian SpectrAA 220. Cold vapour technique was applied, using tin chloride as reductant. Analytical quality control was achieved by using certified reference material BCR 186. Replicate analyses were in the range of certified values. Total of 252 samples of canned tuna from Thailand, Vietnam and Croatia were analysed. Mercury concentrations of Thai samples (112) were in the range of 0.005–0.402 $\mu g g^{-1}$ (mean 0.052 $\mu g g^{-1}$), Vietnamese samples were in the range of 0.015–0.338 $\mu g g^{-1}$ (mean 0.072 $\mu g g^{-1}$), while Croatian canned tuna (68 samples) contained mercury in the range of 0.014–0.502 $\mu g g^{-1}$ with the mean value of 0.155 $\mu g g^{-1}$. One-Way ANOVA test showed significant differences in mercury concentration of samples originating from Croatia and other two countries. In the case of canned sardines, total of 154 samples from four countries were analysed. Mercury concentrations of Thai samples (34) were in the range of 0.011–0.068 $\mu g g^{-1}$ (mean 0.026 $\mu g g^{-1}$), Philippine samples (35) were in the range of 0.016–0.040 $\mu g g^{-1}$ (mean 0.026 $\mu g g^{-1}$), samples from Morocco (37) were in the range of 0.005–0.049 $\mu g g^{-1}$ (mean 0.017 $\mu g g^{-1}$), while Croatian canned sardines (48 samples) contained mercury in the range of 0.007–0.209 $\mu g g^{-1}$ with the mean value of 0.087 $\mu g g^{-1}$. ANOVA test showed significant differences in mercury concentration of samples originating from Croatia and other three countries. Significant differences in mercury concentration were obtained between all combination of pairs excluding samples from Philippines and Thailand where differences were not significant. All samples contained mercury below the maximum level fixed by the Serbian national regulation (0.8 $\mu g g^{-1}$ for canned sardines; 1.5 $\mu g g^{-1}$ for canned tuna); fish products originating from Adriatic sea have highest mercury content.

Keywords: Mercury, Canned fish, Contaminants

F-17
STUDY OF BENZO(A)PYRENE PHOTOOXIDATION PROCESS IN NON POLAR LIQUID MEDIA IN THE PRESENCE OF FOOD ANTIOXIDANTS

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Polycyclic aromatic hydrocarbons (PAH) include the largest class of known environmental carcinogenic compounds. Some of them, even though not carcinogenic, may act as synergists. PAH are extensively found in various foods as a result of technological procedures such as grilling, drying, frying and mainly smoking. An environmentally relevant aspect of PAH toxicity is that it can increase by solar radiation. As known, PAH contain two or more conjugated benzene rings that facilitate the absorption of ultraviolet A (UVA) radiation (320–400 nm), ultraviolet B (UVB) radiation (290–320 nm), and in some instances, visible light (400–700 nm). However, this leads to photoactivation of PAH and increase their toxicity via the photosensitized production of singlet oxygen, and photomodification of original molecules, that results in formation of the products, so called oxy-PAHs. Many of the photoproducts generated through environmental photomodification exhibit greater toxicity than the parent PAHs and have the potential to generate toxic compounds that could impact living systems negatively on human health. On the other hand, photodegradation is an important transformation pathway for most PAH, because this process preferentially attacks the same tertiary carbon atoms that tend to block biodegradation. As already proven, PAH deposited on the surface of smoked food are partially oxidised due to presence of light and oxygen. However, the influence of light and antioxidants on PAH has not been studied so far. So, the aim of this work was to study behaviour of BaP as a reference compound of PAH in non polar liquid media at different wave lengths and in the presence of food antioxidants such as butylhydroxytoluene and guaiacol. Photodegradation of benzo[a]pyrene (BaP) was studied at two different light wavelengths, i.e. 254 nm and 365 nm in a non-polar medium (n-hexane) at concentrations 50, 100 and 150 $\mu g \cdot l^{-1}$. At chosen time intervals, BaP concentration was measured by HPLC using fluorescence detection. Comparing rate constants k and half-lives $\tau_{1/2}$ it was found that decomposition at 365 nm was 15.3 times faster in comparison with the decomposition at 254 nm. The decompositions obey the first order kinetics. Considerable effect had addition of food antioxidants such as 2,6-di-tert-butyl-4-methyphenol (BHT), o-methoxyphenol (guaiacol) when both accelerated BaP decomposition – BHT by 1.17 times and guaiacol even 1.45 times, it means that both antioxidants had prooxidant effects on BaP. These findings could bring new philosophy in attempts to decrease PAH content in foods, where their presence is due to applied production technology ordinary.

Keywords: benzo[a]pyrene, photolysis, kinetics, antioxidants, prooxidant effects

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F-18
APPLICATION OF QUECHERS METHOD FOR DETERMINATION OF PAHS AND CHLOROBENZENES IN SELECTED FOOD SAMPLES

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Aromatic organic compounds, including polycyclic aromatic hydrocarbons (PAHs) and chlorinated benzenes due to its persistence and toxicity to biota are the most dangerous contaminants in the environment. Therefore, it is particularly important to monitor their levels in food. Recent trends in food analysis focus on the development of multiresidue methods that allow determining contaminants belonging to different chemical groups. One of them is QuEChERS method, developed originally for the determination of pesticide residues. The effectiveness and popularity of this method made it to apply for other organic contaminants such as drugs and veterinary medicines. The aim of this study was to evaluate the application of QuEChERS method for determination of PAHs and chlorobenzenes in selected food samples of plant and animal origin. In the experiment the different types of extraction solvents and standard types of sorbents were used. The final extracts were analysed using gas chromatography-mass spectrometry. The usefulness of the method was verified basing on the recovery ratio of analysed compounds. In the samples of plant origin the recovery ratios for chlorobenzenes were very low (about 33%) or they were not achieved at all. The highest result was obtained for hexachlorobenzene – 89.6%. In the group of PAHs the best recovery ratio was noticed for biphenylene (101.4%) and phenanthrene (99%). For fluorene and anthracene the recovery ratio significantly exceeded the established limits (70–120%), which was presumably caused by the influence of the plant matrix. For four-ring PAHs satisfactory results were obtained only for pyrene (80.8%). For the rest of the compounds the recovery ratio did not exceeded 30%. In most cases the recovery ratio was better in the samples where ethyl acetate was used for the extraction. In the samples of animal origin the recovery ratio was much lower. For chlorobenzenes it was established at 19.1%, for hexachlorobenzene – 34%. In the group of PAHs the recovery ratios were ranged from 21.8% for biphenylene to 62.4% (chrysene). The highest value was obtained for anthracene (53.2%). Higher results were achieved in the samples extracted with ethyl acetate. Low values of the recovery ratio were probably influenced by the use of sorbents that might have removed some compounds, especially heavy PAHs, from the samples. The results show that the QuEChERS method can be successfully applied for the determination of selected aromatic compounds in plant matrices. For matrices of animal origin it was found the necessity of modification of the method, especially the selection of an appropriate sorbent for the purification of extracts.

Keywords: QuEChERS, PAHs, chlorobenzenes, GC-MS

F-19
DETERMINATION OF PB, CD, HG, AS AND CU IN ALMOND AND PRODUCTS OF ALMOND BY ICP-MS

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Almond is one of the most nutritious of all nuts. It is low in saturated fat and contain many other protective nutritiens – Ca, Mg, Cu, Mn, Zn, P and rich in the vitamins, especially vitamin E and compounds called phytochemicals, which may help in protection against cardiovascular disease and even cancer. Almond kernel is rich source of energy, protein and fiber. The edible kernel can be eaten raw and cooked, either blanched or unblanched. It can be also combinated with chocolate or as ice cream mixes and combinations with vegetable in many various shapes in bakery products.Tree nuts are considered as one of the most frequent causes of food allergy, but almond allergy seems rather unusual. In SP Laboratory, Bečej, during 2011, were analyzed more than 50 samples of raw almond from different geographic areas (Serbia, Italy, USA, Greece) on heavy metals content (Pb, Cd, Hg and As) and contents of Pb, Cd, Hg, As and Cu in more than 150 products of almond from the Serbian markets. The analysis was carried out in accordance with method BS EN 15763:2009. Samples were prepared by microwave digestion (Multiwave 3000 by Anton Paar) and analyzed by Inductively Coupled Plasma–Mass Spectrometry (Elan 9000/DRC-e by Perkin Elmer). Almost all of analyzed samples had concentration of metals (Pb, Cd, Hg, As and Cu) in accordance with Serbian legislation. Concentration of Pb was in interval between LOQ (0.001 mg/kg) to 0.02 mg/kg, Cd between LOQ (0.001 mg/kg) to 0.015 mg/kg, As between LOQ (0.001 mg/kg) to 0.065 mg/kg, Hg less than LOQ (0.001 mg/kg) and Cu between 6mg/kg to 9.5 mg/kg. Only in one sample (blanched almond) we have found Cu in concentration higher than in Serbian legislation (more than 10 mg/kg).

Keywords: food, almond, ICP-MS

F-20
LEVELS OF PFASS IN SELECTED FOOD COMMODITIES AND FOOD CAULDRONS COLLECTED IN VARIOUS REGIONS OF EU

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In this contribution, the study within the FP7 EU project PERFOOD (PERFluorinated Organics in Our Diet) focused on the distribution of perfluorinated alkyl substances (PFASs) in individual food stuff together with cauldrons is presented. PFASs have been analysed in samples of food cauldrons, whole meals, fast foods and selected foodstuff, which are commonly consumed in different European regions, represented by the Czech, Italian, Belgian and Norwegian markets. For these purposes, a new sample preparation method, including extraction based on the alternative QuEChERS procedure, for the determination of 25 representatives of PFASs group in matrices mentioned above was developed and validated. For final determination (separation and detection), ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) employing electrospray ionisation (ESI) was applied. 1 The target PFASs monitored in sample extracts were: 13 perfluorocarboxylic acids (PFCAs), 4 perfluorosulfonic acids (PFSAs), 3 perfluorophosphonic acids (PFPAs), 3 perfluorooctanesulfonamides (FOSAs) and 2 perfluorooctanesulfonamidoethanols (FOSEs). Limits of quantification (LOQs) 3–60 ng/kg for PFCAs, 3–13 ng/kg for PFSAs and FOSAs, and 6–60 ng/kg for PFPAs and FOSEs were achieved. Only 11 from the target PFASs were detected in any of various food samples. Among them PFOS was the most frequently detected analyte that was presented in approx. 50% of samples (in range of 1–711 ng/kg). Other detected analytes, PFCAs with longer carbon chain C8-C14, were presented in approx. 20% of samples. The concentration ranges of individual compounds in the respective group of PFASs were as follows: 3–71 ng/kg for PFSAs (without PFOS), 6–1999 ng/kg for PFCAs, and 3–292 ng/kg for FOSA. Comparing the individual food samples, seafood was the most contaminated foodstuff with levels of sum of all detected PFASs in range 1805–4688 ng/kg. Significantly lower levels of PFAS sum (in range of 71–579 ng/kg) were found in offal of domestic animals (pork liver, rabbit liver and kidney) collected in the Czech Republic and in samples of butter from Italy. On the other hand, samples of food cauldrons, whole meals and fast foods were almost not contaminated or at very low levels, as was expected.

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Keywords: PFAS, LC-MS/MS, food

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F-21
DETERMINATION OF MUSK COMPOUNDS IN FISH (LEUCISCUS CEPHALUS) FROM THE RIVER SVRATKA

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This work deals with actual issues of the contamination in the environment with synthetic fragrances. Musk compounds are infiltrated to many environmental components (especially an aquatic ecosystem) because of their biological persistence and the ability of accumulation. This study is focused on the selection and the optimization of method for the determination of musk compounds in real biotic matrices (Leuciscus cephalus, fish muscle). It was chosen these compounds for the analysis: nitro musk compounds - musk ketone, musk xylene, musk moskene, musk tibetene a musk ambrette; polycyclic musk compounds - galaxolide, tonalide, traseolide a phantolide. The isolation of analytes was realized by PSE method and the purification of extract was realized by the method of the adsorption column chromatography. Identification and quantification of individual musk compounds was carried out by GC-MS (SIM). In conclusion were results and the contamination of fish from the river Svratka disscussed and evaluated. These fish were caught at the inflow and at the outflow of the wastewater treatment plant (Brno-Modřice, Czech Republic). It was identified ambrette galaxolide and tonalide at the inflow, phantolide, galaxolide and tonalide at the outflow. Concentrations of other analytes were below LOD or LOQ. It was not found the effect of the wastewater treatment plant on the concentration of musk compounds in the fish muscle.

Keywords: musk compounds, PSE, GC/MS, fish

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F-22
DETERMINATION OF METALS IN FOOD ADDITIVES BY MEANS OF LASER ABLATION WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY AFTER ELECTRODEPOSITION

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A novel method for determination of heavy metals in high saline matrix is based on the electrodeposition of metals and subsequent analysis by means of laser ablation coupled to inductively coupled plasma mass spectrometry (LA-ICP-MS). Electrodeposition on the nickel electrode under conditions of controlled current in a stationary mode is proposed for the determination of cadmium, lead, copper and chromium in food additives. Three arrangements for electrodeposition were studied. The use of different working electrode materials and suitable conditions for electrodeposition of Cd, Pb, Cu, and Cr (pH, deposition current, time of electrolysis) from high salt matrices were studied. After electrodeposition the metals accumulated on the surface of electrode were evaporated/ablated by Nd:YAG laser into ICP-MS spectrometer. The measurements were carried out using Nd:YAG laser ablation system UP-213 (New Wave Research, USA) operating at wavelength of 213 nm and quadrupole ICP-MS spectrometer Agilent 7500CE (Agilent, Japan). Intensity of isotopes ^{53}Cr, ^{63}Cu, ^{65}Cu, ^{111}Cd a ^{208}Pb was measured. The detection limits were 14 μg l^{-1} Cr, 16 μg l^{-1} Cu, 0.5 μg l^{-1} Cd and 8 μg l^{-1} Pb. The accuracy of measurement was checked by using Certified Reference Material KCl II – K03 (Slovak Institute of Metrology). This technique was used for determination of Cd, Pb, Cu and Cr in different food additives (table salt, mixture of phosphate salt – E450, E451, E452). Table salt is one the mostly used food additive with unique place in food consumption. The concentration of Pb, Cr and Cu was 26.4 ± 4.0 μg kg^{-1}, 25.9 ± 4.0 μg $kg^{-1}$and 103.9 ± 7.9 μg kg^{-1}, respectively. The content of cadmium was below limit of detection (5 μg kg^{-1}). The concentrations of tested heavy metals were well below the maximum levels set by Codex [1]. In samples of phosphate salt (min. 95% of phosphate) was measured only cadmium. The concentration of cadmium was in the range from 0.043 to 0.045 mg kg^{-1}. The Czech legislation sets up the maximum level for cadmium content in food additives 1 mg kg^{-1}. This limit wasn´t exceeded.

[1] Codex standard for food grade salt, CX STAN 150-1985, Rev. 1-1997, Amend. 1-1999, Amend.2-2001

Keywords: electrodeposition, laser ablation, ICP-MS, additives

F-23
QUANTITATION AND IDENTIFICATION OF PHTHALATES IN FOOD AND BEVERAGE SAMPLES USING HIGHLY SELECTIVE LC–MS/MS

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Recent issues with phthalates in tainted soft drinks have highlighted the need for both food manufactures and regulatory agencies to utilize fast and accurate analytical techniques to proactively ensure product quality and consumer's safety. A fast and sensitive LC-MS/MS method was developed for the analyzes of 22 phthalates utilizing simple extraction, fast LC separation, and MS/MS detection using a triple quadrupole based mass spectrometer operated in highly selective Multiple Reaction Monitoring (MRM) mode. In addition the method provides an extra degree of confidence through the use of MRM ratios and library searchable full scan MS/MS spectra for compound identification Major challenges of LC-MS/MS analysis were high background interference and matrix interferences from beverage samples. To overcome these challenges we successfully applied MRM3 scanning and differential mobility spectrometry (DMS) for an added degree of selectivity. MRM3 uses filtering of secondary fragment ions to enhance method selectivity. DMS uses an asymmetric waveform applied to a planar cell to separate species based on their high field and low field mobility as they traverse the cell. In addition chemical modifiers are used to adjust separation characteristics of targeted analytes. Here we used acetonitrile to separate phthalates from interfering matrix signals and to separate isobaric species, such as BMPP and DHXP (Bis (4-methyl-2-phenyl) phthalate and Dihexyl phthalate).

Keywords: LC-MS/MS, phthalates, quantitation, identification, ion mobility

F-24
DETERMINATION OF PCDDS, PCDFS, DIOXIN-LIKE AND NON DIOXIN-LIKE PCBS IN FISH SAMPLES – A COMPARISON BETWEEN PRESSURIZED SOLVENT EXTRACTION AND SOXHLET EXTRACTION

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The extraction process for the determination of 17 PCDD/Fs, 12 dioxin-like and 6 non dioxin-like PCBs in fish samples is normally time and solvent consuming, especially if the classical Soxhlet method is applied. Pressurized Solvent Extraction (PSE) is a well-established alternative to the conventional Soxhlet method. PSE has many accurate, precise and robust methods which have been developed for POPs. At elevated temperatures, penetration of the matrix, diffusion rates as well as the ability to disrupt matrix-analyte interactions are increased, thus enhancing the extraction efficiency. The elevated pressure is mainly used to keep the solvent in a liquid state and to make sure the extraction cell is quickly filled with fresh solvent. In this study PSE was performed with BUCHI's SpeedExtractor E-914. The purpose of this study was to compare PSE results with those gathered by the application of the classical Soxhlet extraction method, and an interlaboratory round robin test, respectively. The isolation of PCDD/Fs and PCBs was performed in fish samples with different fat content; eel and trout. The samples were cut into pieces, freeze-dried, ground and homogenized using a knife mill. The extraction was carried out with DCM:Hexane (1:1/*v:v*) and the fat content was determined gravimetrically. For the concentration of the analytes and the separation of unwanted matrix components the extracts were purified by a multi-step chromatographic column clean-up. PCDD/Fs and PCBs were analyzed by means of high resolution mass spectrometry (HRGC/HRMS). The results of PCDD/Fs and PCBs of the trout reference material show an accurate reproduction of the data for both, Soxhlet and PSE. Samples were determined in triplicate yielding excellent reproducibility with very low RSDs which is the result of highly comparable conditions due to the parallel set-up of the SpeedExtractor. Blank runs performed in parallel and subsequent to the fish extractions showed neither cross contamination nor carryover of analytes. PCDD/F and PCB data of the fish samples obtained with PSE are closely comparable to the results obtained with classical Soxhlet extraction and indicate an equivalence of both techniques. This study indicates that the time period of two to four days using Soxhlet extraction is reduced by one day using the SpeedExtractor during the determination of PCDD/Fs and PCBs. This is a result of inherent time savings of PSE vs. classical extraction and the parallel extraction set-up of the SpeedExtractor which is perfectly designed for batch workflows.

Keywords: PCDD, PCDF, PCB, Soxhlet, PSE

F-25
RESULTS OF FIRST WORLDWIDE UNEP INTERLABORATORY STUDY ON POPS

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Within the framework of the Stockholm Convention on Persistent Organic Pollutants (POPs) a various capacity building activities are being organized at all five continents. One of the activities was an interlaboratory study on POPs, which took place between December 2009 and March 2011. More than 60 laboratories from all continents participated. Matrices analysed included fly ash, human milk, sediment and fish. The target compounds were indicators PCBs, chlorinated dioxins and furans, dioxin-like PCBs and organochlorine pesticides (OCPs). All participants were allowed to use their own methods. A substantial number of laboratories in developing countries had received training in POP analysis, both on site and a few days in expert laboratories. This study offered the unique possibility to compare the performance of laboratories to analyse POPs from Africa, Asia, the Pacific, and South-America. In addition, a number of laboratories from OECD countries joined this study. The experience with dioxin analysis and the use of high resolution mass spectrometry (HRMS) made the results from several Asian countries better compared to those from laboratories just starting this kind of analysis. In spite of the training, several laboratories from Africa still struggled with general difficulties in POPs analysis such as problems with gas chromatography, extraction and clean up. Clearly, further steps can be made to improve the quality of the POP analysis worldwide. The process of improvement is basically similar to that observed in the 1980s in European and North-American laboratories with the difference that conditions in the laboratories in Africa and South-America are less favorable. Investments in housing, training, instrumentation and glassware are needed to achieve acceptable results.

Keywords: POPs, Interlaboratory study

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EFFECTS OF THE COLLISION INDUCED DISSOCIATION (CID) VOLTAGE AND THE DAMPING GAS FLOW ON CO-PLANAR POLY CHLORINATED BIPHENYLS (CO-PCBS) DETERMINATION BY QUADRUPOLE ION TRAP MASS SPECTROMETRY

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The typical method for the determination of co-Planar PCBs in environmental samples is high resolution mass spectrometry in combination with high resolution gas chromatography as described in US EPA Method 1668A. Recently, a quadrupole ion trap (QIT) mass spectrometer has been introduced to determine PCBs in sewage effluents and food matrices because HRMS is very expensive to purchase and maintain. This method obtained by MS/MS with the QIT contains four step process involving ionization (EI or CI), parent ion isolation, collision induced dissociation (CID) and mass analysis of the daughter ions. The process can be repeated many times in order to obtain the information of $(MS)^n$. The aim of the present study is to optimize the collision induced dissociation (CID) voltage and damping gas flow for co-planar PCBs, which has known Dioxin-like compounds, by using MS-MS techniques of QIT mass spectrometer and to apply the method to the PCBs analysis in food packaging. The main features of PCBs in MS/MS spectrum is fragmentation under loss of Cl, 2Cl, 3Cl ions. For the MS/MS scan function, the optimization was performed by selecting the highest ion (parent ion) of the molecular isotopic cluster for each PCBs, which were $[M+2]^+$ion for the tetra- to hepta-compounds. The main fragments (daughter ion) of PCB molecular ions is mostly $[(M+2)-2Cl]^+$ ion. The optimization of CID voltage is to obtain the highest yield of the daughter ion by dissociation. The CID voltages of 12 PCB congeners were optimized to 3.8 4.0 V for the parent ion dissociation (MS/MS) and 3.6–3.8 V for the daughter ion dissociation (MS/MS/MS). The effect of damping gas on yields of the product ions showed that the total product ions was increased in proportion to the flow rate of the damping gas(He). The damping gas was optimized to 1.5 ml/min for 12 PCB congeners. The calibration curves were obtained using solutions of six different levels of concentration ranging from 0.5 to 1000 pg for 12 co-planar PCB congeners. Calibration curve was based on the signal areas due to $[(M+2)-2Cl]^+$+$[(M+2)-3Cl]^+$+$[(M+2)-4Cl]^+$ for PCB congeners with the MS/MS/MS scan function. The correlation coefficients of each calibration curves were all higher than 0.999.

Keywords: co-Planar PCBs, quadrupole ion trap (QIT), mass spectrometer, collision induced dissociation, damping gas

F-27
ANALYSIS OF PERFLUORINATED ALKYLATED SUBSTANCES IN BIOTA SAMPLES BASED ON FAST AND SIMPLE ACTIVATED CHARCOAL CLEAN–UP PROCEDURE FOLLOWED BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY: METHOD INTERLABORATORY STUDY

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The European project CONffIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure) aims to improve food safety in Europe by the development of faster and more cost-efficient methods for the detection of three target perfluorinated alkylated substances (PFASs) – perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide (FOSA) in fish fillets, milk and feed commodities, since most of currently used analytical procedures is laborious, time consuming and require sophisticated and expensive instrumentation. Within the project, simple, fast and cheap analytical approach for determination of PFOS, PFOA and FOSA in fish fillets was developed. The sample preparation procedure was based on extraction of target analytes using methanol and subsequent clean–up of crude extract was realized using carbon powder (activated charcoal). After centrifugation, the aliquot of supernatant was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). In comparison with other traditionally used methods for PFASs, this sample preparation procedure needs only cca 60 minutes for 10 samples. In this study, the analytical approach was validated in accordance with the Commission Decision 2002/657/EC. Recoveries and repeatability of the overall method were obtained by the analysis of six replicates of samples fortified at levels 0.25, 0.5, 1, 1.5 and 2 $\mu g.kg^{-1}$. Recovery (calculated as the ratio between levels measured and spiked amount) were in the range 85–110%, that is in agreement with 2002/657/EC where recovery from 70 to 120% is required. Repeatability was expressed as relative standard deviation (RSD) and ranged from 2 to 15%. Decision limits (CCα) and detection limits (CCβ) were calculated according to the international standard ISO 11843-2, based on a linear regression model analysing fortified material at different concentration levels. The obtain values for CCα were 0.015, 0.14 and 0.18 $\mu g.kg^{-1}$ and for CC_β 0.21, 0.47 and 0.49 for PFOS, PFOA and FOSA, respectively. Limit of quantifications of PFOS, PFOA and FOSA were 0.15, 0.3 and 0.3 $\mu g.kg^{-1}$, respectively and fulfil the request of the European Commission for 1 $\mu g.kg^{-1}$ (2010/161/EU). In addition to the validation study, the interlaboratory study was organized to assess the transferability of the developed procedure. The real-life contaminated fish fillets containing mainly the PFOS and FOSA (above LOQ) were employed. Together 5 laboratories were participated. No false positive result for PFOA was reported by participating laboratories. For results distribution illustration the Z-scores were calculated, which were for both PFOS and FOSA between -2 and 2.

Keywords: CC_α; CC_β; method validation; perfluorinated alkylated substances; 2002/657/EC

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TRACE MICROELEMENT CONTENT IN EDIBLE FISH FROM BULGARIAN BLACK SEA COAST

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Fishes are one of the major biological groups used for the classification of ecological status of surface waters according to the European Union Water Frame Directive as well as fishes are representative organisms in the process of development of environmental quality standards for the assessment of chemical status of water bodies. From the other point of view the European Commission directive 1881/2006 specifies maximum levels for several highly toxic elements in a variety of foodstuffs, including fishes as widely consumed product. Edible fish samples (fillets and gills), typical for Bulgarian Black sea cost have been analyzed for microelement content by ETAAS, HG AAS, ICP-AES and HG ICP-AES. Different analytical approaches as digestion procedures, sample solubilization (teramethylammonium hydroxide) or slurry technique have been compared and discussed. As a forward step to understand metal bioavailability and assess the potential impact on aquatic biota, a study of trace element speciation of sediments and water were performed. Total dissolved metal content in Black sea water has been determined by solid phase extraction followed by ETAAS, HG AAS or HG ICP-AES and free metal ion concentrations in Black sea water have been determined by DGT technique. Total element content in sediment samples has been determined after aqua regia digestion and ICP-AES measurements. Bioavailable metal species in sediment samples has been determined by using different leaching reagents (Black sea water, 0.11 mol L^{-1} acetic acid, 0.05 mol L^{-1} EDTA). Correlation coefficients for the systems Black sea water/fish gills/fish fillets taking into account total dissolved and free ion concentrations were presented. Analogous, correlation coefficients for system sediment/ fish gills/fish fillets taking into account mobile metal concentrations in various sediment fractions were defined. Bioaccumulation factors were expressed at environmentally relevant conditions for bioavailable elemental concentrations in Black sea water and sediment and compared with published bioconcentration factors defined under controlled laboratory conditions. Conclusion for the safety and quality of various fish species from Bulgarian Black sea cost indented for human consumption in comparison with defined ecological and chemical status of Black sea water and sediments were presented.

Keywords: fish, Black sea, sediment, bioaccumulation, microelements

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MERCURY DETERMINATION AND SPECIATION IN WINE BY NEW ION–IMPRINTED SORBENTS

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Wine is widely consumed beverage and strict control for toxic element content is required according to national and international legislation. The presence of toxic elements in wine results from deposition of airborne particulate matter on grapes and the intake of microelements by the grapevine from groundwater and soil. Typical Hg concentrations in wine are at sub-μg/L levels and their reliable and accurate determination calls for preliminary preconcentration and separation of mercury species. This step most frequently permits also selective determination of inorganic and organic Hg species in connection with high differences of their toxicity and moblility. The solid-phase extraction using ion-imprinted sorbents is most popular analytical procedure for this purpose. In this study the preparation and characterization of Hg(II) ion-imprinted polymer layer-coated silica gel particles (Hg(II)-IIP) are presented and tested for determination and speciation of Hg in wine. To induce the selective performance of surface polymerization, the polymerizable double bonds were first grafted at the surface of silica gel particles by the silylation. Hg(II)-imprinted polymer layer was prepared by copolymerization of methacrylic acid as monomer, trimethylolpropane trimethacrylate as crosslinking agent and 2,2'-azobisisobutyronitrile as initiator, in the presence of Hg(II) compexes with three different chelating agents: (i) 1-pyrrolidinedithiocarboxylic acid (Hg-PDC), (ii) 1-(2-thiazolylazo)-2-naphthol (Hg-TAN) and (iii) dithizone (Hg-DTZ). The separation and preconcentration characteristics of the Hg(II)-IIPs for inorganic mercury were investigated by batch procedure. The optimal pH values for the quantitative sorption were determined. The adsorbed Hg(II) was easily eluted by 0.1 M thiourea in 0.1 M HCl. The selectivity of the Hg(II)-IIPs toward inorganic mercury (Hg(II)) ion was confirmed through comparison of the competitive adsorptions of CH_3Hg^+, Cu(II), Cd(II), Fe(III) and Pb(II) and high values of the selectivity and distribution coefficients were calculated. There is also evidence indicating that the Hg(II)-IIPs compared with non-imprinted sorbent show a higher selectivity and affinity toward Hg(II). Experiments performed for selective determination of inorganic mercury in wine samples showed that the interfering matrix does not influence the extraction efficiency of Hg(II)-IIPs. The detection limit for inorganic mercury is 0.05 μg L^{-1} (3 s), determined by CV AAS or ICP-Ms. The relative standard deviation varied in the range 5-9% at 0.05 to 2 μg L^{-1} Hg levels. The new Hg(II)-IIPs were tested and applied for the speciation of Hg in wines: inorganic mercury has been determined selectively in nondigested sample, while total mercury e.g. sum of inorganic and organic mercury, has been determined in digested sample.

Keywords: wine, mercury, speciation, ion-imprinted sorbens

F-30
DETERMINATION OF METALS AS MARKERS OF OIL CONTAMINATION IN SEAFOOD

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Concern about fin fish, shell fish and bivalve contamination as a result of the Gulf oil spill and others has become an increasingly important question. Polynuclear aromatic hydrocarbon (PAH) content has been used as an indicator of exposure and may help to define food safety. However, PAHs are rapidly metabolized and may not be detectable when the measurement is made, even though exposure has occurred. Metal determination, particularly nickel and vanadium, has been used to characterize oil for identification and to assess its ability for emulsification. These elements have also been shown to bioaccumulate in mollusks and may be used as a watch ± to monitor contamination. This work will demonstrate improved methodology to efficiently measure nickel, vanadium, and other elements in a variety of sea creatures at low concentrations. Sample preparation, detection limits, and interferences will be discussed.

Keywords: Seafood, metals, contamination, ICP-MS

F-31
OCCURENCE OF PERFLUORINATED COMPOUNDS IN FOODSTUFFS IN SWITZERLAND: PRIMARY FOOD AND PACKAGING CONTRIBUTION

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Whilst human exposure to perfluorinated compounds (PFCs) is significant and well known, (mainly due to surfactants, waterproofing agents, fire-fighting foams and stain repellants), Swiss data on human exposure through the food chain involving primary food contamination and food packaging materials contribution are still scarce. Indeed, direct contamination of primary food can easily occur and oil and water repellant food packaging materials commonly used as for example in the fast-food area are also able to migrate into foodstuffs. Thus, the present study aims at estimating PFCs in various food products as they might be a significant route of human exposure. An analytical approach was developed and validated based upon an LC-MS/MS method for the targeted screening of 25 perfluoroalkyl chemicals including perfluoroalkyl carboxylates, perfluoroalkyl sulfonates, perfluoroalkyl sulfonamides and perfluoroalkyl sulfonamidoethanols, all at concentrations down to about 10 µg/kg. The procedure involved a microwave-assisted extraction, followed by a SPE clean-up and finally a "core-shell" based chromatographic separation. The use of a partially porous packing reduced analysis time while maintaining an efficient separation. The method was applied to the analysis of about 200 samples of all common food types from the Swiss market covering indirect environmental contribution as well as possible packaging contamination. Amongst primary food analyzed, 13 fish samples were positive for PFOS with concentrations ranging from 16 to 74 µg/kg and one of them also contained N-MeFOSE at 14 µg/kg. These measured amounts and corresponding human intakes fall below the tolerable daily intake (TDIs) recently estimated by the European Food Safety Authority at 0.15 and 1.5 µg /kg bodyweight/day for PFOS and PFOA, respectively. On the one hand, all positive samples were fishes from Swiss lakes demonstrating that the major contribution of PFOS contamination is conveyed by the environmental route. On the other hand, samples covering possible packaging contribution were free of significant concentration of PFCs. Finally, by comparing PFOS values obtained from ten years old lyophilised samples of fishes from the same Swiss lakes, it was observed that residue levels did not increase during the last decade.

Keywords: Perfluorinated compounds (PFCs), survey, primary food, packaging

F-32
METHYLMERCURY DETERMINATION IN FISH AND SHELLFISH BY GOLD AMALGAMATION – DIRECT MERCURY ANALYZER

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Continuously, it has been worried about food safety and especially caused controversy on heavy metals, lead, cadmium, mercury and so on. In spite of steady attentions, methylmercury, organic compound of mercury, haven't standed out among others. In 2006, KFDA established standard regulation of methylmercury in fishes eventually and has tended to study actual conditions of methylmercury exposures. In the study, I suggest methylmercury analysis in fish and shellfish by Gold Amalgamation - Direct Mercury Analyzer (DMA) and perform risk assessments. To set up for methylmercury analysis in fish and shellfish is based on analyte and matrix characters, then put the steps of digestion and extraction. Digestion conditions for leaching methylmercury from matrix are classified into alkaline and acidic digestion and compared in study. After acidic condition - HCl digestion, extract with toluene, back - extract with L-cysteine solution. L-cysteine sol. leads to aqueous phase from organic phase, improves stability of methylmercury and clean-up from matrix. extracting test solutions get into DMA. To perform validation of optimized method, examine validation factors, linearity, sensitivity, selectivity, accuracy, limit of detection & limit of quantification in certified refernece materials and spiked sample. and participates in FAPAS international quality control program. Korean and international official methods are gas chromatography - electron capture detection (GC-ECD) for determination of methylmercury. They have many disadvantages, long analysis time, low accuracy and precision because of matrix effects, hard to derivatization as pH change, and so on. But on the other hands methylmercury determination by DMA is possible to analyze trace level in a short time as results of more low detection limit, high accuracy and precision. In addition, DMA has been use of total mercury analysis only, but it will be a chance to share methylmercury determination after extraction. In basis on the analytical method, perform risk assessments for 200 Korean fish and shellfish samples. As the results of assessments, the upper group in food chain, deep - sea fishes, has higher contents of methylmercury than others. And analyze total mercury, study relation between them. Methylmercury forms 49.0~80.7% of total mercury in fish and shellfish, is similar to preceding studies. In comparative study of methylmercury determination by GC - ECD, it has a close affinity with 78.5~113%. As establish methylmercury analysis in fish and shellfish by Gold Amalgamation - Direct Mercury Analyzer (DMA), it will be possible to gather more reasonable results and useful application. In constant monitoring methylmercury exposure transitions, we need to build databases in the inside and outside.

Keywords: Methylmercury, Direct mercury analyzer, Fish and shellfish, L-cysteine

F-33
VALIDATION OF HEAVY METALS ANALYSIS BY ICP–MS FOR REGISTRATION OF KOREAN FOOD CODE

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Analytical methods of Food Code are traditionally applied in evaluation of all food in Korea and implements objective methods in accordance with an international standard. However, the analytical methods of Food Code demand rapid development and implement many technologies and more efficient method. In addition to having declined steadily the regulation in domestic and foreign trend, we set up and validate the analytical method for heavy metal in foodstuffs by ICP-MS after microwave digestion for registration of Korea Food Code. We have to classify according to matrix for systematic heavy metal analysis, so divide into 7 groups with reference to 7th Food Ingredients Table of Korean Rural Development Administration. Considering contents of water, protein, lipid and carbohydrates, we choose representative foods, pork, frozen pollack, bean oil, rice, sugar, seaweed, salt, radish, and canned goods. Pretreatment methods for digesting samples are wet digestion (H_2SO_4 - HNO_3 digestion, microwave digestion) and dry digestion, we performed a comparative study of the conditions. The best is microwave digestion, others are easy to apply in relatively restrictive range. We perform validation of established method, recovery 82.6~110%, LOQ (limit of quantity) Pb 0.03~0.13 mg/kg, Cd 0.06~0.29 mg/kg, As 0.02~0.09 mg/kg, Sn 0.03~0.13 mg/kg, Cu 0.06~0.29 mg/kg. We participated in international proficiency test, FAPAS, acquire Z-score Pb -0.4, Cd -0.2 in cereal, Cd -1.0, As -0.3 in canned fish. In basis on the analytical method, perform risk assessments for 119 kinds, 3820 Korean foodstuffs. As the results of lead in samples, polished rice (0.10~135 μg/kg, 8.30 μg/kg, 1.15 μg/kg, range, average, median), onion (0.10~87.7 μg/kg, 11.9 μg/kg, 0.10 μg/kg), pork (0.10~178 μg/kg, 13.5 μg/kg, 9.33 μg/kg), tuna (0.10~64.3 μg/kg, 9.95 μg/kg, 1.61 μg/kg). Cadmium in samples, polished rice (3.35~ 84.0 μg/kg, 21.0 μg/kg, 16.3 μg/kg), onion (0.10~15.2 μg/kg, 5.04 μg/kg, 4.95 μg/kg), pork (0.10~4.88 μg/kg, 0.45 μg/kg, 0.10 μg/kg), tuna (0.34~43.3 μg/kg, 16.2 μg/kg, 10.3 μg/kg). Mercury in samples, polished rice (0.10~104 μg/kg, 13.6 μg/kg, 3.42 μg/kg), onion (0.10~16.6 μg/kg, 2.09 μg/kg, 0.37 μg/kg), pork (0.10~83.7 μg/kg, 7.06 μg/kg, 3.43 μg/kg), tuna (33.5~1.56E+03 μg/kg, 486 μg/kg, 268 μg/kg).

Keywords: Heavy metals, Korean Food Code, ICP-MS, Validation

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REGIONAL VARIATION OF MEHG RATIO TO TOTAL HG IN FISHES FROM KOREAN CITIES

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Total mercury and methylmercury in 14 kinds of fishes from six Korean cities in 2010 were determined with DMA (direct mercury analyzer) and GC/ECD. No pretreatment is needed in DMA but for methylmercury with GC/ECD and methods were validated. Correlations of methylmercury (MeHg) with total mercury (Hg) were investigated in tuna, shark, whale, cutlassfish, mackerel and mackerel pike. Ratio of MeHg to total Hg were 0.45, 0.62, 0.16, 0.38, 0.38 for tuna, cutlassfish, shark, whale and mackerel/mackerel pike, respectively. Linearity of correlation was relatively good for tuna and cutlassfish (R^2=0.75, 0.51, respectively) which means that ratio of MeHg to total Hg can be the marker for the species of fishes. Ratio of MeHg to total Hg in fishes were almost same from Seoul, Busan, Incheon cities (0.41~0.47). Ratio were high at Gwangju (0.68) and Daegu (0.55) and low at Gangneung (0.36). Concentration of total Hg and MeHg in tuna at Daegu was very high compared to tuna concentration of other cities. Linearity of correlation was very good at Gangneung and Gwangju (R^2=0.82) which indicates that ratio of MeHg to total Hg can be the marker for the origin of mercury contamination. Further investigation for more samples and the correlation with the blood sample from cities will be needed.

Keywords: Total mercury, Methylmercury, DMA (direct mercury analyzer), GC/ECD, Fishes from Korean cities

F-35
SIMULTANEOUS DETERMINATION OF 1,4-DIOXANE AND FORMALDEHYDE IN WATER BY SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY–TIME OF FLIGHT MASS SPECTROMETRY

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Simultaneous determination of 1,4-dioxane and formaldehyde in water by solid phase microextraction (SPME)-gas chromatography–time of flight mass spectrometer (GC-TOFMS) was investigated. Two types of SPME fibers, polydimethylsiloxane (PDMS)/divinylbenzene (DVB) and 100 Ąěm–polydimethylsiloxane (PDMS) were investigated. The aim of this study was to develop a method that allows the determination of 1,4-dioxane and formaldehyde simultaneously. Before the direct SPME extraction, 0.3 w/v % of 2,4-dinitrophenylhydrazine was used as a derivatizing agent for the analysis of formaldehyde. Three extraction parameters (extraction time, temperature, and salting effect) were observed. PDMS/DVB fiber was found to give the better response. Optimum extraction condition was found at 30°C for 15 min in 5% NaCl solution. The method showed a linear correlation (R^2>0.99) in the calibration range of 0.03–3.33 Ąěg/ml for 1,4-dioxane and 0.03–1.67 ng/ml for formaldehyde.

Keywords: 1,4-Dioxane, Formaldehyde, water, SPME-GC-TOFMS

F-36
PHTHALATE INTAKE OF INFANTS BASED ON THE RESULTS OF A DUPLICATE DIET STUDY IN GERMANY (INTEGRATED EXPOSURE ASSESSMENT SURVEY, INES II)

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Due to their widespread use as plasticizers, today phthalates are ubiquitous environmental chemicals with potential health effects on different endpoints, especially on reproduction. The INES study created a framework for an integrated exposure assessment approach including data of different organic pollutants in various environmental media, in food, and the body burden of humans. The exposure of adults, older children and breastfed and formula-fed babies has been relatively well described in the meantime, but there are still no such data for children following the lactation period. The aims of the study were (1) to estimate the daily dietary intake on the basis of duplicates, (2) to determine the excretion of primary and secondary phthalate metabolites and (3) to compare the dietary intake with the overall intake. Here we present the data for the dietary intake. The study group consisted of 25 individuals, 9 girls and 16 boys. All of the participating children were born in Munich or the surrounding area and were aged between 15 and 21 months (median: 18 months). Over a seven-day period the parents collected duplicates of all of the solid and liquid foods consumed by their children each day (a total of 175 samples). The foods were prepared as for consumption. Phthalates were detected using low resolution GC/MS in SIM-mode. We were able to detect the target analytes in more than 98 % of the 175 single samples. We estimated the “average” and “high” daily intakes for infants using medians and 95th percentiles of the measured phthalates combined with the consumption data. The “average” (“high”) daily intake via food was 2.4 (6.4) µg/kg b.w. for Di-(2-ethylhexyl) phthalate (DEHP), 0.4 (1.0) µg/kg b.w. for Di-n-butyl phthalate (DnBP), and 0.5 (1.4) µg/kg b.w. for Di-isobutyl phthalate (DiBP). Overall, the dietary phthalate intake of infants was in the same range as for adults but higher than that of breast-fed infants, aged from 1 to 5 months. For risk assessment purposes our intake estimates could be compared to tolerable lifetime intake levels at which no appreciable health risks would be expected over a lifetime (Tolerable Daily Intakes, TDI) recommended by EFSA. The “average” (“high”) intake from diet was about 1% (3%) for DEHP, 0.04% (0.1%) for DnBP, and 0.05% (0.14%) for DiBP of the TDI value.

Keywords: Dietary intake, phthalates, DEHP, exposure

F-37
DETERMINATION OF ARSENOSUGARS IN ALGAL EXTRACTS BY HIGH-TEMPERATURE LIQUID CHROMATOGRAPHY – INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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The accurate determination of arsenosugars is very important for two reasons: environmental, since they play a central role in the metabolism of arsenic in marine organisms; and toxicological, since these compounds are bioaccumulated in marine organisms such as algae and fish that can be used as human food. Therefore, speciation studies are necessary for this purpose. In this work, a new approach based on high-temperature liquid chromatography (HTLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) has been applied for the determination of these compounds in biological samples. The hyphenation HTLC-ICP-MS combines the advantages of HTLC as a separation technique (good and faster separations without the need to use salts or organic solvents as the mobile phase) with the advantages of ICP-MS as a powerful detector (the possibility to determine organometallic compounds at low concentrations). The main goal of the present communication was to demonstrate that the hyphenation HTLC-ICP-MS is a competitive technique for the determination of arsenosugars in biological samples. For this reason a comparison between HTLC-ICP-MS with conventional methods was also discussed. A porous graphitic carbon column (hypercarb) was used and temperatures ranged from 40 up to 140°C. This is the first attempt to use temperatures higher than 100°C for speciation purposes. At an optimal temperature of 120°C, we obtained a good separation of the analytes, free of interferences from all the major As compounds. Precision was good (< 3%) and the limits of detection were comparable with those obtained with conventional techniques (0.3–0.4 ng/g). The developed method was finally applied to biological samples such as Antarctic algae, crustaceans and mollusks with good results.

Keywords: HTLC, ICP-MS, arsenosugars

F-38
MERCURY IN ORGANIC AND CONVENTIONAL BABY FOODS MARKETED IN THE REGION OF LISBOA, PORTUGAL: OCCURRENCE AND EXPOSURE ASSESSMENT

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Baby foods have special functions to play in diets of infants because they are major source of nutrients (1) and a unique source of food during the first months of their life. Fish and seafood products are the major source of exposure to mercury (2. A growing part of the European population gives preference to organically produced agricultural products due to the alleged absence of chemical contaminants within this mode of production. In this study, commercial baby food labeled as from organic and conventional origin, including processed-cereal based foods, infant and follow-on formulae and dinners (vegetable/meat/fish and fruits) (n=87) were analysed for total mercury content by US EPA 7473 method based on the principle of thermal decomposition, amalgamation and detection by atomic absorption spectrometry. A limit of detection of 0.14 ng, a limit of quantification of 0.38 ng and reference materials Z-scores ranging between -1.56 and 1.54 were obtained for mercury determination. Mercury contents ranged from 0.19 to 19.56 μg kg^{-1} with median concentrations of 0.55 μg kg^{-1}, 0.50 μg kg^{-1} and 0.70 μg kg^{-1} for processed cereal based foods, infant and follow-on formulae and dinners, respectively. The highest mercury content was observed in two dinners containing fish, as also reported by other authors (3,4). There is a significant difference (p=0.038) for total mercury considering the two farming methods. The estimated mercury intake through baby foods was calculated using consumption information provided by the DONALD study (5). For infant formula, exposure ranged between 0.016 μg kg^{-1} bw $week^{-1}$ (0.98% of PTWI) and 0.060 μg kg^{-1} bw $week^{-1}$ (3.74% of PTWI) for a mean mercury concentration of 0.47 μg kg^{-1}. For processed cereal based foods, exposure ranged between 0.021 μg kg^{-1} bw $week^{-1}$ (1.32% of PTWI) and 0.031 μg kg^{-1} bw $week^{-1}$ (1.93% of PTWI) for a mean mercury concentration of 1.09 μg kg^{-1}. For dinners, exposure ranged between 0.199 μg kg^{-1} bw $week^{-1}$ (12.44% of PTWI) and 0.469 μg kg^{-1} bw $week^{-1}$ (29.33% of PTWI) for a mean mercury concentration of 2.46 μg kg^{-1}. The PTWI level for the different exposure scenarios (90^{th}, 95^{th} and 99^{th} percentiles of mercury concentration) was only exceeded for dinners in 95^{th} and 99^{th} percentiles. Occasionally exceeding the PTWI does not per se indicate a health risk, since the assessment should be based on chronic dietary exposure (6). However, a risk to some infants who are high consumers of dinners containing fish cannot be excluded.

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Keywords: baby food, mercury, exposure assessment, organic foods

F-39
CHLOROPROPANOLS EXTRACTION FROM WATER AND FRUIT JUICE BASED ON DISPERSIVE LIQUID–LIQUID MICROEXTRACTION

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Chloropropanols are contaminants formed during the processing of different foodstuffs. Mechanisms of formation in HVPs and model systems have been studied (1). Cloropropanols releases to the aquatic environment can occur via numerous waste streams. For instance, they can enter natural waters as a consequence of the use of epichlorohydrin for production of wet-strength resins, chlorine bleaching of paper pulps, or as a contaminant of polyamine flocculants used in the treatment of drinking water. Moreover, they have also been identified in drinking water samples after chlorination (2,3). A rapid and sensitive method has been established for the determination chloropropanols in water and juices samples by using simultaneous dispersive liquid-liquid microextraction (DLLME) and derivatization combined with gas chromatography-mass spectrometry. The method uses 1,3-DCP-d_5 and 3-MCPD-d_5 as internal standard. Parameters potentially affecting the performance of the sample preparation method (sample pH, ionic strength, type and volume of dispersant and extractant solvents) were evaluated using experimental designs. Under the optima extraction conditions (extraction solvent: trichlorometane: 60 µL; dispersive solvent: acetonitrile: 0.9 mL; derivatizante volume: 50 µL; addition of salt; extraction temperature 40°C and ultrasound extraction time of 5 min), the method was evaluated. The linearity of the method was obtained in the range of 5−100 ng mL^{-1} (1,3-DCP and 2,3-DCP) and 5−100 ng mL^{-1} (3-MCPD in waters samples) and 50−200 ng mL^{-1} (3-MCPD in juices samples) with the correlation coefficients (R^2) of 0,999. The method detection limits were 0.15−1.8 ng mL^{-1} (water samples) and 0.5−15.1 ng mL^{-1} (juices samples). The precision varied from 1.3−4.9 % RSD (n=6) in water and 1.3−4.8%RSD (n=12) in juices. The recoveries of chloropropanols at spiked level of 10.0 ng mL^{-1} were 98-101.1% from water samples and 97−101% from juices (1,3-DCP and 2,3-DCP). The recovery of 3-MCPD at spiked level of 100.0 ng mL^{-1} was 100%. As a result, this method can be successfully applied for the rapid and convenient determination of chloropropanols in real water and juices samples.

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Keywords: chloropropanols, dispersive liqui-liquid microextraction, water, juices

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F-40
VALIDATION OF A METHOD FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN CEREALS AND VEGETABLES BY GC-MS

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Polycyclic Aromatic Hydrocarbons (PAH) are contaminants that may enter the food chain either by environmental impact or as by-products of certain steps of food processing, e. g. smoking techniques. Benzo[a]pyrene, widely used as a marker substance for PAH, is classified as carcinogenic to humans, while further PAH are classified as possibly carcinogenic. Based on scientific assessment, the European Commission recommended the monitoring of 16 selected PAH, among these Benzo[a]pyrene (BaP), Benzo[a]anthracene (BaA), Benzo[b]fluoranthene (BbF) and Chrysene (CHR) [1]. The analytical method established and validated in our laboratory for the analysis of these 16 PAH in unprocessed cereals and vegetables consists of three basic steps: (1) pressurised liquid extraction (PLE) with cyclohexane and silica added to the extraction cell; (2) clean-up with SPE on a polymeric sorbent; (3) determination by GC-MS with isotopically labelled standards. Unprocessed cereals and vegetables are not yet covered by European Community legislation with respect to PAH, but the requirements set for other categories of food [2,3] were considered as a suitable benchmark for the design of the validation study. The method was validated according to the matrix-comprehensive alternative approach laid down in Commission Decision 2002/657/EC [4]. During the validation study, certain experimental conditions ("factors") were varied systematically: the matrix type itself (cereal or vegetables), different lots of SPE sorbent, two operators, different instruments (low-resolution MS vs. high-resolution MS). Four different kinds of grain (rye, linseed, spelt, wheat) and four kinds of vegetables (red cabbage, leek, carrots, green cabbage) were included in the study to account for variations of the matrix. The validated range covers a concentration interval of 0.4 µg/kg to 2.0 µg/kg for each analyte. The dispersion of results appears to be acceptable: the within-laboratory reproducibility of the four key analytes ("PAH4") - BaA, BaP, BbF and CHR - ranges between 8.5% and 11.8 % at the lowest validated level of 0.4 µg/kg and is declining when the concentration rises. The method appeared to be robust against variations of the experimental conditions and no major interferences of matrix components occurred. The limits of detection (LOD) and limits of quantification (LOQ) were determined as foreseen in Regulation (EC) No 333/2007 (calculated from the standard deviation of the mean of blank determinations), and the requirements of LOD < 0.30 µg/kg and LOQ < 0.90 µg/kg were met for all analytes of the PAH4 group.

[1] Commission Recommendation 2005/108/EC OJ L 34, 08.02.2005, p. 43-45
[2] Commission Regulation (EC) No 1881/2006, OJ L 364, 20.12.2006, p. 5–24
[3] Commission Regulation (EC) No 333/2007, OJ L 88, 29.03.2007, p. 29-38
[4] Commission Decision 2002/657/EC, OJ L 221, 17.8.2002, p. 8–36

Keywords: PAH, Benzo(a)pyrene, validation,

F-41
ENHANCEMENT OF PRODUCTIVITY FOR THE ANALYSIS OF FOOD SAMPLES WITH THE 7700X ICP-MS

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The evaluation of the food samples on the human health is passing through the characterization of their elemental composition. Indeed, elements can mainly be classified as essential or toxic for the health. As the ranges of concentrations where the elements can be found are quite various, different techniques are in use for the sample characterization. As an example, major elements are mainly measured using an ICP-OES instrument when the trace element concentrations are determined by ICP-MS or even by mono-elemental techniques, such as AAS. In consequence, different analyses need to be performed for the full characterization of only one sample. In this work, we investigated the possibility to measure all the elements, either the trace or the major constituents, with only one analysis run on the 7700x ICP-MS proposed by Agilent Technologies. Thanks to the 9 orders of magnitude provided by the detector, the measurements of low concentrated elements but also major constituents are possible on this system in the same run and with only one configuration, i.e no need to run extra dilution of samples or to change the ICP MS parameters for the high concentrated elements. One issue link to the use of an ICP-MS is the spectral interferences generated by the plasma. To eliminate those artifacts, many types of gas can be used into the system. In this study, we investigated the use of only one gas, the helium, to simplify the method, but also to avoid new issues generated with the use of reactants gases, such as hydrogen. During the study, we also evaluated the use of a discrete sampling system to decrease the time required for one analysis. On the 7700x instrument, the system called "ISIS-DS" is proposed for such purpose and it authorizes the reduction of the dead times link to one analysis, i.e sample uptake or washout between samples. Thanks to all of those improvements, we were able to characterize the elemental composition of one sample with only one analysis in less than 60s.

Keywords: ICP-MS, elemental analysis, inorganic contaminants

F-42
DETERMINATION OF POLYCHLORINATED BIPHENYL CONGENERS IN FOODSTUFFS AND ANIMAL FEED USING GC–MS/MS

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A study was undertaken to compare the quantitative performance of a gas chromatograph - triple quadrupole mass spectrometer (GC-MS/MS) to that of a gas chromatograph - high resolution mass spectrometer (GC-HRMS) for both dioxin-like Polychlorinated biphenyl congeners (dl-PCBs; #77, 81, 105, 114, 118, 123,126, 156, 157, 167, 169 and 189) and non-dioxin like PCBs (ndl-PCBs; #28, 52, 101, 138, 153 and 180). Eighty samples of four different foodstuffs and animal feeds - Cows' milk (n=11), Meat (n=19), Liver (n=5) and Animal Feed (n=45), were extracted and analyzed using GC-HRMS. The same sample vials were then transferred to the GC-MS/MS system and reanalyzed. Samples of foodstuffs and animal feed were extracted and cleaned up with the final extracts prepared in Toluene. The extracts were analyzed by HR-GCMS using a Waters Autospec M472 at a resolution of R=10,000 and then transferred to an Agilent Technologies 7890A-7000B GC-MS/MS system where the dl- and ndl-PCBs and their 13C-labelled internal standards (ISTDs) were analyzed using multiple reaction monitoring (MRM) mode using two MS/MS transitions from two different pre-cursor ions for each analyte and its associated ISTD. The mass spectrometer was operated in electron impact (EI) ionization mode with electron energy set at -78 EV. The agreement between the results (upperbound concentrations) obtained for the total of the 12 dl-PCB congeners on the GC-HRMS and the GC-MS/MS system for foodstuffs and animal feed samples at levels above 1 pg TEQ/g were within the range of +/- 10 %. The agreement between the results (upperbound concentrations) obtained for the sum of the 12 dl-PCB congeners on the GC-HRMS and the GC-MS/MS system for foodstuffs and animal feed samples at levels between 0.1 and 1 pg TEQ/g was within the range of +/- 15%. Only those animal feed samples with total dl-PCB congener concentrations below 0.1 pg TEQ /g gave some results with percentage differences greater than 15%. This is due to the lower limit of quantitation provided by the GC-HRMS system, but these levels are well below the current action limits and maximum residue limits set for dl-PCBs in foodstuffs and animal feed by EU legislation. The agreement between the sum of the results obtained for the 6 ndl-PCB congeners on the GC-HRMS and the GC-MS/MS system for foodstuffs and animal feed samples at levels between 0.5 and 10 ng/g was within the range of +/- 10%. The GC-MS/MS system demonstrated the ability to determine ndl-PCB concentrations below 1 ng/g product. Some animal feed samples with total ndl-PCB congener concentrations below 0.5 ng/g gave results with percentage differences greater than +10%. The performance of the GC-MS/MS system meets the requirements for the determination of ndl-PCBs in animal feed as given in the draft amendment to Commission Regulation (EC) No 152/2009 Annexe V letter B of 27 January 2009, scheduled for October 2011.

Keywords: Polychlorinated biphenyls, GC-MS/MS, Food, Animal feed

F-43
UNCERTAINTY APPROACH APPLIED TO THE DETERMINATION OF ORGANIC AND INORGANIC CONTAMINANTS FOR QUALITY MANAGEMENT SUPPORT

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Quality Management has become essential in laboratories of residues and contaminants aiming food analysis, especially because of the importance to assure the analytical results and their impact in society. Thus, these laboratories have applied different tolls, as validation process, evaluating if the analytical method is fit for purpose and can be considered an evidence of reliable results; and uncertainty measurement that characterizes the dispersion of the values being attributed to a measurand based on the information used. Different approaches for uncertainty calculation are available, leading to many discussions about the best way to obtain reliable results in a quick way. The purpose of this study is to develop an uncertainty approach applied to the determination of organic and inorganic contaminants for Quality Management support. The literature guides the application of two approaches to calculate uncertainty: bottom-up (a hard calculation that consider all steps of the methodology) and top-down (a simplified method that consider the validation results). Results showed that the uncertainties associated with the validation process are the most important parameters contributing to at least 90% of all uncertainty value. Therefore, in the organic and inorganic contaminants determination, the uncertainty of all other analytes was calculated from validation process (top-down approach), which can be used during the routine analysis in a simpler, faster and fit for purpose uncertainty calculation.

Keywords: uncertainty, validation, contaminants, Quality Management

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LEVELS OF PERFLUORINATED COMPOUNDS IN HUMAN MILK AND FOOD SAMPLES

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Perfluorinated compounds (PFC) are synthetic substances widely used in several fields for covering plastic tissues, plastic materials and electric products, antispot compounds, non-stick coverings and photographic films. PFC include compounds based on perfluottan sulphonylfluorur (POSF) that, upon being degradated, e.g., by environmental factors, or metabolized by the organisms, entail the formation of composed of toxicological importance such as, among the others, perfluoctane sulphonate (PFOS) and perfluoroctanic acid (PFOA). The high stability of the molecules makes these able to accumulate in the organisms, for which they turn out to be toxic. PFOA and PFOS are part of EDC (endocrine disrupting chemicals) list and presently some researchers are trying to highlight possible correlations between PFOS and PFOA exposure and the presence of some diseases. Despite the environmental importance of PFC and the detection of these compounds in many countries around the world, little is known on their occurrence and distribution in Italy. In this work are reported the results of a study on distribution and levels of PFOS and PFOA in human milk and food samples from the Sienese area (Central Italy). The analytical procedure for the extraction of PFOS and PFOA from samples was similar to that described by Corsolini (1). PFOS and PFOA are extracted using an ion-pairing extraction procedure and are determined using high performance liquid chromatography (HPLC) with electrospray tandem mass spectrometry. LOD= 1 ng/g w.w. 41 milk samples (within a week after delivery) from women living in the city of Siena and its province and 70 samples of food from a supermarket of the city of Siena were analyzed for this study. PFOS was determined in 13 of the breast milk samples analyzed (mean value ± SD: 0.76± 1,27 ng/g w.w.) while PFOA was detected in only one sample (8.04 ng/g w.w.). Basing on the results achieved, we have assessed the intake of pollutants by newborn infants: PFOA: range 0-1.21 µg/day PFOS: mean 0.1 µg/day range 0-0.65 µg/day. In the food samples analysed we have only found the presence of PFOS. Fish and fish products were the most contaminated samples among foodstuff, even if with low levels (mean value ± SD: 7.65±34.21 ng/g w.w.). Mean concentrations of PFOS in meat and milk and dairy products were similar: 1.43±7.21 ng/g w.w. and 1.35±3.45 ng/g w.w., respectively). In all the samples analyzed among the categories of cereal based foods, eggs, vegetables and beverages PFOS and PFOA were below the LOD.

[1] Corsolini S, Guerranti C, Perra, G, Focardi S. Polybrominated diphenyl ethers, perfluorinated compounds and chlorinated pesticides in swordfish (Xiphias gladius) from the Mediterranean Sea. Environ Sci Technol 2008; 42:4344-9.

Keywords: Perfluorinated compounds, Food samples, Human milk

F-45
LEVELS OF ENDOCRINE DISRUPTORS IN ORGANIC AND CONVENTIONAL FOOD

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The consumption of organic foods versus conventional ones is presented by producers, and considered by most consumers, as bearer of many healthy effects. In fact, in organic breeding, using synthetic growth promoters, such as antibiotics, is not allowed, feed and fodder administered to animals should be organic and pasture should be the basis of diet. However, often the health benefits of organic products consumption are supported more by ideological convictions and emotional factors than from results of scientific studies, creating the conditions for incorrect information. In 1991 the European authorities, through the Regulation 2092/91 on organic products marketing, had established that neither advertising nor label should suggest to the buyer that their consistency with organic production method is a guarantee of superior organoleptic, nutrient or healthy quality. Regarding the contaminants transmitted mainly through the environment, such as organohalogen compounds, organic and conventional foods are equally at risk. From this point of view, food from organic production is not better and healthier than conventional. In this study, 6 milk, 12 cheese, 4 eggs and 32 meat pools of samples, from both conventional and organic production, were analyzed for their levels of PCBs, chlorinated pesticides, PCDDs, PCDFs and PBDEs. Results of PCDDs, PCDFs and dioxin-like PCBs are expressed as WHO-TEQ. The results showed that also organic products are contaminated by the compounds of interest. In some cases the levels of contamination were higher for organic products compared with conventional, even though there were no statistically significant differences between the two kinds of products (Mann-Whitney-Wilcoxon test). Among meat samples, for instance, the levels of PCBs, DDTs and HCB, were higher in the organic products than in conventional ones (mean levels in meat were: PCBs 4.48 ng/g l.b. in organic and 1.39 ng/g l.b. in conventional; DDTs 1.88 ng/g l.b. in organic and 0.05 ng/g l.b. in conventional; HCB 0.84 ng/g l.b. in organic and 0.05 ng/g l.b. in conventional). In eggs samples, on the contrary, the mean levels of the all the compound of interest were higher in conventional products than in organic ones (mean levels in eggs were: PBDEs < LOD in organic and 0.29 ng/g l.b. in conventional; HCB < LOD in organic and 0.34 ng/g l.b. in conventional; PCDDs, PCDFs and dioxin-like PCBs expressed as WHO-TEQ 0.27 pg/g l.b. and 0.17 pg/g l.b.). The results obtained support the hypothesis that organic foods may be potentially contaminated in the same way as conventional ones and despite the many benefits associated with organic food consumption, the presence of endocrine disruptors still represents a danger.

Keywords: Endocrine disruptors, Organic food, Conventional food.

F-46
COMPREHENSIVE ANALYSIS OF PESTICIDES, HERBICIDES, MYCOTOXINS AND OTHER EXOGENOUS CHEMICALS IN FOODSTUFFS USING HPLC HIGH-RESOLUTION TOF

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Introduction: High resolution mass spectrometry has grown appreciably in use in the recent past with availability of new instrumentation being a significant contributor to that growth. Accurate mass analysis and accurate relative isotope abundance provided by a ultra high resolution TOF serves as the framework for the development of a comprehensive method for the analysis of diverse compounds of environmental interest. The ability to simultaneously detect compounds of interest (targeted) and survey other possible contaminants offers substantial opportunity in environmental and food/safety efforts.
Experimental: Plant, vegetable and food extracts were spiked at various levels with a mixture of 247 compounds covering the range of 0.3 through 300 ng/mL. Samples were analyzed by UHPLC interfaced to an LC-HRT high-resolution time-of-flight mass spectrometer. The LC-HRT was operated in high resolution mode (R(FWHM) = 50,000)and in ultra-high resolution mode (R(FWHM) = 100,000)with positive ionization.
Results: Accurate mass analysis has provided a powerful tool for the trace analysis of compounds in complex matrices. High resolution MS provided the means to selectively detect and quantify a broad range of analytes in a sensitive and selective fashion. The acquisition rates and chromatographic analyses were accelerated to test the ability of the resolution and dynamic range to provide strong coverage and detection limits under accelerated acquisition conditions. The implementation of accurate mass analysis and relative isotope abundance permit the clear identification and separation of closely related single analytes.
Conclusions: The analysis of trace level analytes in complex matrices has been investigated using high performance time-of-flight mass spectrometry. Speed of acquisition and high performance capabilities have led to the detection and confident identification of over 200 analytes in complex food-based matrices. Resolving power in excess of 100,000 in conjunction with mass accuracies of less than 1 ppm combined to detect analytically challenging compounds. The resolution and mass accuracy also lend to a more robust deconvolution of peaks.

Keywords: High performance TOF MS,Food-based matrices, Ultra-high resolving power, Accurate mass data and isotope abundance, Compound identification

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F-47
DETERMINATION OF CHLOROPROPANOLS IN MILK USING ULTRASOUND-ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION WITH DERIVATIZATION AND GC-MS DETECTION

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Chloropropanols are contaminants that may be formed during the processing of different food products and they were originally associated with the savoury food ingredient acid-hydrolysed vegetable protein (acid-HVP) (1). It has since been found in a number of other foods and ingredients as a result of processing, storage or migration (2-3). Simultaneous ultrasound-assisted dispersive liquid–liquid microextraction (DLLME) and derivatization combined with gas chromatography- mass spectrometry was used to determinate 1,3-dichloro-2-propanol (1,3-DCP), 3-chloropropane-1,2-diol (3-MCPD) and 2,3-dichloro-1-propanol (2,3-DCP) in milk samples. The method uses 1,3-DCP-d5 and 3-MCPD-d5 as internal standard. Some parameters that affect the extraction efficiency such as sample pH, salt effect, volume of acetonitrile as dispersant solvent, type and volume extractant solvents and extraction/derivatization temperature has been studied and optimizated. Heptafluorobutyrylimidazole (HFBI) in extraction solvent (chloroform) was used as derivatization reagent. Under the optimum extraction conditions, the linearity of the method was obtained in the range of 5–100 ng mL^{-1} (1,3-DCP and 2,3-DCP) and 50–200 ng/mL^{-1} (3-MCPD) with the correlation coefficients (R2) of 0.999. The method detection limits were 1.11–9.64 ng mL^{-1}. The relative standard deviations varied from 1.01% to 1.73% (n=6). The relative recoveries of chloropropanols from milk samples at spiking levels of 10.0 ng mL^{-1} (1,3-DCP and 2,3-DCP) and 100.0 ng mL^{-1} (3-MCPD) were 100–103 % and 100.5%, respectively. The proposed method has been successfully applied to the analysis of chloropropanols in milk samples.

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[3] Hamlet Coling G., "Cap. 12: Chloropropanols and their Fatty Acid Esters" Bioactive Compounds in foods Edited by John Gilbert and Hamide Z. Senyuna Blackwell Publishing (2008) 323-351.

Keywords: Chloropropanols, dispersive liquid-liquid microextraction, milk

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F-48
ADVANCED APPROACHES OF CELL BASED SCREENING METHOD HTPS DR CALUX FOR DIOXINS AND DL-PCBS AND INTERNATIONAL APPLICATION EXAMPLES (GERMANY, CHILE, KUWAIT)

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Since more than a decade yearly dioxin/PCB crises occur in Europe. The last one in 2011 in Germany did prove that not enough testing is routinely done as well as the testing capacities from the existing laboratories are too low – leading to long turn-around-times. Over the past decade, several official governmental authorities as well as feed/food companies have moved from the traditional chemical method HRGC/HRMS to easier-to-learn, low cost, high-through-put capacity, faster and regulatory compliance cell based screening methods called dioxin responsible chemically activated luciferase expression (DR CALUX). Cell based screening methods have to fulfil strict QA/QC requirements such as the ones listed in EU or other Directives, but also has to comply with ISO 17025 accreditation and/or procedures of quality systems (GMP+, QS or FEDIOL). To optimise the effectiveness of the CALUX method now high-through-put robots and stackers are in routine used to keep pace with the demands of the international feed/food chain. The new tools lead to the possibility to extract, clean-up, seed/dose and measure more than 30 samples/hour (per each step) for separate dioxin- and PCB-TEQ analysis. The presentation will highlight various aspects of some recent investigations of automatisation steps for the measurement of dioxins and dl-PCBs by DR CALUX in all kinds of food/feed. Application examples of the DR CALUX will be presented from three different continents: from the last dioxin crisis in Germany, an industrial feed/food monitoring program in Chile and a National Surveillance Monitoring Program in Kuwait. In case of the recent German dioxin crisis the reported levels of dioxin and dioxin-like PCBs measured separately by DR CALUX for mostly pig meat, poultry meat, eggs and all kinds of feed materials have been more than 99% compliant. During this time the measured confirmative analysis results with HRGC/HRMS proved to be compliant. In this crisis situation for the here analysed matrices, the DR CALUX has proven to have a lower than 1% false negative and false positive rate. Advancements of luminometer-based measurement tools combined with cell based biological assays have allowed cell culture providers to develop new testing methods, in addition to the standard regulated testing. Now the technology provider offers also highly sensitive, reliable and easy-to-learn/adapt test systems for obesogens (PPAR CALUX), hormones (Cancer Biomarker), painkillers (Dexamethason; GR CALUX), pharmaceuticals (Antibaby-pill), plastic additives (Bisphenol A; ERα and anti-AR CALUX)), car exhaust gas (PAH CALUX), waste recycling (unknown pollutants), eco-toxicological endpoints (such as genotoxicity; P53 CALUX), UV-sun oils (TR and PR CALUX), oil spills (Risk Rapid Biomarker) and for healthy soils, wildlife reproduction, mother/baby health biomarkers, anabolic steroids (Doping; CALUX panel) and many more for wildlife and human health relevant issues.

Keywords: dioxins, dioxin-like PCBs, cell based screening, CALUX, obesity

F-49
ANALYSIS OF PERFLUORINATED COMPOUNDS IN FISH TISSUE: A PILOT STUDY FROM THE CZECH REPUBLIC

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For humans, food represents one of the important exposure sources to potentially toxic perfluorinated compounds (PFCs). One of the research tasks within the EU CONffIDENCE project has been focused on a development of a rapid test for control of three major PFC representatives 1 in food of animal origin: perfluorooctansulfonate (PFOS), perfluorooctanoic acid (PFOS) and perfluorooctanesulfonamide (PFOSA). A new simple sample preparation strategy is based on methanol extraction followed by dispersive solid phase extraction clean-up realized by activated charcoal. For subsequent instrumental analysis, ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) is employed. The method has been developed and validated not only for dairy products, fish and fish product, but also for fish feed. The performance characteristics of this really fast and easy procedure determined for 3 target PFCs are as follows: recoveries 85–110%, repeatabilities 2–15% and LOQs 0.1–0.7 µg/kg, what is fully in agreement with the current European Commission recommendation 2010/161/EU from March 2010 for the monitoring of various groups of PFCs together with their precursors in food (recovery in range 70–120% and LOQs below 1 µg/kg). For the pilot study on contamination of the Czech aquatic ecosystem by these PFCs, fish was used as a suitable bioindicator organism. 9 sampling sites located at two main Czech rivers Vltava (3) and Elbe (6) were examined. The major compound detected in all tested samples was PFOS, followed by PFOSA; on the other hand PFOA was measured only in 40% of fish samples. In addition to 3 targeted PFCs, also other perfluorocarboxylic acids, especially those with the longer chain (C9 – C14) were found in most of fish.

1 The EFSA Journal (2008). Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain 653:1–131.

Keywords: PFCs, fish, PFOS, PFCAs

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F-50
IMPLEMENTATION OF GC×GC–TOFMS FOR THE SIMULTANEOUS DETERMINATION OF PCBS, PBDES AND PAHS IN ENVIRONMENTAL SAMPLES

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Comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) represents a powerful tool for simultaneous determination of different types of contaminants that considerably increases the separation efficiency of GC analysis. Since the whole system comprises two capillary columns with different polarities, the total peak capacity for GC×GC is the outcome of the individual column capacities. Moreover, peak compression effect occurring at the interface of the 1st and 2nd dimension columns allows achieving lower limits of detection needed for the trace analysis. One task of the European project CONffIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure), is the development and validation of a comprehensive profiling strategy using GC×GC-TOFMS technique for the simultaneous determination of 18 polychlorinated biphenyls (PCBs), 7 polybrominated diphenyl ethers (PBDEs) and 16 polycyclic aromatic hydrocarbons (PAHs) was optimized to obtain the best chromatographic resolution and quantification limits (LOQs) of target analytes. Two injection techniques, pulsed splitless and large volume programmable temperature vaporization (LV-PTV), and several capillary column systems were tested within the experiments (BPX-5 and BPX-50 in the 1st dimension and BPX-50, Rt-LC35 and HT-8 in 2nd dimension). Any of tested column combinations enable separation all target PCBs and PBDEs. Selection of the column system was therefore mainly influenced by its ability to separate the critical groups of PAHs (1st group: B[a]A, CP[cd]P and Chr, 2nd group B[f]F, B[k]F and B[b]F, 3rd group DB[ah]A, I[1,2,3-cd]P and B[ghi]P). Although none of the column combinations evaluated in the present study allowed a complete separation of target PAHs, a combination of BPX-50 × HT-8 columns showed the best ability to separate the 2nd group of PAHs. To achieve desired LOQs, the LV-PTV injection (8 µL) was implemented allowing to obtain LOQs for almost all target analytes by approximately one order of magnitude lower as compared to hot splitless injection (1 µL). The LOQs achieved using LV-PTV-GC×GC were as follows: PCBs 0.1–0.25 µg/kg, PBDEs 0.5–2.5 µg/kg, PAHs 0.05–0.25 µg/kg.

Keywords: PCB, PBDE, PAHs, GCxGC-TOFMS, fish

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F-51
FAST SCREENING FOR NUTRITION-RELEVANT AND TOXIC TRACE ELEMENTS IN PLANT AND FISH MATERIAL BY TXRF SPECTROSCOPY

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The accurate analysis of nutrition-relevant and toxic trace elements is of crucial importance because of many reasons. The human dietary micronutrients, which are required in very small amounts, include trace elements as V, Cr, Cu, Mn, Fe, Ni, Zn, Se, As as well as several vitamins. In order to ensure that dietary intake is providing adequate levels of micronutrient elements, these trace elements must be determined accurately. In addition, contamination by toxic elements like Hg, Pb and Tl, e.g. from industrial or mining sources or plant treatment with herbizides and fungizides, is an analytical task for trace elemental analysis. The most common analytical techniques for the analysis of trace elements in plant and fish material are Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) [1] and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) [2]. But as these analytical techniques demand a laborious and time-consuming sample preparation, their suitability for a fast screening of large sample batches is limited. In addition, the requirements for the laboratory environment and the need for costly consumables may not allow the routine use in laboratories with limitations in facilities, supplies and budget. In this paper the possibilities and limitations of TXRF analysis for the analysis of nutrition-relevant and toxic trace elements are summarised. When analysing four plant reference standards (NIST 1515; apple leaves, NIST 1547; peach leaves, NIST 1572, citrus leaves, NIST 1573a, tomato leaves) and one fish reference standard (DORM-3), the measured concentrations were in good concordance with the reference values. The recovery of the micro- and macronutrients in the plant standard samples was in the range of 85 to 115%. For the fish standard satisfying recoveries were achieved for Cr, Fe, Ni, Cu, Zn, As and Pb. Concentrations and detection limits of other elements will be also shown. In addition different sample preparation methods for fast element screening of food material will be discussed.

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[2] Koplík, R., Čurdová, E., Suchánek, M. (1997), Fresenius J. Anal. Chem. 360, 449-451

Keywords: contamination, nutrient, trace element, plant, fish

F-52
A NOVEL SPECIATION ALTERNATIVE FOR THE DETERMINATION OF INORGANIC ARSENIC IN MARINE SAMPLES

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Arsenic (As) is bioaccumulated from seawater to concentrations in the mg/kg range in marine animals. More than 50 naturally-occurring arsenic containing species, both inorganic and organic forms, have been identified in marine animals. The organic forms are mainly considered to be non-toxic, whereas inorganic arsenic is highly toxic and exposure may lead to severe adverse effects including cancer. Since seafood is the major dietary source for arsenic exposure in the European population, arsenic speciation analysis of marine samples is highly relevant for food safety. However, most data collected in the official EU food control today are reported as total arsenic. High Performance Liquid Chromatography Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) is a useful but expensive tool for metal speciation analysis. Our novel, simple and inexpensive method for determination of inorganic arsenic in marine based food is based on microwave extraction, species separation by strong anion solid phase extraction (SPE) and hydride generation atomic absorption spectrometry (HG-AAS) detection. Separation organic arsenic compounds (e.g. MA, DMA and AB) and inorganic arsenic in the form of As(V) is possible due to different charges (pKa values) of the arsenic species at a specific pH. SPE method development and sample extraction was evaluated using HPLC-ICP-MS. No degradation or conversion of organic arsenic species such as AB, MA or DMA were observed under the chosen extraction conditions. In brief: The sample is heated with a hydrochloric acid and hydrogen peroxide solution (20 minutes at 90°C with 0.06 M HCl, 3% H_2O_2). Hereby the sample is solubilised and As(III) is oxidised to As(V). Inorganic arsenic is selectively separated from other arsenic compounds using strong anion exchange SPE. The procedure include first pre-condition of the column, then loading of the buffered samples (pH 5.0-7.5), washing with 0.5 M acetic acid and finally elution of the sample from the column by 0.5 M HCl. The concentration of arsenic is determined by HG-AAS using external standards. The method SPE-HG-AAS was in-house validated by spiked and naturally incurred marine samples. Mean recoveries of the spiked samples were 101–104%. The limit of detection was determined to 0.08 mg/kg and was calculated as three times the standard deviation at intra-laboratory reproducibility conditions divided by the average recovery, both at the lowest spike level (0.5 mg/kg). The in-house reproducibility standard deviations were less than ≤13% for samples containing 0.2 to 1.5 mg/kg inorganic arsenic. The results obtained by SPE-HG-AAS and HPLC-ICP-MS detection were not significantly different (95% confidence).

Keywords: Inorganic arsenic, speciation, solid phase extraction, atomic absorption spectroscopy, validation.

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MERCURY SPECIATION ANALYSIS IN MARINE SAMPLES BY HPLC–ICPMS

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Mercury (Hg) is a naturally occurring element, which is found in the earth's crust and can be released into the environment through both natural and anthropogenic processes. Mercury exists as elemental mercury (metallic), inorganic mercury and organic mercury (primarily methylmercury). Methylmercury is highly toxic, particularly to the nervous system, and the developing brain is thought to be the most sensitive target organ for methylmercury toxicity. Methylmercury bioaccumulates and biomagnifies along the food chain and it is the most common mercury species in fish and seafood. Human exposure to methylmercury is mainly from fish and other seafood consumption. A simple method for the determination of methylmercury in marine based foods and feeds has been developed and in-house validated. The applied HPLC-ICPMS method was inspired by Vallant et al (2007). Samples were extracted with 5 M hydrochloric acid by sonication. Hereby the protein-bound mercury species are released. The extracts were then centrifuged (10 min at 3170 x g) and the supernatant decanted (extraction step was repeated twice). The combined extracts were added 10 M sodium hydroxide to increase pH, following further dilution in the mobile phase and filtering prior to analysis. Analysis of mercury species were performed using HPLC-ICPMS equipped with a MicroMist nebuliser. Typical plasma conditions were 1500 W RF power, 15 l/min, 0.97 l/min and 0.17 l/min for plasma, carrier and makeup gas, respectively, Analysis was performed in the time resolved analysis mode monitoring the 202Hg, 198Hg, 35Cl (m/z) with 1 s (Hg) and 0.01 s (Cl) integration time per data point. Separation of inorganic mercury and methylmercury was obtained on a polymer-based cation-exchange column (150×2.1 mm id, 10 µm) using isocatic elution (0.2 ml/min at 40°C). The mobile phase (pH~3) consisted of L-cysteine (0.5% w/w), pyridine (50 mmol/L), methanol (5% v/w) and formic acid (0.8% v/w). Total run time 10 min. External calibration standards (0–10 µg/L) were run before and after the samples in order to quantify the methylmercury species by peak height (*m/z* 202). The methylmercury method was validated by triplicate analysis of certified reference materials (DORM-2, TORT-2 and DORM-3) and 4 other fish and feed samples of marine origin, repeated on 3 different days. The limit of detection and quantification were 0.027 and 0.054 mg/kg, respectively. The limits were calculated as three and ten times the standard deviation at intra-laboratory reproducibility conditions of a natural fortified sample with low content (0.06 mg/kg) divided by average recoveries for certified reference materials. Mean recoveries of the reference materials were 94–102%. The in-house reproducibility standard deviations were less than ≤12% for samples containing 0.15 to 4.47 mg/kg and less than ≤20% for samples with 0.06 mg/kg.

Vallant B, Kadnar R and Goessler W (2007) J Anal Atom Spectrom 22, 322–325.

Keywords: Methylmercury, inorganic mercury, speciation, HPLC-ICP-MS, validation.

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DETECTION OF CONTAMINANTS IN CEREALS BY NEAR INFRARED HYPERSPECTRAL IMAGING

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In the last years, hyperspectral imaging has proven its performance for quality and safety control in the cereal sector by allowing the collection of spectroscopic images at single kernel level, which is of great interest for cereal control laboratories. Contaminants in cereals concern, among others, impurities such as straw, grains coming from other cultures or insects but also undesirable substances such as ergot (Claviceps purpurea). For the cereal sector, the presence of ergot involves high toxicity risk for animal and human due to its content in alkaloids. To reduce the risk of poisoning, the European directive 2002/32/EC on undesirable substances in animal feed fixed a limit of 0.1% for ergot in all feedingstuffs containing unground cereals. The regulation EEC No 689/92 restricted to 0.05% the concentration of ergot bodies in cereals for humans. The current work, performed in the framework of the CONffIDENCE project (http://www.conffidence.eu), aims to detect and quantify the presence of ergot bodies in cereals using NIR hyperspectral imaging. For this study, several instrumentation approaches (plane and line scan), softwares and chemometrics tools have been tested at the laboratory level and further transfer to an industrial setting for testing, validation in practice and demonstration. The aim was then to show the advantages of such hyperspectral imaging system in order to try to integrate it in an automatic cereal control scheme. The conclusions obtained from both at the laboratory level and at the industrial level have shown that NIR hyperspectral imaging and chemometric tools could be used as control method to assess the presence and the quantity of contaminants such as ergot bodies in cereals. Together with this poster, a diaporama will show a video with the instrument in action during the CONffIDENCE project Open Day held on 3rd November 2011 at RAFA 2011.

Keywords: ergot, contaminant, kernel analysis, hyperspectral NIR imaging, feedsafety

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F-55
ANALYSIS OF PERFLUORINATED COMPOUNDS (PFCS) IN FISH: A COMPARISON BETWEEN FARM AND OPEN SEA FISH

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Perfluorinated compounds (PFCs) comprise a large group of compounds widely used in industrial applications since 40s that are characterized by a fully fluorinated hydrophobic linear carbon chain attached to one or more hydrophilic heads. They have unique properties to make materials stain, oil, and water resistant, and are widely used in several applications such as stain and water resistant textiles, food packaging, in fire extinguishing formulations, pesticides, paints, personal care products and surfactant agents [1], among others. PFCs are resistant to breakdown, ubiquitous environmental contaminants, which persist and may be accumulated attached to proteins and biomagnified through the food chain. In recent years, an increasing scientific interest has raised due to their widespread distribution. The main direct routes of exposure of PFCs to humans are in their diet and drinking water. Although fish is one of the main sources of PFCs in diet [2], the levels found in farm fish and sea fish could differ due their distinct sources of food and exposure to environmental PFCs. This work presents the study of different fish species, each of them purchased from the market, from farm and open sea (the same specie). The analytical procedure consisted in the extraction of 2 g of fish by methanol (shaking 1 min), followed by clean-up by activated charcoal, developed under the frame of the project CONffIDENCE [3]. The method was validated according to Commission Decision 2002/657/EC [4] for three of the most used PFCs: perfluorooctanoic acid (PFOA), ion perfluorooctanesulfonate (PFOS) and perlfuorooctanesulfonamide (FOSA). The analyzed samples corresponded to three different species: salmon, turbot and gilthead bream. The samples were from two origins: I) farm fish (n=9) and, II) open sea fish (n=9). A total of 54 samples were analyzed in triplicate.

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Keywords: Perfluorinated compounds, wild fish, farm fish, active charcoal, LC-MS/MS

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GENERAL FOOD ANALYSIS

(G-1 – G-75)

G-1
DETERMINATION OF PHENYLALANINE CONTENT IN LOW PROTEIN FLOUR MIXTURES BY LC–MS

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Phenylketonuria (PKU), caused by an error in amino acid metabolism, is the most prevalent metabolic disease. It results from the mutations in phenylalanine hydroxylase (PAH) gene [1]. PAH catalyzes the hydroxylation of phenylalanine (Phe) to generate tyrosine. Deficiency in the activity of this enzyme causes increased level of Phe in blood and brain. Untreated PKU causes severe mental retardation accompanied by symptoms such as eczematous rash, autism, seizures [1]. There are several treatment strategies for PKU such as dietary treatment, gene therapy and enzyme substitution with phenylalanine ammonia lyase [1, 2]. A low Phe diet is very restrictive for patients [3]. Natural food sources suitable for patients are fruits and vegetables and there is an increasing range of manufactured foods in low amount of protein. Acid hydrolysis is widely used for the determination of Phe. However, this technique is problematic due to high amounts of starch in low protein flour mixtures; side reactions such as Maillard reactions can occur. There is not an accurate method for the determination of low Phe levels in foods. In addition, low concentrations of Phe are difficult to detect by conventional UV-Vis detection. It is necessary to use highly sensitive mass spectrometry coupled to liquid chromatography (LC-MS) to analyze appropriately hydrolyzed samples for Phe. This study aimed to develop an analytical method for the determination of Phe in low protein and gluten-free flour mixtures. The method includes enzymatic hydrolysis of sample prior to LC-MS. In detail, 100 mg flour sample was suspended with 4 mL water and 100 µL Alcalase (2.4 LG) was added. The mixture was incubated at 60°C for 12 hrs. The hydrolysate was centrifuged at 10000 rpm for 5 min. Supernatant was diluted tenfold with a mixture of acetonitrile: water (80:20, *v/v*). It was filtered through 0.45um membrane and analysed by LC-MS. Chromatographic separation was performed on a Merck ZIC-HILIC column (150 × 4.6 3.5 micron) using isocratic mixture of acetonitrile: 100 mM formic acid (80:20, *v/v*) as the mobile phase at a flow rate of 0.5 mL/min (30°C). Mass spectrometer was operated in single ion monitoring mode under positive ionization conditions. The signal having *m/z* of 166 was monitored for the quantitation of Phe. There was a linear correlation between Phe concentration and signal response (r^2=0.96) in a concentration range between 1 and 10 mg/l. The analytical method was applied to various low protein flour samples with a certain level of success.

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Keywords: Phenylalanine, LC-MS, phenylketonuria, low protein flour mixture

G-2
CE-AD APPLICATION IN FOOD ANALYSIS FOR NUTRIENTS, LEGAL ADDITIVES AND HAZARDOUS CONTAMINANTS

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In recent years, people have paid more and more attention to the safety of foodstuffs for the sack of public health and security. Accordingly a simple, fast and reliable analytical method is urgently needed to analyze numerous foodstuffs for nutrients, legal food additives as well as food contaminants. In the work reported here, a capillary electrophoresis with amperometric detection (CE-AD) method was developed and introduced to fulfill this task. In particular, this CE-AD method in its conventional, small-sized, and miniaturized versions has been developed to analyze numerous real-word food samples for either nutrients including flavones and flavonoids, or legal additives including anti-oxidants and food preservatives, or food contaminants (illegal food additives) including Â-agonists and melamine. The experimental results of this analytical method are quite positive, proving itself as a useful and competitive analytical tool in analyzing numerous and diverse food products.

Keywords: Capillary Electrophoresis, Amperometric Detection, Food Analysis, Additive, Contaminant

G-3
OPTIMIZING SAMPLE PREPARATION TO SPEED UP THE ANALYTICAL WORKFLOW PROCESSES

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The typical workflow in most analytical laboratories includes sample collection, extraction, and preparation for analysis (e.g. solvent exchange, cleanup and concentration, etc.) and, finally analysis. Typically this can involve Gas chromatography (GC), High Performance Liquid Chromatography (HPLC) and/or Ion Chromatography (IC) often with Mass Spectrometry (MS or MS-MS). Recent food safety issues (E Coli, Gulf Oil Spill, dioxin contaminations, etc) have again demonstrated the need for fast and accurately obtained data. In the last few years, chromatograms have been reduced to a few minutes. Modern data handling software has automated data collection and reporting. Sample Preparation remains a persistent bottleneck in the analytical workflow process. This presentation will demonstrate how Accelerated Solvent Extraction (ASE) can rapidly extract pollutants while retaining un-wanted co-extractables such as lipids. High lipid content samples can be exhaustively extracted and cleaned in less than 10 minutes. ASE can work both as a screening or an exhaustive extraction technique and while eliminate post-extraction clean up. Data will be presented showing the improvements in sample preparations for a variety of food matrices including milk, consumer products and fish.

Keywords: Extraction, lipids, PAH's, fish, foods

G-4
COMPARISON OF POLARIMETRY AND HPLC METHOD-USING CROWN ETHER BASED HPLC CHIRAL STATIONARY PHASE (CSP), FOR THE DETERMINATION OF (L)-AMINO ACIDS OPTICAL PURITY

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Although an assay method for the content of amino acids is provided as titration which could not distinguish (L)-amino acid with (D)-amino acid, definitions of content are expressed as (L)-amino acid in the KP and USP. We want to compare specific rotation (a) using polarimeter with enantiomeric excess (ee) using chiral crown ether HPLC column for (L)-amino acids. And we find out m/z of the front peak on the chromatogram from LC/MS/MS whether amino acid becomes degradation or not. Main advantage being that amino acids of concentration as low as ppm level can be analyzed using the HPLC-CSP, which is far better than polarimetry analysis which required higher concentration (20-110 mg).

Keywords: optical purity, amino acids, chiral resolution, chiral crown ether

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G-5
DETERMINATION OF AMINO ACIDS IN TEA BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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Methods used for amino acid analysis are usually based on a chromatographic separation of the amino acids present in the sample. Current chromatographic techniques require postcolumn derivatization, unless the sample is analyzed using precolumn derivatization. Derivatization of free amino acids is necessary either to increase their detection sensitivity on ultraviolet-visible and fluorescence detectors, or to improve their chromatographic separations on conventional reversed phase columns. Amino acids are polar molecules that can be separated better using hydrophilic interaction liquid chromatography (HILIC) without precolumn derivatization. Underivatized amino acids can be sensitively detected by means of mass spectrometry. Analysis of free amino acids in foods by using HILIC coupled to mass spectrometry offers great advantages over the existing chromatographic methods in terms of analysis speed, accuracy, and cost. In this study, a liquid chromatography system (Thermo Scientific Accela) coupled to an orbitrap high-resolution mass spectrometry (Thermo Scientific Exactive) operated in positive electrospray ionization mode was used for the analysis of amino acids. Chromatographic separations were performed on an Atlantis HILIC column using a gradient mixture of acetonitrile and 100 mM aqueous formic acid solution. High-resolution mass spectrometry was used to scan ions between *m/z* 50 and 300 with ultra-high resolving power. Ground tea (1 g) was extracted three times with 10 ml of hot water. The aqueous extract was diluted with acetonitrile (1:1) and injected onto HILIC column. The analytical conditions successfully resolved the peaks of amino acids in a total runtime of 6 min. Retention and molecular mass data were used to confirm the presence of amino acids in samples. Orbitrap mass spectrometry analysis verified the molecular masses of identified amino acids in tea samples with very high mass accuracy (<2 ppm). These amino acids detected in tea include theanine, glutamic acid, glutamine, serine, alanine, aspartic acid, asparagine, arginine, tyrosine, valine, phenylalanine, leucine, isoleucine, tryptophan, proline, lysine, histidine, and glycine. In principle, the method described here is applicable to any food matrices for high throughput analysis of amino acids.

Keywords: Amino acids, HILIC, high-resolution mass spectrometry, tea

Acknowledgement: Thermo Fisher Scientific

G-6
CHEMICAL CHARACTERIZATION OF A TRADITIONAL FISH PREPARATION (MISSOLTINO) OBTAINED FROM SALTED AND DRIED TWAITE SHAD

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Missoltino is a traditional Italian fish product obtained from salted and dried twaite shad (Alosa fallax lacustris), an endemic fish of northern Italian lakes. A large amount of these fish is caught near its reproductive period, during the end of spring. This reason had led local professional fishermen to find a way to preserve the fish for all the year using an old processing techniques. Briefly, the freshly-caught twaine shad, weighing about 80 g, are eviscerated and then salted using fine sea salt. The exceeding salt is removed by water washing and the fish are naturally dried in a room for 3-5 days. Afterwards fish are arranged in layers in metallic containers and pressed at ambient temperature for a long period, nearly 3 months. These containers are closed with a wooden lid and the pressure is progressively increased with a crank handle. The aim of this work was to chemically characterize this fish product, prepared using two different levels of salt and at different time of ripening, in order to identify the optimal processing technique that resulted in best quality properties. Thirty six samples of twaite shad caught in Como lake were collected and used to make missoltini with 2 different salt concentrations, 80 g kg^{-1} (A) and 40 g kg^{-1}. (B). Four samples were sampled: after catching, during salting, after 30 days, after 60 days and after 90 days of pressing. Chemical composition, TBARS value, histamine, fatty acid composition and flavor volatile compounds were determined in all samples. Fresh twaite shad showed a lipid percentage of 6.7±2.41. This percentage increased progressively in missoltini while the moisture content decreased during drying and pressing. The salt percentage had a direct influence on the lipid content. In missoltini made using 80 g kg^{-1} of salt lipids were lower than in missoltini made using 40g kg^{-1} of salt. This difference was statistically significant

Keywords: Twaite shad, processing conditions, volatile compounds, fatty acids, salt

G-7
ASSESSMENT OF THE LEVEL OF THERMOTOLERANT COLIFORMS AND TRACE METALS IN BIVALVE MOLLUSCS COMMERCIALIZED IN THE PUBLIC MARKETS FROM PERNAMBUCO, BRAZIL

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Like most cities located along the Brazilian coast, the city of Recife, Pernambuco, Brazil has a large number of public and street markets which sell fruits, vegetables, fish, meat and others. The public markets and fairs are establishments where the supply of fish is very high due to the informality of this activity and the easiness of these products purchase. In these establishments, the lack of proper hygienic practices can compromise the quality of the fish, bringing serious risks to the population that uses them as a food source. In most markets, the procedures for the conservation of mollusks are inadequate, exposing the product to a series of microbiological contaminants. In addition, these mollusks have filtering habits, which can also accumulate inorganic contaminants such as trace metals that can cause adverse reactions in organisms causing mutagenic, teratogenic, carcinogenic effects amongst others. Given the importance of this subject, this work aimed to evaluate the concentrations of trace metals (Cu, Zn, Cd and Pb) and fecal coliform in shellfish (Mytella charruana, Anomalocardia brasiliana and Crassostrea rizophorae) sold in the public markets (St. Joseph, Itapissuma and Rio Formoso) located in Recife city, Pernambuco, Brazil. The samples were collected in the months of December 2010 and February 2011, packed in plastic bags (refrigerated) for further treatment and chemical analysis. The optical emission spectrometer Inductively Coupled Plasma (ICP-OES) method was used to quantify the concentration of trace metals and to measure the fecal coliform bacteria the rapid plate count 3M® method was used. The results of trace metals indicate that all shellfish studies were within the range allowed by current legislation in Brazil. The market for Rio Formoso had better sanitary conditions and the values found for fecal coliform bacteria indicate that the market of San Jose has rates above the maximum allowed under Brazilian law.

Keywords: Bivalve Molluscs, Trace metals, Food analysis, Thermotolerant coliform

G-8
QUANTITATIVE DETERMINATION OF SUGAR CONTENT IN SOFT DRINKS BY ATR-FTIR SPECTROMETRY AND CHEMOMETRICS

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It is convenient to measure the food properties by the nondestructive methods. The main method for determination of sugars in food are based on refractive index measurements, which provide information about the amount of dry matter existing in soft drinks and give a quick estimation about the total sugar content [1]. There are several methods in determination of carbohydrate drinks. Modern industrial method such as GC, HPLC, capillary electrophoresis or spectrometric methods can provide information about the total content of sugars and the specific concentration of each carbohydrate [2-4]. In this research a simple analytical procedure has been proposed for rapid determination of sugars in soft drink samples, using infrared spectrometry and chemometric multivariate data processing techniques. Direct measurements were made by ATR-FTIR spectrometry and partial least squares technique (PLS1) was used to create the calibration models, being cross-validated (leave-one-out approach) [5]. Root mean square error of calibration (RMSEC) for the constructed models was 0.3072, 0.6509 and 0.1552 for sucrose, fructose and glucose respectively. Accurate and precise prediction of the sugar levels in soft drinks by ATR-FTIR spectrometry was comparable with standard method. Also this method is a non-destructive analysis approach which may be useful for juices analysis and could be applied in quality control of beverages or to monitor for adulteration or contamination.

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Keywords: sugar content, ATR-FTIR, chemoemtrics, quantitative, non-destructive

G-9
SOLID–PHASE EXTRACTION APPROACH IN COMPREHENSIVE ANALYSIS OF WORT AND BEER SAMPLES

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The determination and identification of biomolecular composition of brewing samples play a crucial role in beer quality assessment and in the monitoring of technological processes. To obtain the reliable results a specific sample pretreatment addressed to target species is required. In this work, we dealt with the development of solid-phase extraction method for selective purification of oligosaccharides in the presence of peptides and proteins. Due to heterogeneity of the carbohydrates with respect to degree of polymerization, monosaccharide and branching pattern, the selectivity towards molecular structure was included. For enrichment of compounds of interest, a wide variety of sorbents based on porous graphite, reverse phase (on polymeric and silica supports), and hydrophilic interaction chromatography (amino, amido, diol, cyano) phases were evaluated. The special attention was paid to promising affinity chromatography techniques utilized immobilized phenylboronic acid, as well titanium dioxide particles. The particular extractions were improved in detail by the optimization of conditioning, washing and elution steps, especially in pH, buffer composition, number a of washing solvents, and duration of interaction time between oligosaccharides and stationary phase. The methods were optimized for the best recovery of selected oligosaccharide mixtures and then extended to wort and beer samples. The developed approaches facilitated the differentiation of beer samples produced by the different technologies. The recovery, efficiency of extraction and purification steps were performed on high performance liquid chromatography technique using refractive index and/or mass spectrometry detection. During the experiments, oligosaccharides were more efficiently retained on graphitic carbon and aminopropyl phase in accordance with the experiences from liquid chromatography. Cartridges filled with the polyamide show a unique ability to separate disaccharides from the higher molecules. Introducing of titania and other covalent interactions required careful buffer selection and subsequent desalting step. Those methods showed only weak retention with no apparent discrimination of structure features.

Keywords: solid-phase extraction, oligosaccharides, sample preparation, beer

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G-10
SPECTROPHOTOMETRIC DETERMINATION OF NITRITE IN CURING MEAT SAMPLES

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The determination of nitrite in various types of samples, particularly environmental samples and foods, is growing in significance as a result of the increasing eutrophication of natural waters and in salinisation of aquifers, as well as the health problems caused cyanosis and the formation of nitrosamines in living organisms from amines and nitrites used as food additives. Alkali nitrites and nitrates, along with sodium chloride have long been used in the curing of meat products to prevent bacterial spoilage and to enhance the flavor, color and texture of these food products. A rapid and sensitive method for the determination of nitrite ion based on the diazo-coupling reaction was studied. Nitrite was reacted with acidified 2-aminobenzoimidazole to yield a water-soluble diazonium ion, which subsequently coupled with orcinol to form an azo dye, having maximum absorption at 305 nm. The calibration graph showed that Beer's law is obeyed over the concentration range of 0.05-3.0 µg/ml of nitrite, with the detection limit of 0.03 µg/ml and the molar absorptivity was 3.06×104 l/mol. cm. The precision and the accuracy were acceptable depending upon the values of relative standard deviation and error percentage. The influence of common interferences was studied and the method was applied for the determination of nitrite ion in cured meat samples. The results were agreed with those obtained by the NEDA standard method.

Keywords: Determination, Nitrite, Spectrophotometry, Meat samples

G-11
PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF HONEY FROM NORTH-WESTERN REGIONS OF IRAN

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In the present study, the microbial (yeast and enterobacterial) contaminations and physicochemical properties (pH, ash, commercial glucose, starch, reducing sugars and moisture) of 263 honey samples from north-western regions of Iran in a 2 years period in different seasons of 2009 and 2010. Results showed that moisture and ash content (0.4±0.01%) of all samples were in the required standard range Levels of reducing sugars and sucrose content of 1.52% and 6.84% samples were unacceptable, respectively. All pH values were in acidic range (4.44±0.02). No commercial glucose or starch was detected in any of samples. Only 7 samples 2.66 (%) contained yeast and 2 samples (0.76%) were contaminated bacteria from family Enterobacteriaceae (2 samples with both contaminations). None of isolates were found to be of major pathogenic importance.

Keywords: Honey, Physicochemical, Microbial, North-Western, Iran

G-12
MERCURY SPECIATION IN WILD MUSHROOMS

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Mushrooms are able to accumulate relatively high concentrations of mercury and, organic compounds are of special concern because of their efficient bioaccumulation, and its marked tendency to be biomagnificated through the food chain. The goal of this work was to develop a simple and low cost procedure based on a non-chromatographic speciation approach. The organic mercury (o-Hg) was calculated as the difference between the total mercury (t-Hg) and the selectively extracted inorganic mercury (i-Hg). Lyophilized samples were extracted with HCl at room temperature and resulted solution was divided in two portions. One portion was reserved to i-Hg determination and the other one was treated with a mixture $KBr/KBrO_3$ in order to carry out the t-Hg determination. The final analysis was performed by cold vapor atomic fluorescence spectrometry (CV-AFS) after dilution in HNO_3 medium. A direct analysis of untreated samples by employing atomic absorption spectroscopy after oxygen combustion-gold amalgamation was also applied for comparative purposes. Previous studies were focused on the evaluation of the extraction capability of various reagents (HCl, HNO_3, H_2SO_4, TMAH, KOH...) under different conditions (microwave, ultrasounds, room temperature), and the studies of the quantitative degradation of organic species into inorganic ones through a simple reaction (with $KBr/KBrO_3$, $KMnO_4$ or $K_2Cr_2O_7$). Special attention was plaid on assuring any inter-species conversions and to overcome matrix interferences at the detection step. The concentrations found in 9 samples of wild mushroom (Lactarius deliciosus) from different geographic origins and marketed in Spain, ranged between 20 ng g^{-1} and 50 ng g^{-1} (fresh matter) being 70% i-Hg and 30% o-Hg.

Keywords: non-chromatographic speciation, mercury, wild mushrooms, cold vapor atomic fluorescence

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G-13
INCREASING SELECTIVITY IN LC/MS/MS ANALYSIS USING TECHNIQUES SUCH AS MRM^3 (MS/MS/MS), DIFFERENTIAL ION MOBILITY AND HIGH RESOLUTION LC/MS/MS

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The demand for speed and cost reduction in food analysis has meant that sample preparation is often simplified. Techniques such as liquid /liquid extractions or QuEChERS are commonly employed in Food Testing but the resulting extracts are more complex leading to matrix interferences which can in turn lead to increased number of false positives in food testing and issues with quantitation. There is therefore a need to increase the selectivity of detection in LC/MS/MS, to get around the issues of matrix interferences but still maintain speed of analysis and the sensitivity needed to reach the regulatory limits. This talk will discuss several different ways to overcome these issues using various types of mass spectrometry techniques. Techniques including MRM^3 and ion mobility separation using the new differential ion mobility interface will be discussed and examples where these techniques have benefits will be shown. In addition the principles of high resolution LC/MS/MS will be described together with examples of where this technique has been applied to food testing.

Keywords: LC/MS/MS, ION MOBILITY, MRM3,

G-14
SIMULTANEOUS DETERMINATION OF SYNTHETIC COLORANTS IN FOODSTUFFS AND BEVERAGES BY HPLC/DAD AND MONITORING RESULTS

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Synthetic dyes are often added to food and drinks to restore their original appearance when color is affected by processing, storage, packaging and distribution. Furthermore colors are used to make food more visually attractive to consumers. The EU Directive 94/36/CE lists the permitted substances that can be used in foodstuffs. The European Food Safety Authority (EFSA) is in charge to reassess all allowed additives according to the Commission Regulation 257/2010 that includes food colors. In order to investigate the content of some permitted and not permitted dyes in food and drinks, a sensitive and helpful method has been developed to determine simultaneously nine synthetic red colorants by high-performance liquid chromatography coupled with diode array detector. Substances involved are Azorubine (E122), Amaranth (E123), Ponceau 4R (E124), Red 2G (E128), Allura Red (E129), Azocarmine B, Azocarmine G, Ponceau 2R, Ponceau 6R. Solid food matrices, e.g. meat and fishery products, were extracted by an hydro alcoholic mixture, cleaned up on polyamide SPE cartridge and eluted with basic methanol solution. Otherwise a simple dilution and filtration of samples were used for drinks. Chromatographic conditions include a C8 column (150 mm × 4,6mm), a gradient elution with acetate buffer pH 7–acetonitrile (99:1 *v/v*) and detection at 515, 570 and 480 nm. The method has been validated according to the Commission Regulation 882/2004 and can be applied to a concentration range between 5-300 mg/kg (5-100 mg/l in drinks) depending on the dye. The accuracy, intra-day and inter-day precision, specificity and ruggedness was assessed. The overall recoveries ranged from 60.4% to 89.3% for foodstuffs and from 63.1% to 101.1% for drinks. The usefulness of the method has been proved and validated for further 5 synthetic yellow colorants in foodstuffs and drinks (E102 Tartrazine, E110 Sunset Yellow, E104 Quinoline Yellow, Orange II and Metanil Yellow) and for 3 blue colorants in drinks (E131 Patent Blue V, E132 Indigo Carmine and E133 Brilliant Blue FCF). During years 2009-2011 136 food samples for synthetic colorants were analyzed. They included fishes, meat, candies, confectionery and bakery products, fruit juices, additives and diet supplements. Most samples were fishery products like tuna fish where the presence of E124 was confirmed in 7 out of 41 samples. Three of them was considered as not compliant because the admitted food dye was not reported on the label. Others matrices were evaluated as regular except for a single candy sample that showed a concentration of E129 above the allowed limit.

Keywords: Food additives, Synthetic dyes, Foodstuffs, Beverages, HPLC/DAD

G-15
MICROBIOLOGISTS MEET ANALYTICAL FOOD CHEMISTS: THE APPLICATION OF LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY FOR THE QUANTIFICATION OF BACILLUS CEREUS TOXIN CEREULIDE IN FOOD

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The emetic toxin of Bacillus cereus (cereulide) has been responsible for some lethal foodborne intoxications. The toxin is pre-formed in food and, due to its highly resistant profile to heat and extreme pH conditions, it is not destroyed by usual food preparation procedures or degraded upon ingestion of contaminated food. A newly developed LC-MS/MS method (LCQ - deca XP, Thermo Fisher Scientific), allows the quantification of cereulide in food with great specificity and sensitivity. The presented and validated method consists of cereulide determination in foods by LC-MS_{ζ}, using low amount of samples and with valinomycin as a surrogate standard. The method was validated in-house. Compared to the boar semen assay, LC-MS/MS is a more specific and quantitative method, with lower detection limits for cereulide determination in complex food matrixes. The method was applied on different complex food matrices (chili con carne, spices, red beans,…) suspected of Bacillus cereus contamination. A high percentage of analyzed samples were shown to contain cereulide (12.5% of chili con carne, 58.3% of red beans and 83.3% of spices). The bacterial counts found in the contaminated foods were very low or even absent

Keywords: Bacillus cereus, Cereulide, Liquid Chromatography, Mass Spectrometry, Toxins

G-16
PROFILING OF HIGHLY COMPLEX CITRUS JUICE SAMPLES USING UPLC ION MOBILITY TIME OF FLIGHT MASS SPECTROMETRY

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Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties. Such attributes mean many flavonoid-containing plant species may be used as functional foods or phytomedicines. The presence of flavonoids in Citrus juices has attracted attention because of their biological and physiological importance. The use of HPLC-MS and HPLC-MS/MS based methods to profile flavonoids has become more routine. The role of flavonoids compounds as markers is important and is a challenge due to sample complexity. HDMS can provide a route to specific and unambiguous identification. As well as enable the unequivocal distinction of flavonoid isomers. High definition mass spectrometry has been utilised to profile citrus juice products and this techniques offers some unique advantages to profiling complex mixtures. It is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility (IM) mass spectrometry is a rapid orthogonal gas separation phase technique that technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape and charge, as well as mass. The study undertaken investigates the use of Ion Mobility separation in combination with UPLC (Ultra High Performance Chromatography). The profiling study undertaken clearly shows the benefits of using HDMS. The results obtained show that isomer/conformational analysis can be performed. It is possible to separate co-eluting analytes and increase peak capacity. This enables single component accurate mass spectra of chromatographic co-eluting components to be obtained. These were used to generate elemental composition information. The enhanced peak capacity enables more information to be extracted from fragmentation studies and the individual MSe fragmentation spectra have been obtained for flavonoid isomers which are co-eluting, from which structural elucidation has been performed. The enhanced peak capacity brought about by profiling using UPLC HDMS can be visualised rapidly using the MSe data viewer. Co-eluting compounds and unknown isomers can to be resolved rapidly.

Keywords: Ion, Mobility, UPLC, Flavonoids, Profiling

G-17
EXPANDING SELENIUM SPECIATION IN WATER AND FOOD

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There are an extraordinary number of selenium compounds which exist in the environment and foods. Although selenium has only two primary oxidation states (Se^{+4} and Se^{+6}), the wide variety of selenium-containing compounds are differentiated by other atoms or organic constituents associated with the selenium, thereby possessing different chemical characteristics. Our previous work focused on separating Se(IV), Se(VI), and SeCN- through the use of reversed-phase ion pairing chromatography using a C8 column. This study extends that work by looking at additional selenium compounds, as well as different separation strategies. Extracted food samples will be examined for selenium species.

Keywords: metals, speciation, HPLC, ICP-MS, selenium

G-18
READING NATURE'S BARCODE: HOW FOODS AND INGREDIENTS CAN BE AUTHENTICATED IN THE SUPPLY CHAIN

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Stable isotope measurements have been proven, over decades, to provide important information about food origin and authenticity, mostly in the academic environment. The amount of isotopes such as carbon-13, hydrogen-2 (deuterium) and oxygen-18 in any plant, compared to their more common alternatives (carbon-12, hydrogen-1 and oxygen-16) is related to plant species (and variety), growing region and growing conditions. This chemical record constitutes Nature's Barcode and can be used as a profile of any food or ingredient. Drying, fermenting, alkalizing, or otherwise processing a food crop can change the signature, but the concept of a testable profile is retained. In addition, long dead plants and organisms have generated oil, coal and gas reserves with discriminating isotopic profiles formed by eons of temperature, pressure and bacterial action. These profiles can be used to distinguish synthetic ingredients such as fragrances and flavors from natural and also profile products from different manufacturing sites. Cavity Ring-Down Spectroscopy (CRDS) is a high precision, high sensitivity device that enables bench-top, fast and easy stable isotope measurements. The key to this optical technique is that it is time-based; which provides the raw performance characteristics for demanding stable isotope measurements. The availability of CRDS is revolutionizing stable isotope analyses; it is an instrument that can be used by any food lab in the world. In this paper we will describe the technology and show data describing the breadth of potential applications. Further, we will use this forum to discuss significant future enhancements that will greatly aid food analyses, such as additional isotopes and off-line and in-line separations for component analysis.

Keywords: isotopes, CRDS, origin, traceability, carbon13

G-19
CHARACTERIZATION OF NITROGENOUS COMPOUNDS OF DIFFERENT TYPES OF BOVINE WHEY

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Beta-lactoglobulin, alpha-lactalbumin and bovine serum albumin, all major constituents of the bovine whey protein fraction, as well as small nitrogenous compounds (peptides, amino acids etc.) are marginally affected during the production of fresh-cheese, thus remain soluble and migrate into the drained-off whey. Given the variable production protocols in cheese making and dairy technology, the qualitative patterns of these whey-intrinsic nitrogenous compounds may vary indeed e.g., sweet whey features an unique macropeptide fraction as result of the enzymatic cleavage of kappa-casein by the specific activity of rennet during the curdling of milk. The major objectives of this work were to characterize the nitrogenous fraction of different bovine whey samples (sweet, sour/acid, and thermo-quark whey, and their respective UF/NF-permeates) based on electrophoretic and chromatographic techniques. Protein/Peptide patterns were assessed by means of classical electrophoretic methods (Native-, SDS-PAGE) as well as by high-resolving peptide mapping using UPLC™. Additionally, the corresponding free amino acid profiles were analyzed using an UPLC™ adapted protocol of the Waters HPLC-AccQ.Tag™ amino acid method. Given that whey macropeptides are only intrinsic to sweet rennet whey, all analyzed samples, except for the thermo-quark whey, indicated rather similar peptide profiles showing beta-lactoglobulin and alpha-lactalbumin as the predominant whey proteins. As for rennet whey, the performed UPLC™ peptide mapping allowed further separation of whey macropeptides into their glycosylated (glycomacropeptide) and non-glycosylated fractions. Due to the modified thermal processing, thermo-quark whey exhibited an extensive decline in both beta-lactoglobulin and alpha-lactalbumin which were thermally coagulated, hence enriched in the resulting fresh-cheese. Additionally, the respective peptide profiles were highlighted by significant variations compared to acid or sweet whey showing an intensively enhanced peptide quantity in the low to medium hydrophobic range. Since the thermo-quark process is characterized by a coagulation using both lactic acid bacteria and rennet, the obtained peptide profiles might result from the non-specific activity of natural rennet and bacterial peptidases at a lower pH. Moreover, consistent results were also obtained for the corresponding free amino acid analyses at which the thermo-quark samples showed a nearly fourfold increase in total amounts compared to that of sweet whey (340 vs. 85 mg/L), thus implying an extended proteolytic process. In general, whey is mostly considered as an accruing dairy by-product with rather little commercial interest. However, in-depth analyses of such products might highlight distinct product characteristic, particularly regarding the nitrogen fraction intrinsic to thermo-quark whey, hence emphasizing the possibility for novel approaches in whey utilization.

Keywords: whey, proteins, peptides, amino acids, UPLC™

G-20
DEVELOPMENT OF NEW CERTIFIED REFERENCE MATERIALS FOR FOOD ANALYSIS BY CONTENT ASSIGNMENT WITH HIGH-PERFORMANCE QUANTITATIVE NMR

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Certified Reference Materials (CRMs) for chromatographic investigation of organic compounds in food and beverages were not available until now. This due to fact that only a very limited number of organic CRMs are available from the national metrological institutes. Quantitative NMR spectroscopy (qNMR) has become an invaluable instrument for exact content assignment and quantitative determination of impurities [1]. 1H-qNMR is a relative primary method because the intensity of a signal is direct proportional to the number of protons contributing to the signal of interest [2]. Therefore the signals of a sample compound and a reference substance with known content can directly be compared and the content of the sample compound can be assigned. Based on this approach an unequivocal traceability to international acknowledged reference standards from a national metrological institute, e. g. from NIST, is achieved [3]. Chromatographic techniques such as HPLC or GC usually do not provide this traceability, because they require a highly pure standard of the substance to be investigated which is often not available. This work presents the approach of content assignment by High-Performance Quantitative NMR (HP-qNMR®) and its application on organic compounds for Food Analysis from different classes such as amino acids, polycyclic aromatic hydrocarbons, pesticides, antibiotics, fatty acids and fatty acid esters, resulting in new Certified Reference Materials for chromatography. The high performance qNMR measurements were performed at maximum accuracy with a 600 MHz NMR instrument under ISO17025 and ISO34 double accreditation resulting in expanded uncertainties between 0.1% and 0.5%.

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Keywords: Certified Reference Materials, Food Analysis

G-21
HIGH MASS ACCURACY IDENTIFICATION OF TARGETED AND NON-TARGETED ANTI – FUNGAL COMPOUNDS PRODUCED BY LACTIC ACID BACTERIA USING THE LTQ – ORBITRAP XL HYBRID MASS SPECTROMETER

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Fungal contamination in food causes health and economic concerns. Several strains of Lactic acid bacteria (LAB) have been recognised to provide anti-fungal activity allowing inhibition of spoilage microorganisms. LAB have GRAS-status (Generally Regarded As Safe) allowing them to be safely integrated into food systems to be used as natural food preservatives. Numerous strains have been isolated and found to show anti-fungal activity but the identification of the key compounds providing this activity has been limited. This method allows for the identification and quantification of 25 known anti-fungal compounds through the development of a long chromatographic run that separates these compounds individually. Chromatographic separation was achieved on a Phenomenex Gemini C18 100A column (150 × 2.0 mm; 5 µm) using a gradient mobile phase of methanol / water spiked with acetic acid (0.1%) at a flow rate of 0.3 ml/min. This stepped gradient developed from 10–95 % methanol over 70 minutes. The LC was coupled to a LTQ - Orbitrap XL Hybrid Mass Spectrometer operated in negative ionisation mode. High mass accuracy data (< 2 ppm) was obtained using the high resolution (60,000K) Fourier Transform (FT) MS, this allowed the unequivocal identification of known targeted compounds. Fragmentation studies (MSn) on targeted compounds was achieved using the Linear Ion Trap (LIT) which along with high mass accuracy data from the FT-MS can aid the development of spectral libraries. This methodology permits the comprehensive profiling and comparison of various LAB strains and is also proved beneficial for the identification of unknown compounds produced by these strains.

Keywords: lactic acid bacteria, anti-fungal compounds, natural food preservative, LTQ – Orbitrap

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G-22
PACKAGING RAW TURKEY SKEWER IN MODIFIED ATMOSPHERE

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Packing foods under modified atmosphere, in a convenient container, can offer extended shelf life and improved product presentation making products more attractive to the retail customer. This packaging systems allows products shelf life extension, by slowing biochemical deteriorative reactions and slowing growth of spoilage organisms. When CO_2 is required to control the bacterial and mould growth, a minimum of 20% is generally used. Optimal levels appear to be in the region of 20–30% (v/v). Packaging fresh meat is carried out to delay spoilage, permit some enzymatic activity to improve tenderness, reduce weight loss, and, where applicable, to ensure an oxymyoglobin or red colour in red meats at retail or customer level. The main objective of this study was to find the best gas mixture to introduce in the packaging system without affecting products organoleptic properties over storage time. Laboratory assays were performed using different gas mixtures. In the first assay, in order to evaluate the effect of O_2 on storage, raw turkey skewers were packed under three diferent gas mixtures 0/40, 10/40 and 40/40 (%O_2/%CO_2, and N_2 to complete) and stored over 15 days at 5°C. Other two assays, used mixtures with 0/40 and 20/40 O_2/CO_2 were performed this time storing over 18 and 13 days. Analyzed quality parameters were: overall appearance of the packaging system, products weight loss, pH, color, microbial growth and texture analysis (hardness, elasticity, cohesiveness) over storage time. Sensory evaluation was performed by a 8 member semi-trained panel, taking into consideration manly defects and evolution of organoleptic properties during 0, 5 and 12th of storage. Acceptability evaluation was also performed by 122 consumers of ESTG canteen. Results and discussions: Overall appearance indicates that 0/40 O_2/CO_2 is the best mixture that also maintains other organoleptic properties (color and smell) and bacteriostatic effect (microbial inhibit). Results show that turkey skewer can be safely consu! med up t o 12 days, using MAP. Acceptability show that samples packaged in 0/40 O_2/CO_2 were the preferred ones with acceptability between 6 and 7 in a 9 point hedonic scale, being the ones packed under 20% O_2 the less preferred. Significant differences between samples packaged in 0/40 O_2/CO_2 and the other samples were verified by T-student test. The presence of O_2 in mixtures with 10/40, 20/40 and 40/40 O_2/CO_2 leads to color changes (yellow), off flavors and pack collapse. Microbiological analysis indicates that breast meat seams more susceptible to microbiological contamination than turkey thigh meat products. Although, no pathogens were found after performing a microbiological analysis at 12th days to turkey skewers. Canonical variate analysis showed significant differences between samples from the 2 assays. This is mainly due to the different meat used influencing texture and color properties, in spite of used gas mixtures being the same in both assays.

Keywords: Shelf life, sensory evaluation, modified atmosphere packaging, poultry meat

G-23
DEVELOPMENT OF A SPECTROPHOTOMETRIC QUALITATIVE AND QUANTITATIVE METHOD TO DETECT THE AMOUNT OF CARBON MONOXIDE IN FRAUDULENTLY TREATED MEAT AND FISH PRODUCTS

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The freshness of any kind of meat is normally judged by its bright red colour due to the oxy-myoglobin complex [MbO_2], present in the red muscle fibres. Upon aging, the red colour of meat gradually changes into various shades of brown due to oxidation and conversion of ferrous MbO_2 to the brown ferric metMb. In order to preserve the appealing colour of fresh meat, carbon monoxide (CO) is commonly used in the Modified Atmosphere Packaging (MAP) system in the United States and in the Netherlands [1]. CO combines with Mb to form a cherry-like coloured complex, MbCO, that is much more resistant towards oxidation than oxy-Mb. However, CO treatment can mislead the consumers, who often evaluate the freshness of the fillets exclusively by their colour. In fact, although CO-treated food consumption does not represent a risk for the consumer, prolonged shelf-life can be associated to toxicological risks due to growth of pathogenic bacteria (e.g. C. botulinum, Salmonella and E. coli), or due the presence of high level of histamine formed by the histidine decomposition in histidine-rich fishes (e.g. tuna). CO treatment is not admitted in the European Community, and CO is not included in the list of the allowed food additives (Directive 95/2/EC). Simple and rapid spectrophotometric procedures for the determination of CO-treatment of perishable food are presented. These methods allow us to detect (i) qualitatively and (ii) quantitatively the presence of MbCO in CO-treated tuna fish and beef meat. The qualitative method is based on the combined analysis of electronic absorption spectra (UV-Vis) in their normal and second derivative modes [2]. Treated and untreated samples could be distinguished by their peak positions and relative intensities, since the wavelength maxima vary according to the oxidation, spin, and coordination states of the heme iron [3]. The quantitative method allows one to determine quantitatively CO concentration in the meat drip by simply measuring three different absorbance values in the UV-Vis spectrum of an unknown sample. This method is presently used in the official laboratories of food control of the Italian Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana. The spectrophotometric method has been compared with the HS-GC–MS techniques by analysing untreated and treated tuna fish samples. The comparison has revealed that both methods are able to discriminate between treated and untreated samples, although the quantitative analysis cannot be directly compared [4,5]. The origin of this discrepancy will be discussed.

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Keywords: CO, UV-vis, tuna fish, HS-GC-MS, meat

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G-24
MONITORING OF IMPORTED IRRADIATED LIVESTOCK PRODUCTS USING ELECTRON SPIN RESONANCE SPECTROSCOPY AND GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Statistically randomly selected 290 livestock products; beef, pork, chicken, and processed products imported to Korea in 2010 were analyzed for the detection of irradiation process. Gas chromatographic analysis of specific hydrocarbons was applied to the food containing fat, and ESR spectroscopy was applied to food containing bone. Signals from free radicals induced during irradiation, captured in hydroxyapatite of bone structure, were characterized by their asymmetric shape and g-values. 135 samples (113 beefs, 22 porks) from 12 countries were analyzed by ESR spectroscopy, and the results were all negative. Extracted fat from meats and processed products were analyzed by GC/MS for detecting irradiation induced specific hydrocarbons, 1,7-hexadecadiene and 8-heptadecene from meat and 1,7-hexadecadiene from processed products such as beef jerky and egg york powder. 155 samples (58 beefs, 67 porks, 11 chickens, and 19 processed products) from 17 countries were analyzed by GC/MS and no irradiation specific hydrocarbons were detected. These results showed that tested samples were not treated by irradiation.

Keywords: Irradiation, ESR, GC-MS

G-25
ADVANCED MULTI-TARGET COMPARATIVE SCREENING USING HIGH RESOLUTION AND ACCURATE MASS LC–MS/MS

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LC-MS/MS using Electrospray Ionization (ESI) is a powerful analytical tool for the analysis of a wide molecular weight range of polar, semi-volatile and thermally labile compounds. Especially triple quadrupole based mass analyzers are popular for targeted quantitation of hundreds of food contaminants in a single analysis because of their extra degree of selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode. Advancements in LC-MS/MS technology, including hybrid systems like triple quadruple linear ion trap (QTRAP®) and triple quadrupole Time-of-Flight (TripleTOF™), now provide the ability to perform non-targeted screening on a routine basis. However, since this workflow does not use a target analyte list, compound detection is not based on any a priori knowledge, such as retention times and information on molecular and fragment ions. In addition, full scan chromatograms are very rich in information and contain easily thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Here we present residue results of using a novel approach of comparative multi-target screening using a generic extraction and LC separation procedure followed by high resolution and accurate mass MS/MS detection. TOF-MS and MS/MS data was acquired using an AB SCIEX TripleTOF™ 5600 system. TOF-MS information was used to screen for targeted food contaminants. Quantitative information was achieved by performing single concentration standard addition at the level of the Maximum Residue Level (MRL). Identification was based on retention time, accurate mass quasi-molecular ion, isotopic pattern and MS/MS fragmentation pattern.

Keywords: LC-MS/MS, pesticides, accurate mass, high resolution, software

G-26
ESTIMATION OF TOTAL EXPOSURE TO ALUMINIUM

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Despite of the presence of aluminium (Al) in small amounts in all organisms, there is still no known functional value to it in any of them. Quite the contrary, it is now apparent in certain pathological states that the body burden of Al may be markedly increased with resulting toxicity. Its absorption is very low independently of the way of exposure (< 1% for oral and cutaneous exposure and < 3% for respiratory exposure). An estimation of the total exposure to Al of the adult Belgian population is the aim of this study. Different ways of exposures are taken into account: (1) alimentation (Al content of food samples and migration from food contact material); (2) inhalation from air and ingestion of dust/soil; (3) beauty products, and (4) pharmaceutical products. A representative food list of the foods normally consumed by the Belgian population was established from the National Food Consumption Survey with 499 individual foods (as composites). Design of experiment (DOE) is used to establish a model determining the influence of temperature, pH and contact time on the Al migration from 7 possible food contact materials. The average surface of food contact materials used by the Belgian population is retrieved from the survey on the use of the kitchen ware. Aluminum exposure from air, dust and soil, personal care and pharmaceutical products (buffered aspirin and antacids) are estimated from literature. All data will be available at the conference.

Keywords: risk assessment, aluminium in food, migration, total exposure

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G-27
ION MOBILITY SPECTROMETRY AS NOVEL TECHNOLOGY FOR THE QUALITY CONTROL IN FOOD INDUSTRIES

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Since years, ion mobility spectrometry is known as a powerful technique for the detection of traces of volatile organic compounds especially in the security field like the detection of explosives on airports. The ion mobility spectrometry (IMS) is a very sensitive physical measuring method for chemicals containing a heteroatom (e.g. aldehydes, ketones), which are common compounds in flavours and odours. The use of IMS could be beneficial for any kind of sample and task, resp., where the focus of interest is on the samples flavour and its composition. The technology offers advantages such as speed and simplicity and, beyond that, it allows to reliably characterize products with respect to quality, authenticity etc.. By analysing the headspace of the solid or liquid samples IMS systems can also be applied as online process monitor e.g. for the off-flavour detection. The system that will be presented is a combination of an ion mobility spectrometer (IMS) and a gas chromatographic column (GC) that greatly overcomes the limitations of selectivity of the IMS in complex matrices. To achieve an improved reproducibility and make the operation of sampling easy and transparent the FlavourSpec® is set up with an automatic headspace sample injector. Due to the dual-separation by GC and by IMS and the compound detection (IMS), a measurement reveals a sample headspace composition in a three dimensional dataset. Besides the classical analytical evaluation, like e.g. the quantification of individual compounds, an automated software for relating samples under investigation to freely selectable, custom-made sample libraries is available. Sample identification and classification thereby is achieved without operator effort. The device is successfully used for a wide range of applications, like e.g. the determination of the freshness of fish, differentiation of different brands and charges of tea and the detection of different aroma compounds. Next to the theory of IMS the working principle of the device, data handling procedures as well as measurements results will be presented.

Keywords: ion mobility spectrometry, gas chromatography, flavour, automated data analysis

G-28
EVALUATION OF SOME OF THE MAIN INORGANIC IONS IN BRINE SOLUTIONS USED FOR SEA SALT PRODUCTION BY FLOW INJECTION ANALYSIS AND FOURIER-MID INFRARED SPECTROSCOPY

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In Portugal the production of 'traditional' sea salt is seen as a sustainable activity with a production increases during the last years [1]. Today, this type of salt is considered a 'gourmet' product due to its distinctive physico-chemical characteristics. The extraction of the 'traditional' sea salt is a hand-labour activity which requires the correct management of brine flows coming from the sea which travels through well defined sea pans areas until sodium chloride (NaCl), together with other types of salts (i.e. $MgSO_4$, $MgCl_2$ and others), crystallizes. The determination of inorganic constituents in the brine solutions which are used for the production of 'traditional' sea salt is of vital importance as the presence of trace elements in those brines will determine the final quality of 'traditional' sea salt. The main objective of the presented work was to ascertain whether the application of Fourier-mid-infrared spectroscopy in combination with flow injection analyses are well suitable for the rapid and simple evaluation of brine solutions in terms of inorganic constituents (i.e. sulfates and nitrates) and other parameters like the salinity and how these parameters are also influencing the final quality of sea salt.

[1] INE (2010) Statistical Yearbook of Portugal – 2009. Instituto Nacional de Estatística, I.P. Ed. p. 367. ISBN 978-989-25-0047-8

Keywords: traditional-sea-salt, sea-water, trace-elements, flow-analysis, infrared-spectroscopy

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G-29
THE DETECTION OF ARTIFICIAL SWEETENERS BY LC/MS/MS

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Introduction As we aim to eat less sugar, many of us are turning to alternative sweeteners. Intense sweeteners such as Acesulfame-K are very low in calories but as with additives, sweeteners are thoroughly assessed for safety, and are only permitted to be used in a limited range of products. The European Parliament has set out guidelines for the labeling of food with regards to sweeteners and the presence of aspartame and aspartame-Acesulfame salt should state that the food 'contains a source of phenylalanine'. At present standard methods for the detection of sweeteners in food use HPLC with evaporating light scattering detection. This work shows some initial data where LC/MS/MS has been used to detect artificial sweeteners in diet drinks.
Methods Samples of soft drinks such as cola, orange flavoured fizzy drink and lemonade were just diluted in water. Extracts were analysed by reversed-phase HPLC on a polar end capped column (4 µm, 2.1 mm × 150 mm) using a Shimadzu UFLC System over a 6 minute gradient from 1% to 99% Acetonitrile in water. Mass Spectrometry Analysis was performed on an ABSCIEX 3200 QTRAP® system with Turbo V™ source in negative ion electrospray mode At present standard methods for the detection of sweeteners in food use HPLC with evaporating light scattering detection. This work shows where LC/MS/MS can be used to detect 7 commercially available artificial sweeteners in diet drinks and baby food which were obtained from local supermarkets.
Preliminary Data At present standard methods for the detection of sweeteners in food use HPLC with evaporating light scattering detection1. This work shows where LC/MS/MS can be used to detect 7 commercially available artificial sweeteners in diet drinks and baby food which were obtained from local supermarkets. In all cases due to the sensitivity of the technique and the level of artificial sweeteners in the products tested the samples had to be diluted 1000 fold before analysis thus reducing the effects of matrix on the analysis and simplifying sample preparation. The method is greater than 5 times quicker than non LCMS methods currently available.

Keywords: artificial sweeteners, LC/MS/MS, dilute and shoot approach

G-30
HIGH RESOLUTION TOF-MS PROFILING OF LISTERIA MONOCYTOGENES

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Introduction Food-borne pathogens pose serious health risks to the general population. As such, a great deal of emphasis has been placed on the development of methods for screening and profiling microorganisms causing food-related illnesses. The Listeria species group has received a great deal of focus due to recent events. Mass spectrometric methods for the differentiation of Listeria species and subspecies involve the analysis of biomolecules and offer distinct advantages to currently-used serological or PCR methods. This work describes the profiling of Listeria bacteria using high-resolution time-of-flight mass spectrometry analysis of bacterial metabolites obtained directly from cultures by simple extraction, followed by principal component analysis.
Methods Cultures from 12 strains of Listeria monocytogenes were inactivated by treatment with ethanol. Cultures were centrifuged and the cell pellet resuspended in 70% formic acid/acetonitrile. After vortexing and centrifugation the supernatant was removed, lyophilized and resuspended in 5mM ammonium formate. Samples were analyzed by LC-MS using a hybrid quadrupole/time-of-flight mass spectrometer. Samples were analyzed in both polarities over the mass range 100-2000 Daltons using generic 40 minute gradients. Samples were run in triplicate and in random order to minimize LC/MS drifting effects. The data was processed via principal-component analysis (PCA) and subsequent principal component variable grouping to find correlated variables. After removing any variables arising from apparent LC/MS drift, the remaining variables were analyzed to find potential species-specific markers. Preliminary Data Over 121,000 data points, corresponding to 3102 peaks (unique *m/z* at a specific retention time after peak finding and alignment) were identified from the data set. Principal component variable grouping was applied to remove variables that were found to be the result of experimentally-derived drift over time. After removal of these variables, principal component analysis was applied and a smaller number of variables was found showing clear separation of the various strains of Listeria monocytogenes. The *m/z* of the features could be extracted directly from the data. Due to the high resolution and high degree of mass accuracy achievable on the MS system used (< 2 ppm RMS) and the fast acquisition speeds enabling large numbers of MS/MS per scan cycle, software tools were used to match the precursor m/z and the fragmentation profiles to predict the chemical formulas and identities of these strain-distinguishing features. Future MRM assays could be developed using these markers to potentially screen other samples in complex matrices.

Keywords: High resolution TOF-MS, Listeria monocytogenes, bacterial metabolites

G-31
AUTOFLUORESCENCE SPECTRAL TECHNIQUE FOR MONITORING MEAT DEGRADATION AND DETECTION OF CONTAMINANTS

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Several noninvasive techniques have been developed for the quality analysis of meat products. One of them is optical technique. The optical technique is sensitive to the chemical compositions and can be more portable than other techniques, such as X-ray technique. There are many levels in the quality control but here we have investigated an optical technique to monitor the degradation of the meats and the contamination by the animal hair on the meat surface. The optical technique will enhance the detectability of the visual inspections and give a quantitative measure of the analysis. In this research, we have focused on the autofluorescence analysis of flesh meat by the excitation of violet to near-ultraviolet light. The autofluorescence from the meat with the excitation of violet and near-ultraviolet region gives the valuable information on the native chromophores in tissue, such as Flavins, NAD(P)H, Collagens and Porphyrins or other compounds. These spectrum are partly masked by very strong absorption band of hemes due to the internal absorption effect. Thus, the autofluorescence spectrum carries not only the information on such fluorescent internal markers but also the absorption by hemes. Further, the spectrum may be modified by the other chromophores of the contaminants. Herein, we aim to find out the spectrum change with respect to the degradation of meats. The autofluorescence spectrum from beef, pork and ground pork, with an excitation at 445nm, were measured at the several different regions on the surface of the meat. Then, the spectrum change of the meat, which was stored in refrigerator at 4-5 degree up to one week, was monitored. Initially, the spectrum from the fresh meat shows a peak at about 515nm and two dips at about 556 and 580nm, which can be attributed to the fluorescence of flavins and the absorption by oxy-myoglobin, respectively. With the passage of time, these two dips in the spectrum were gradually disappeared and a smoothed single spectrum was observed. This change was more clearly evaluated by the intensity ratios. The intensity ratios at 557nm to 515nm and at 580nm to 515nm were increasing almost monotonically with time. This time trend can be found in all tested meats although the ratios were considerably varied with individual measurement regions in case of beef and ground pork. The relative change of the ratio might be used as a scale of the degradation of meats. We have also tested the detection of white body hairs of swine on the meats as an example of screening of the contamination. Usually, the white hair is difficult to see on the pork meat but it emits rather strong fluorescence, which shows a slightly shorter shoulder in the fluorescence spectrum. The hair on the meat could be distinguished by using the intensity ratio at 520-580 nm to 466-500 nm. These results indicate that the fluorescence technique will be useful to monitor the degradation and detection of the contamination in meat processing.

Keywords: Meat, degradation, contamination, autofluorescence, imaging

Acknowledgement: This work is supported by A-step by Japan Science and Technology Agent.

G-32
ANALYSING BEVERAGE STABILITY IN THE BOTTLE: DEVELOPMENT OF AN IN-SITU DATA LOGGER SYSTEM

Stefan Castritius[1], **Damian Wyrobek**[2], **Mirko Geier**[3], **Jana Gierds**[4], **Nina Baumjohann**[5], **Phuc Nguyen**[6], **Diedrich Harms**[7*]

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During storage and transport of beverages in bottles and containers, environmental conditions such as temperature, light and mechanical impact affect the quality of the beverage [1]. Gas diffusion, chemical reactions of phenolic substances, oxygen and other reactions in the bottle may result in undesirable haze formation, changes in beverage colour or pressure lost [2]. Changes in beverage stability during storage and transport highly depend on the environment conditions, the chemical composition of the beverage and the packaging [3]. Investigations according the influence of temperature, light and mechanical impact on the stability of beverages have shown high interactions. The influence of environmental conditions on the beverage stability is of considerable interest for the beverage industry to understand the chemical and physical stability of beverages under certain conditions. To study the interactions in detail, an in-situ Data Logger System was developed, to monitor time-resolved the chemical and physical stability of beverages in the package. The sensor system comprises of an optical sensor, measuring the beverage turbidity in transmittance and 90° scattering angle at a wavelength of 640 nm and the optical density in transmittance at a wavelength of 430 nm as a measurement of the beverages colour. An additional sensor for pressure and temperature measurement is integrated into the sensors system. In order to be usable in the proposed application, the system requires a very low power consumption. Due to this requirement, the sensor system has a clocked measuring and standby cycle. During the measurement cycle, all measurements are performed and the results are saved on a flash memory card. All sensors, the micro controller and the flash memory card are integrated in a cylindrical stainless steel housing with the measures 16 mm × 100 mm. The Data Logger System enables in-situ investigations of the beverages turbidity, colour, temperature and pressure every 30 minutes over a period of four months to study the chemical and physical stability of beverages time resolved.

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Keywords: haze, stability, beverage, data logger, colour

Acknowledgement: This study was supported by the German Federal Ministry of Economics and Technology (BMWi) via the AiF - German Federation of Industrial Research Associations.

G-33
A NEW SCREENING METHOD WITHIN THE FRAMEWORK OF QUALITY MONITORING FOR BEVERAGES USING HPLC–ESI–MS/MS

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With the aid of modern analytical methods it is possible to evaluate the quality of beverages like beer, spirits or mineral water in detail. Carbonyl compounds play an important role regarding the quality of different kind of beverages. A differentiation has to be made between carbonyls, which are volitional or undesired in the product. In spirits there are both existing, carbonyls which account for the flavour like benzaldehyde, and carbonyls, that present contaminations (e.g. acrolein). The aging compounds in beer belong to the undesired carbonyls. Due to their very low odour and taste threshold, for instance the odour threshold of 2-(E)-nonenal is 0.04-0.5 µg/L [1], low concentrations affect the quality of beer. Mineral water in PET-bottles can be contaminated with acetaldehyde, which can migrate from the PET-material into the beverage [2]. These carbonyl compounds can be determined using an appropriate screening-method and the results can be used for the evaluation of the quality as well of food safety. It is possible to determine these carbonyls with the use of a reagent (Dinitrophenylhydrazine = DNPH), that converts them into yellow to orange coloured derivatives. By stripping the carbonyls with a gas stream and slight temperature, they are trapped on a DNPH-impregnated silicagel-cartridge and eluted afterwards. Normally these derivatives are analyzed by UV-detection after liquid chromatography. To increase the sensitivity and selectivity of the method the derivatives are determined by high performance liquid chromatography coupled with electrospray-ionization and tandem mass spectrometry (HPLC-ESI-MS/MS). The method described can be extended for further carbonyl compounds, for aldehydes as well as for ketons. Additionally, it is possible to search for unknown carbonyls and to generate finger prints.

[1] Schieberle P, Komarek P (2002) Freshness and Shelf Life of Foods, Ch. 5: 70-79.
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Keywords: carbonyls, HPLC, Dinitrophenylhydrazine, beverages

G-34
THE ADVANCED APPROACHES TO NUTRITIONAL AND BREADMAKING QUALITY DETERMINATION OF WHEAT, BARLEY AND RYE FLOUR AND THEIR BLENDS

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Starch, protein and non-starch polysaccharides are main components of cereals. The content and quality of proteins and content of damaged starch are important by reason of the technological quality of flours. The high content of high molecular weight proteins are considerable for bread technology especially, while soluble protein fractions and non-starch polysaccharides, dietary fibre, are important for nutrition. The set of wheat, barley and rye flours and their blends were analyzed and their qualitative parameters were determined (fractionation of protein, Zeleny test, solvent retention capacity, wet gluten content, gluten index, falling number, determination of soluble, insoluble and total dietary fibre). Fourier transform-infrared spectroscopy screening and principal component analysis correlated to various nutritional and breadmaking parameters.

Keywords: cereals, quality, FT-IR spectroscopy, PCA

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G-35
IDENTIFICATION AND STRUCTURAL ELUCIDATION OF TWO NOVEL GLUCOSINOLATES IN AUBRIETA DELTOIDEA USING UPLC QTOF MS WITH ION MOBILITY

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Here we present the use of the Waters SYNAPT® G2 HMDSTM system in the identification of two novel glucosinolates (GLS), methylthio-3-oxononyl-GLS and methylsulfinyl-3-oxononyl-GLS in Aubrieta deltoidea. While rock cress is not a food plant it was apparent that it contained new GLS and was considered suitable as a food model. This ultra performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-QTof-MS) system in combination with MassFragmentTM software provided a high level of specificity for rapid structural elucidation of bioactive glucosinolates in complex plant matrices. The nature of GLS with their endless range of side-chain modification makes identification and structural elucidation challenging. Freeze dried samples were extracted with 70% methanol. GLS were isolated by weak anion exchange solid phase extraction cartridges to produce extracts for analysis that were free of non-GLS components. Rapid chromatographic separation was achieved using ACQUITY UPLC® HSS T3 2.1 × 100 mm, 1.8 µm column. GLS were detected by negative electrospray QTof- MS. The extracts were initially screened for known GLS compounds. During this process two unidentified peaks were detected in the total ion chromatogram at 3.08 and 5.18 minutes. Extraction of MS spectra showed major deprotonated ions at *m/z* 520.0981 and 504.1032. When these exact masses were analysed by the elemental composition calculator the formulas $C_{17}H_{31}NO_{11}S_3$ (methylsulfinyloxononyl-GLS) and $C_{17}H_{31}NO_{10}S_3$ (methylthiooxononyl-GLS) were each top hits, using iFITTM, with < 1 ppm mass accuracy. The high energy MSE spectra were processed using MassFragment™ to assign the fragment ions to the new GLS structures. A total of 26 accurate mass fragments were assigned to methylsulfinyloxononyl-GLS and 18 to methylthiooxononyl-GLS, providing significant structural information to support the proposed structures. The predominant fragmentation ions were from desulfation and removal of the terminal methyl-sulfinyl groups. The MSE fragments *m/z* 182.9660, 228.0331, 312.0212 and 344.0103 suggest that the [absent] oxygen function is further along the alkyl chain, i.e. not on C1-C2. Further experiments using ion mobility provided specific fragmentation information of the first generation fragments to support the identifications. Fragments such as *m/z* 262.0749 provided an unambiguous diagnostic ion at *m/z* 191.9967, where the mechanism of the complete removal of the C5-C9 chain positions the oxo-function on C3. An analogous ion at *m/z* 241.0019 provided evidence of the positioning of the 3-oxo group in methylthio-3-oxo-nonyl-GLS.

Keywords: Glucosinolates, UPLC-QTof-MS, Structural Elucidation, Aubrieta deltoidea

Acknowledgement: Although no financial support was provided by the UK Food Standards Agency (FSA), this work arose from the outcomes of contract E01086, and that support is gratefully acknowledged. The conclusions and opinions expressed are the views of the author alone.

G-36
RELATION BETWEEN LOT SIZE AND SAMPLE SIZES IN SAMPLING PLANS FOR FOOD INSPECTION

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Food inspection at border has essential role in protecting people's health from food related pathogens. Most countries operate such inspection system. The procedure includes collecting samples from imported lot and analysis of the sample. Recently, it is more and more acknowledged that the uncertainty arising from sampling is often the major source of total measurement uncertainty. In the recent Codex Committee of Method on Analysis and Sampling (CCMAS), sampling uncertainty is discussed in a scientific basis. Indeed, improper sampling may lead to improper results and therefore improper judgment about acceptance/rejection of the lot. Therefore, there are a lot of documents regarding the method of sampling. For example, food sampling methods in EU is described in a EC directiveon with a table of sample size and lot size. In food sampling practice,, it is often the case that sample size is changed depending on the lot size with a relation. Codex guideline (CAC/GL 50) indicates that it is not necessary to increase the sample size depending on the lot size to judge a meet the specification more correctly. Namely, there is no relation between lot size and sample size in statistical point of views. At the same time, the guideline justifies the increase of sample size by lot size for considering the economic and health impact if the lot was accepted/rejected basing on the incorrect judgment. Codex guideline (CAC/GL 54) notices that the uncertainty comes from various stages of sampling and how to consider those uncertainties. The primary source of sampling uncertainty is population variance and the sample size has a essential role to determine the sampling uncertainty. We have studied the relation between sampling design and uncertainty. In this study, we analyzed the relation between sample size and lot size, and the result shows that sample size are often determined as proportional to square root of the lot size. We found the proportion coefficient between the sample size and square root of lot size changes by 30% for the samplesize to lot size relation described in FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (AQL - 6.5) (CAC/RM 42-1969), which lists tables on required sample size for a certain lot size. The table covers lot-size from less than 4800 to exceeding 240001, in the case where container size is less than 1 kg. The table also shows the case where container size is between 1kg to 4.5 kg and container size is over 4.5 kg. This relation between lot size and sample size is cited or used in codex adopted method of testing wide ranges of foods including agricultural and fishery products, especially where the purpose of testing is about the quality of food. We conducted same analysis for the case of other container size.

Keywords: sampling, uncertainity, sample size, food, codex

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G-37
AUTOFLUORESCENCE SPECTRAL TECHNIQUE FOR MONITORING MEAT DEGRADATION AND DETECTION OF CONTAMINANTS

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Several noninvasive techniques have been developed for the quality analysis of meat products. One of them is optical technique. The optical technique is sensitive to the chemical compositions and can be more portable than other techniques, such as X-ray technique. There are many levels in the quality control but here we have investigated an optical technique to monitor the degradation of the meats and the contamination by the animal hair on the meat surface. The optical technique will enhance the detectability of the visual inspections and give a quantitative measure of the analysis. In this research, we have focused on the autofluorescence analysis of flesh meat by the excitation of violet to near-ultraviolet light. The autofluorescence from the meat with the excitation of violet and near-ultraviolet region gives the valuable information on the native chromophores in tissue, such as Flavins, NAD(P)H, Collagens and Porphyrins or other compounds. These spectrum are partly masked by very strong absorption band of hemes due to the internal absorption effect. Thus, the autofluorescence spectrum carries not only the information on such fluorescent internal markers but also the absorption by hemes. Further, the spectrum may be modified by the other chromophores of the contaminants. Herein, we aim to find out the spectrum change with respect to the degradation of meats. The autofluorescence spectrum from beef, pork and ground pork, with an excitation at 445nm, were measured at the several different regions on the surface of the meat. Then, the spectrum change of the meat, which was stored in refrigerator at 4-5 degree up to one week, was monitored. Initially, the spectrum from the fresh meat shows a peak at about 515nm and two dips at about 556 and 580nm, which can be attributed to the fluorescence of flavins and the absorption by oxy-myoglobin, respectively. With the passage of time, these two dips in the spectrum were gradually disappeared and a smoothed single spectrum was observed. This change was more clearly evaluated by the intensity ratios. The intensity ratios at 557nm to 515nm and at 580nm to 515nm were increasing almost monotonically with time. This time trend can be found in all tested meats although the ratios were considerably varied with individual measurement regions in case of beef and ground pork. The relative change of the ratio might be used as a scale of the degradation of meats. We have also tested the detection of white body hairs of swine on the meats as an example of screening of the contamination. Usually, the white hair is difficult to see on the pork meat but it emits rather strong fluorescence, which shows a slightly shorter shoulder in the fluorescence spectrum. The hair on the meat could be distinguished by using the intensity ratio at 520-580 nm to 466-500 nm. These results indicate that the fluorescence technique will be useful to monitor the degradation and detection of the contamination in meat processing.

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G-38
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Stefan Castritius[1], **Damian Wyrobek**[2], **Mirko Geier**[3], **Jana Gierds**[4], **Nina Baumjohann**[5], **Phuc Nguyen**[6], **Diedrich Harms**[7,*]

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G-39
A NEW SCREENING METHOD WITHIN THE FRAMEWORK OF QUALITY MONITORING FOR BEVERAGES USING HPLC–ESI–MS/MS

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With the aid of modern analytical methods it is possible to evaluate the quality of beverages like beer, spirits or mineral water in detail. Carbonyl compounds play an important role regarding the quality of different kind of beverages. A differentiation has to be made between carbonyls, which are volitional or undesired in the product. In spirits there are both existing, carbonyls which account for the flavour like benzaldehyde, and carbonyls, that present contaminations (e.g. acrolein). The aging compounds in beer belong to the undesired carbonyls. Due to their very low odour and taste threshold, for instance the odour threshold of 2-(E)-nonenal is 0.04-0.5 µg/L [1], low concentrations affect the quality of beer. Mineral water in PET-bottles can be contaminated with acetaldehyde, which can migrate from the PET-material into the beverage [2]. These carbonyl compounds can be determined using an appropriate screening-method and the results can be used for the evaluation of the quality as well of food safety. It is possible to determine these carbonyls with the use of a reagent (Dinitrophenylhydrazine = DNPH), that converts them into yellow to orange coloured derivatives. By stripping the carbonyls with a gas stream and slight temperature, they are trapped on a DNPH-impregnated silicagel-cartridge and eluted afterwards. Normally these derivatives are analyzed by UV-detection after liquid chromatography. To increase the sensitivity and selectivity of the method the derivatives are determined by high performance liquid chromatography coupled with electrospray-ionization and tandem mass spectrometry (HPLC-ESI-MS/MS). The method described can be extended for further carbonyl compounds, for aldehydes as well as for ketons. Additionally, it is possible to search for unknown carbonyls and to generate finger prints.

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Keywords: carbonyls, HPLC, Dinitrophenylhydrazine, beverages

G-40
THE ADVANCED APPROACHES TO NUTRITIONAL AND BREADMAKING QUALITY DETERMINATION OF WHEAT, BARLEY AND RYE FLOUR AND THEIR BLENDS

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Starch, protein and non-starch polysaccharides are main components of cereals. The content and quality of proteins and content of damaged starch are important by reason of the technological quality of flours. The high content of high molecular weight proteins are considerable for bread technology especially, while soluble protein fractions and non-starch polysaccharides, dietary fibre, are important for nutrition. The set of wheat, barley and rye flours and their blends were analyzed and their qualitative parameters were determined (fractionation of protein, Zeleny test, solvent retention capacity, wet gluten content, gluten index, falling number, determination of soluble, insoluble and total dietary fibre). Fourier transform-infrared spectroscopy screening and principal component analysis correlated to various nutritional and breadmaking parameters.

Keywords: cereals, quality, FT-IR spectroscopy, PCA

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G-41
NEW SOFTWARE FOR THE IDENTIFICATION AND CHARACTERIZATION OF PEPTIDES GENERATED DURING FONTINA CHEESE RIPENING USING MASS SPECTROMETRY DATA

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The microbiological profile in raw milk cheese is typically characterized by a multitude of microbial groups; during cheese ripening a wide range of enzymes interact, hydrolyzing caseins into peptides and free amino acids. Although a number of microbial enzymes are common to many cheese varieties, the final peptide composition of a cheese reflects its characteristic ripening process. The peptide profile composition differs according to different stages of aging, type of manufacturing, and territory, resulting in the flavour and texture characteristic of the particular variety. The peptide profile may cover thousands of peptides derived from the four original casein molecules of different genetic and chemical variants and other, recently discovered, of non-proteolytic origin but synthesised de novo in cheese by enzymatic activities. An effective way of acquiring more information on the proteolytic process in cheese is to identify the peptides produced throughout ripening. Some proteolytic peptides have been identified in exploratory studies mainly using amino acid sequencing and mass spectrometry, but little information is available on the peptide profile of Fontina cheese, an Italian semi-hard and semi-cooked cheese, marked with the quality P.D.O.label, typically produced in Aosta Valley, made from raw cow milk and ripened for at least 3 months in natural caves. Nowadays various software, available online, allow users to identify proteins but all of them are focused on human or human model proteins data sets. Furthermore the peptides data-base used for molecular weight matching are generated by in-silico digestion with only few proteolytic enzymes. The aim of this work was to design and implement a new bioinformatics software which is able to identify the protein peptides from the peaks arising from in-source or MS/MS fragmentation. The oligopeptide fraction was extracted from Fontina cheese at different stages of ripening and subsequently analyzed by LC-MS/MS. The peptides were identified on the resulting total ion chromatograms by a method based both on the in-source fragmentation detectable with a single-quadrupole mass analyzer and by a new software that we developed. This software performs an in-silico digestion of the major milk proteins, it calculates all the possible peptide fragments generated by the loss of the first N- or C-terminal amino acids, and finally, it matches the experimental ion chromatogram with the in-silico generated theoretical spectrum to identify the exact amino-acid protein sequence of the unknown oligopeptide. With this tool we obtained useful insight into the proteolytic processes which occur during Fontina cheese aging, arriving at a better understanding of the functional features of the proteolysis end product.

Keywords: Cheese ripening, LC-MS/MS, new bioinformatics software.

G-42
IN VITRO ANTIFUNGAL EFFECT OF THYMOQUINONE AGAINST DAIRY SPOILAGE YEASTS IN MILK MEDIUM

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Thymoquinone (TQ) has been identified as the one of the chief quinone constituent of Black cumin (Nigella sativa L.) seeds volatile oil, which is also responsible for the pharmacological properties of the plant [1]. Thanks to the biological activity, TQ plays also an important role of the potential preservative agent in food processing. The inhibition effect of TQ against the dairy spoilage yeasts is presumed due to the N. sativa seeds utilization to flavor a variety of foods including milk products such as Mediterranean cheeses and the well know antimicrobial nature of the active principles [1; 2]. Six yeasts concerned as the most frequently occurred spoilers, namely Debaryomyces hansenii, Kluyveromyces marxianus, Pichia anomala, Saccharomyces cerevisiae, Yarrowia lipolytica and Zygosaccharomyces microellipsoide [3; 4] were tested using an agar dilution test [5] with Skim milk powder supplemented Sabouraud dextrose agar, which has simulated the dairy medium. The minimum inhibitory concentrations (MICs) of TQ ranged from 25 to 100µg/ml against all tested yeasts, which has been assessed as more effective in comparison with the common dairy preservative potassium sorbate (MICs from 500 to 2000 µg/ml). The strongest inhibitory effect of TQ was detected by inhibition of Z. microellipsoide (MIC = 25 µg/ml). These results are comparable with the previous findings with the same intension using the broth microdilution method without a Skim milk supplement [6]. In summary, the results showed a perspective alternative of the artificial preservative agents enhancing the shelf life of dairy products. However, further research focused on the safety and technological properties of TQ is necessary before its potential practical application.

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Keywords: thymoquinone, spoilage yeasts, dairy products

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G-43
EVALUATION OF THERMAL STABILITY OF COW'S AND DONKEY MILK MAJOR PROTEINS BY SIZE EXCLUSION AND BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Cow's and donkey milks show varying protein composition and, although some of the protein components of donkey and cow's milk are similar, sequence homology, concentrations and thermal stability are often different. In order to study relationship between composition, nutritional and technological properties, it is crucial to develop a method that enables simultaneous quantification of cow's and donkey milk major proteins in native and denatured states. The major whey proteins in cow's milk are β-lactoglobulin (β-lg), α-lactalbumin (α-la), immunoglobulins, blood serum albumin, lactoferrin and lysozyme (lys), which is similar to donkey milk. Compared to cow's milk, donkey milk contains less β-lg and more α-la, lys and immunoglobulins. Cows' milk is widely used as a substitute for human milk but it can lead to an abnormal immunological response. Donkey milk feeding was confirmed as a safe and valid treatment of the most complicated cases of multiple food intolerance. Several methods are used to analyze the protein fractions, but the use of HPLC has resulted in the development of rapid and automated analyses, characterized by good separation, high accuracy and reproducible results. The size, electrophoretic charge and polarity of the various milk proteins can be affected by heat treatment. Therefore, the methods used to separate and quantify native proteins present limitations when used for quantification of denatured proteins which are present in thermally processed milk samples. In the present work, cow and donkey milks were analyzed for quantification major proteins in native and denatured states. Two different chromatographic approaches, SE-HPLC and RP-HPLC both coupled to UV detection were used. The external standard method was used to calibrate the SE-HPLC and RP-HPLC chromatographic systems. Concerning quantification of lys, β-lg, cn, and α-la no significant differences between results obtained by SE-HPLC and by RP-HPLC (t-test, $p > 0.05$) were observed for whole raw milks and whey. Heating of cow's milk reduced native whey proteins, an aggregation of denatured proteins was observed by SE-HPLC. Lys was quantified in donkey whole raw milk and whey, however in thermally processed donkey milk lys was denaturated. α-la and β-lg from donkey milk were stable to thermal processing at 100°C (5 min). β-lg, α-la, and lys concentrations (in mg/mL) of donkey milk at different stages of lactation (15, 30 and 45 days after parturition) were evaluated The β-lg was the major protein (2.50 mg/mL as mean; peak 3.16 mg/mL), while the α-la had a mean concentration of 1.41 mg/mL (peak 1.96 mg/mL) and lys (0.94 mg/mL as mean) showed the highest value equal to 1.08 mg/mL. All these proteins peaked at 15 days, decreasing up to 45 days. This work highlights the great stability of donkey milk α-la and β-lg when compared with cow's milk whey proteins.

Keywords: lysozyme, β-lactoglobulin, α-lactalbumin, donkey milk

G-44
EFFECT OF COOKING PROCESSES ON THE OXIDATIVE STABILITY OF COMMERCIAL PACKED ALMONDS AND SUNFLOWER SEEDS UNDER ACCELERATED CONDITIONS

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The aim of this work was the study of the effect of different cooking processes on the composition and thermal stability of different commercial packed almonds and sunflower seeds since they are food samples highly susceptible to oxidation reactions [1]. The oxidation stability of raw, toasting and deep frying almonds; and toasting and deep frying sunflower seeds was studied under accelerated oxidation conditions (100 ºC for 5, 10 and 20 days). For this purpose, the fatty acid profile by GC-MS was determined. Two quality indicator tests were also obtained: the peroxide value (PV) for assessing primary oxidation products and the p-anisidine value (AV) for monitoring secondary oxidation products. Thermal stability of the samples was studied by using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Significant differences in the fatty acid profile of all samples were observed with increasing time of heat treatment, showing an increase in saturated fatty and oleic acids contents while the linoleic acid content decreased. A decrease in PV and rapid increase in AV were observed under accelerated conditions, as it was expected [2]. These results indicated that peroxides formed at first stages of oxidation lead to the formation of secondary oxidation products as aldehydes, being the first stage faster under accelerated conditions. DSC profiles of studied almonds showed two thermal transitions (an exothermic crystallization and endothermic melting) which disappeared after 5 days of oxidation except for raw almonds, which disappeared after 10 days. Sunflower seeds showed a different initial thermal profile, with toasted samples showing two transitions (a complex crystallization and melting) while deep frying showed only the melting transition, which disappeared after 5 days of oxidation. The results obtained by TGA indicated four stages of thermal degradation for samples. Maximum degradation temperature decreased with increasing time of heat treatment for all samples, except for raw almonds which were stable during the heat treatment. For sunflower seeds, the roasted samples were found to be the less stable. In conclusion, results showed that cooking processes affect the thermal and oxidative stability of the samples, being less pronounced for deep-frying almonds followed by the toasting ones. As it was expected, raw almonds were the most stable samples. Regarding sunflower seeds, deep frying samples showed a different initial fatty acid and thermal profile because of the frying process. During this process some of the evaporated water and other components are replaced by the frying oil, which is absorbed into the product changing its composition and quality. As a result, deep frying samples were less stable than the toasting ones.

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Keywords: Almonds, Sunflower seeds, Oxidative stability, Thermal processes, packaging

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G-45
DETERMINATION OF SYNTHETIC FOOD COLORANTS IN WATER SOLUBLE FOODS AND BEVERAGES BY HPLC AND NOVEL SPECTROPHOTOMETRIC ASSAYS

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Determination of Synthetic Food Colorants in Water Soluble Foods and Beverages By HPLC and Novel Spectrophotometric Assays Food colorants are the most important class of food additives attracting the attention of consumers and give the first idea about the taste and quality of a food product. They are subgrouped as natural and synthetic colorants and controlled by the limitations of European Union. Synthetic colorants are preferred for their low price and high stability. However, some researches about the health effects of these colorants prove them to be the reason of some severe and life threatening cases [1,2]. Today when the risks are evaluated, it is a necessity of making a serious study to examine the synthetic food colours. Ce(IV) ion reducing antioxidant capacity assay originally developed in our laboratories was utilized to determine the synthetic food colorants for the first time. This method which allowed for total antioxidant capacity assay of dietary polyphenols, flavonoids and ascorbic acid in plant extracts, is based on the room temperature - oxidation of antioxidant compounds with Ce (IV) sulfate in diluted H_2SO_4 solution and measurement of the absorbance of unreacted Ce (IV) at 320 nm [3]. The results of the proposed and reference methods were correlated with high performance liquid chromatography findings. Individual standard solutions, synthetic mixtures of synthetic colorants and colorant extracts were identified and quantified with HPLC on a C18 column equipped with diode array dedector.

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Keywords: food colorants, HPLC, spectrophotometry, analysis

G-46
USING MULTIPLE ANALYTICAL TECHNIQUES TO ASSIST WITH FEATURE SELECTION AND IDENTIFICATION IN COMPLEX MIXTURE ANALYSIS

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Chemometric approaches are often used for the analysis of complex data sets, such as those obtained from non-targeted analysis or metabolomic studies. Principal Components Analysis (PCA), for example, generates new variables from existing ones, whereas Genetic Algorithms maintain the existing variables, selecting those most important for discrimination through processes derived from natural selection. Greater between-group discrimination can be achieved by collection of data using more than one analytical technique, yet the data sets are often analysed individually. This fails to exploit the complementarity that exists between the data sets, almost negating the purpose of having initially acquired them. The use of statistical approaches to analyse data sets in conjunction with each other will increase the confidence in the identification and assignment of spectral features, and can be achieved by the combination of, for example, Nuclear Magnetic Resonance (NMR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC-MS). The use of multiple techniques in combination can highlight correlated variables across different data sets, such as chemical shifts and monoisotopic m/z values, and obviate the need for spiking experiments in the assignment of spectral features. The automation of searches that involve multiple databases will improve the reliability of compound identification in non-targeted analysis, with wide-ranging applications, for example, in food safety and authentication. Traditional approaches in the food industry are based on a 'target list' approach, whereby specific methodologies are developed for each compound on the list. Such methods are well suited for routine monitoring, but can fail to detect emerging contamination events. Recent issues, for example, the potentially fatal addition of melamine to infant formula, place in sharp focus the need to develop methods that are capable of detecting compounds in a non-targeted manner. The flagging of cases where no identification can be made will allow further investigations to be conducted by the analyst to provide a continually evolving database. Methods are proposed that illustrate a range of forms of data integration, in combination with multivariate analysis, to help provide a more holistic data analysis paradigm.

Keywords: chemometrics, NMR, LCMS, identification, mixture

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G-47
UNRAVELING THE CHEMICAL COMPOSITION OF CARAMEL

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Caramel is formed upon heating of sugar. While the volatile fraction of caramel has been studied in detail,[1,2] the chemical composition of the non-volatile fraction still remains largely uncharacterised, if not mysterious,[3] and a challenge to food chemists due to its complexity. We report on the analysis of caramel formed from glucose, fructose and saccharose upon heating using a combination of mass spectrometrical techniques. The analytical strategy employed uses high resolution mass spectrometry to identify the most abundant molecular formulas followed by van Krevelen and Kendrick analysis. A resulting structural hypothesis was further substantiated using targeted LC-tandem MS experiments. Here we show that caramel is composed from several thousand compounds resulted by a small number of unselective and chemoselective reactions. Products obtained after the caramelisation of glucose, fructose and saccharose include oligomers with up to six carbohydrate units formed through unselective glycosidic bond formation, dehydration products of oligomers loosing up to a maximum of eight water molecules, hydration products of sugar oligomers, disproportionation products and coloured aromatic products for which molecular formulas could be suggested. Caramel is produced and consumed by humans at a level of several tens of millions tons annually. The work provided here, using novel analytical strategies for complex mixture analysis, provides for the first time a comprehensive account of the chemical composition of one of mankind's oldest, most popular and most important dietary materials.

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Keywords: carbohydrates, browning, caramel, mass spectrometry, complex mixtures

G-48
VISIBLE EVIDENCE FOR THE FORMATION OF COPPER COMPLEXES IN GARLIC EXTRACTS TREATED WITH COPPER SULFATE AND SODIUM NITRITE MIXTURE

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Spectrophotometric evidence is described of the formation of copper complexes when crude garlic extracts were titrated with aliquots of equimolar concentration mixture of sodium nitrite and copper sulfate. Titrations could be monitored of all four different extracts prepared for each of the two garlic varieties used by the regeneration of a single diminished peak of copper (II) at a wavelength of 530 nm. The titration plots conform to the mole ratio and Job's continous variation plots of metal-ligand complexation. The sigmoidal absorbance plots obtained by the mole ratio method showed strong variations in the characteristic minimum, maximum, and the inflection points which were dependent on the type of garlic extract and on the variety of garlic. The similarity with the sigmoidal plot of copper–alanine titration is also provided in support of complexation of copper with components in garlic extract. The visible evidence appeared to mimic the spectrophotometric characteristics of copper (II) complexation with dipeptide bonds and nitrosothiols. Thus, on average there was more than one inflection point per titration plot. The use of Jaccard's similarity relation to determine pairs of extracts of the two varieties of garlic likely to contain complexe(s) common to the two is also provided. This study will help to delineate conditions under which $CuSO_4/NaNO_2$ decomposition reaction can be used for spectrochemical analysis of garlic and garlic products and for the investigation of garlic-derived copper complexes for nutritional and medicinal use.

Keywords: decomposition reaction, copper complexation, jaccards similarity, spectrophotometric, garlic extracts, sigmoidal curves

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G-49
INFRARED SPECTROSCOPY AS A TOOL TO PREDICT A-TOCOPHEROL AND PHENOLIC COMPOUNDS IN VIRGIN OLIVE OILS

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Over the past years, FT-IR spectroscopy combined with discriminant and partial least-squares analysis has been successfully used in order to detect adulteration of extra-virgin olive oils and for geographical discrimination of olive oils [1]. The development of sampling accessories attached to a wide range of infrared spectrophotometers, such as Attenuated total reflectance (ATR) cells, has led to major improvements in routine IR analysis, by simplifying sample handling and allowing sample analysis without pretreatment [2]. In this study, FT-IR/ATR methods were implemented for the determination of several quality parameters in virgin olive oils such as α-tocopherol, total phenolic content, phenolic alcohols like tyrosol and hydroxytyrosol and flavonoids like luteolin and apigenin. The methods implemented could be used as rapid screening methods to predict those parameters since the samples are analysed without pretreatment. Partial least squares (PLS) methods were applied to FT-IR spectral data, obtained from a set of virgin olive oil samples, as a statistical approach to quantitative analysis. UPLC and spectrophotometric methods were used as reference methods to analyse the samples in order to compare results between both methods.

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Keywords: Olive oil, α-Tocopherol, phenolic compounds, FT-IR, PLS Calibration

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G-50
MINERAL PROFILE OF MENU SAMPLES: A TOOL FOR THE EVALUATION OF DAILY INTAKE

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Menu samples from baby foods to children fast food menus and adult canteen menus were homogenized, freeze dried and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) in order to clearly establish their trace mineral element content. The methodology developed involves a previous sample digestion inside a microwave-oven followed by appropriate dilution and measurement in the ICP-OES. The method was fully validated through the evaluation of their main analytical figures of merit like sensitivity, LOD and LOQ, repeatability and accuracy, the last one tested from recovery experiments and the analysis of reference materials. Data obtained in 32 infant foods, 6 children fast food menus and 13 adult menus were evaluated in terms of the consideration of the presence of toxic elements and the requirements of essential elements through their daily intake recommendations. As a preliminary conclusion, from the studies carried out, we have appreciated the absence of toxic elements above the tolerable levels and the presence of essential nutrients at convenient levels; except in the case of alkaline elements in some samples of adult menus which must be reduced to avoid blood overpressure problems. This fact was probably due to the use of an excess of sodium chloride salt added to the cooked foods.

Keywords: fast food, baby foods, adult canteen, mineral profile, daily intake

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G-51
MEASUREMENT OF TRANS FAT IN EDIBLE FATS AND OILS BY FT–IR WITH A HEATED ATR ACCESSORY

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Trans-fats are mono- or polyunsaturated fats in which one or more of the double bonds is in a trans configuration. Trans fats are present in small concentrations (2–5% of total fat) in milk and meat products from ruminants such as cattle and sheep, but otherwise are found only in processed, partially hydrogenated fats such as vegetable shortening and margarine. Consumption of trans-fats has been shown to increase the risk of heart disease, and there is increasing pressure on food manufacturers both to reduce trans-fat levels and to label clearly the trans-fat level. This has led to a need for a rapid, straightforward analytical method to measure the trans-fat levels in fats and oils. Because of the distinctive molecular structure of trans-fats, the infrared spectrum contains a band that is not present in the spectra of other types of fats and oils. This property is recognised by AOCS method Cd $14e^{-09}$, which employs FT-IR spectroscopy to enable a rapid and sensitive measurement of trans-fat in fats and oils down to levels below 1%. The method employs ATR sampling at elevated temperature for convenient measurement of samples that may be solid at room temperature. Second derivative processing is used to remove baseline effects and enhance the selectivity of the method. In this submission we present an implementation of this method emphasizing performance and usability, and show that detection limits of around 0.5% are achievable for real-world samples, with the limitation arising from spectral interferences rather than signal-to-noise performance.

Keywords: FTIR, trans-fat, ATR, oils

G-52
METHOD VALIDATION FOR MULTI-ELEMENTAL ANALYSIS IN WINE BY INDUCTIVELY COUPLED PLASMA – OPTICAL EMISSION SPECTROMETRY

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The determination of elemental composition of wine has a great interest and can be used for different purposes such as: wine manufacturing processes, authenticity and traceability studies. In this application a simple method for the determination of several elements in wines using an inductively coupled plasma coupled to an optical emission spectrometer (ICP/OES) is described. Both major (K, Mg, Ca, Na), and trace levels elements (Fe, Cu, Mn, Zn, Al, Sr, Ba, Li and Rb) were measured simultaneously in a single reading. The method was validated using an internal standard (Sc) and an ionisation buffer (CsCl). The accuracy of the method was evaluated based on the results obtained on 10 samples of different wines from interlaboratory analysis by calculating Z-scores. Linearity, LQ, r, R and recovery using the new standard 90–210 were also evaluated. This method will be proposed to the OIV (International Organisation of Vine and Wine) as a new standard method which should applied in any laboratory running wine analysis on a routine basis.

Keywords: Inductively coupled plasma, optical spectroscopy, ionisation buffer, trace elements, authenticity, wine

G-53
DETERMINATION OF FREE AND TOTAL ELLAGIC ACID IN THREE DIFFERENT RASPBERRY CULTIVARS GROWN IN SERBIA

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Herbs and berry crops have been shown to contain high levels of phenolic compounds and flavonoids, which have antioxidant and anti-carcinogenic properties. Ellagitannins and ellagic acid have been found to have antimutagenic, antiviral, whitening of the skin and antioxidative properties. Accurate quantification of ellagitannnins and ellagic acid conjugates in berries could be considered as the most important due to wide range of bioactivity. Growing raspberries in Serbia has a long tradition. Although raspberries are of strategic importance for the Serbian economy, there is a lack of systematic control of the phytonutrients. Therefore, the aim of the present work was to establish simple and fast method for systematic monitoring of the free and total ellagic acid content. Here results obtained analysing Meeker and Willamette raspberries cultivars, are presented. Several procedures were followed in order to optimize conditions both for the extraction and determination. Amount of free ellagic acid was determined in methanol and acetone extracts. Total ellagic acid concentration was determined after acid hydrolysis with 4 M HCl in both extracts, and results were compared. Quantification was done using HPLC based on an isocratic elution.

Keywords: Ellagic acid, raspberry, HPLC

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G-54
SIMPLE VISUALIZATION TECHNIQUE FOR THE OPTIMAL POSITIONING COUPLING PLANAR CHROMATOGRAPHY WITH DIRECT ANALYSIS IN REAL TIME MASS SPECTROMETRY

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Direct Analysis in Real Time mass spectrometry (DART-MS) is now rapidly emerging, and its main advantage over other mass spectrometric techniques is the minimization or even absence of sample preparation. E.g., its coupling with planar chromatography is very promising, because it does not require any liquids to be used, therefore the shape of a spot is not distorted with a solvent. However, in early studies due to the fixed, horizontally aligned supply of the gas flow from the DART ionization source to the MS inlet, the introduction of many kinds of samples, e.g., HPTLC/TLC plates as cut strips, was very inconvenient and resulted in low repeatability due to the manual positioning. In 2009, a new version of the DART ion source was suggested, which allowed adjusting the angle of the DART gas stream and the use of a motorized rail. The angled source should significantly extend the general capabilities of DART-MS due to the introduction of and access to wide surfaces (pointwise, linearly or even in 3 different dimensions). However, not any experimental research paper was published on the advantages provided by the use of this source until May 2011. In our recent studies we have made a first step towards the unexplored capabilities of DART-MS arising from the possibility of the desorption at an angle: scanning analysis of surfaces, including the coupling of TLC-DART-MS. In order to select the most favorable conditions for DART-MS analysis, proper positioning of samples is important, therefore a simple and cheap technique for the visualization of the impact region of the DART gas stream onto a substrate was developed based on the chemical reaction on a filter paper or TLC plate upon heating. This approach, when employed using filter paper, does not require any high-cost equipment or reagents. A filter paper or TLC plate, previously loaded with the analyte, was immersed in a derivatization solution. On this substrate, owed to the hot DART gas impact, the reaction to a colored product occurred. Especially for scanning a whole sample track by TLC/HPTLC-DART-MS, such a visualization approach is useful due to the possibility for adjusting the coordinates of DART ion source in an optimal way. Until now, no completely satisfying solution is suggested for TLC/HPTLC-DART-MS coupling. The existing setup could be convenient for the scanning of a TLC/HPTLC strip along its track, which means one scan – one separation (one sample). By a given band length typically ranged between 4 and 8 mm, chromatographed bands can more robust be scanned in this direction as perpendicular to them with band width of only 1 or 2 mm. However, for the development of an efficient HPTLC-DART-MS coupling, the important option would be horizontal scanning of a plate along the socalled hRF substance window. This way analyte signals could rapidly be obtained from all samples on the plate. The results on HPTLC-DART-MS analysis of propolis and plant extracts will be reported.

Keywords: DART-MS, thin-layer chromatography, planar chromatography, HPTLC-DART-MS, visualization

Acknowledgement: This work was partially supported by the Erasmus Mundus EMA2 scholarship

G-55
NITROGEN / PROTEIN DETERMINATION IN FISH MEAL BY FLASH COMBUSTION METHOD IN COMPARISON WITH KJELDAHL METHOD

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High quality fishmeal is recognized by animal nutritionists as an excellent source of protein, energy, minerals and vitamins. Worldwide, millions of tons of fishmeal are produced annually. Fish Meal is used as an ingredient in feedstuffs in aquaculture, livestock and poultry industries. The majority of the fishmeal produced is included in commercial diets for poultry, swine, dairy cattle, mink, pigs, ruminants, farmed fish and very important in the diet of younger animals. As Fish Meal is a thick powder obtained from cooking, drying, and grinding raw fish, the freshness of raw material is important in its effect on the quality of the protein in the end product. Process control in the factory is necessary for the manufacture of high quality fish meal. The evaluation of the protein content is through the determination of the Nitrogen concentration. The analysis of Nitrogen in fish meal is critical for daily quality control of production and for specification in contracts. All fish meal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein content. For this reason the use of an accurate instrumental analytical techniques for Nitrogen determination is required and avoid the use of toxic chemicals. An alternative to the classical Kjeldahl method, based on Dumas (combustion) method, has been developed and approved by different associations (AOAC, AACC, AOCS, ASBC, ISO and IFFO). IFFO (International Fishmeal and Fish Oil Organization) has recommended that its members adopt the Dumas method as an Official Method for Nitrogen and Crude Protein determination. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming every day more notable, it is very important to have a simple and automatic technique which allows the fast analysis with an excellent reproducibility. The Thermo Scientific FLASH 4000 Elemental Analyzer, based on the dynamic combustion of the material, requires no sample digestion or toxic chemicals, while providing important advantages in terms of time, automation and quantitative determination of Nitrogen in a large range of concentration. This paper presents Nitrogen/Protein data of different fish meal samples, obtained with the analyzer using large sample weight to demonstrate the availability of the method for this application Some data compared to the results obtained by the traditional Kjeldahl method demonstrate the validity of the new system as the alternative to the traditional wet chemistry procedure.

Keywords: Food, Fishmeal, N/Protein det., Dumas method

G-56
DETERMINATION OF TRACE AMOUNTS OF IRON AND COPPER IN WATER AND FOOD SAMPLES BASED ON ULTRASOUND ASSISTED EMULSIFICATION SOLIDIFICATION OF FLOATING ORGANIC DROP

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Trace amounts of iron and copper in various substances may be vital, objectionable, or perhaps indicative of contamination or malfunction. In addition, these two elements are always together in environmental samples. Therefore, simple, sensitive and selective methods for the simultaneous determination of iron and copper are in great demand. In this study, we report a miniaturize method based on ultrasound assisted emulsification solidification of floating organic drop microextraction (USAE-SFODME) for preconcentration and determination iron and copper in water, chess, rice, tea, honey and milk samples. In this procedure, 2-mercaptopyridine n- oxide was used as chelating agent and 1- dodecanol was selected as extraction solvent. The factors influencing the complex formation and extraction by USAE-SFODME method were optimized. These factors included extraction solvent type as well as extraction solvent volume, time, temperature, pH, the amount of chelating agent, effect of salt and effect of interfering ions. Under optimum conditions, an enrichment factor of 67 is obtained from only 6.7 mL of aqueous phase. The calibration graph using the preconcentration system was linear between 40–800 mg L^{-1} and 20–1200 mg L^{-1} for iron and copper respectively. Based on three standard deviation of the blank, the detection limit were 8.6 mg L^{-1} and 4.1 mg L^{-1} for iron and copper respectively. The relative standard deviations (R. S. D) for ten replicate measurements of 500.0 mg L^{-1} of metal ions were 2.88 and 1.24 for iron and copper respectively.

Keywords: ultrasound assisted emulsification, Copper, Iron

G-57
NON-DESTRUCTIVE SCREENING OF CHILI POWDERS FOR COLOUR VALUES AND CAPSAICINOIDS BY SPECTROSCOPIC TECHNIQUES

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The quality of chili powder or paprika, both of the genus Capsicum, is primarily determined by colour (carotenoids), pungency (capsaicinoids) and flavour. The price depends on the acetone soluble carotenoids in ASTA 20.1 units [1]. The content of capsaicinoids is typically analysed by HPLC with fluorescence detection [2]. These methods are time and organic solvent consuming. Analysing chili powders by near infrared (NIR) and comparing the spectral data with reference data using multivariate data analysis, it is possible to create a model. This can be used to predict the values for extractable colour (ASTA 20.1) and capsaicinoids content, with only minimal sample preparation. The non-destructive NIR measurement is a very effective, rapid and cheap screening method as already described by other authors, e.g. [3,4]. Till now, more than 50 samples were analyzed to create a model to predict the values for the extractable colour. The spectra were recorded with a new UV/Vis/NIR spectrometer (Jasco UV/Vis-NIR Photometer V 670). Using multivariate data analysis (The Unscrambler Camo Inc.®), it was possible to create a calibration model which allows to predict the ASTA 20.1 value. For reference analysis the official ASTA 20.1 method was used. An FT-NIR spectrometer (Perkin-Elmer Spectrum IdentiCheck) was utilized for the development of a calibration model to predict the total content of capsaicinoids. Chili powders were analyzed by HPLC-FD for reference data. With the results of more than 50 different chili samples it was possible to develop a calibration model. Native chili varieties from Peru and Bolivia and also chili powders from local markets in Germany were analyzed with both methods.

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Keywords: Capsicum, chili, near infrared, capsaicinoids, colour values

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G-58
EASY AND FAST METHOD DEVELOPMENT FOR THE MERCURY SPECIATION IN FOOD BY HPLC–ICP–MS

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Mercury speciation in food analysis is required to fully estimate the toxicity of this element on human health. Due to the low detection limits required for such analysis, the use of GC ICP MS coupling is generally preferred. But with the improvements on modern ICP-MS, the use of an HPLC system is relevant for such analysis. This coupling is particularly attractive thanks to the simple and fast connection of both techniques. In addition, contrary to a GC analysis, sample extracts are directly injected into the system and no species derivatization is required, limiting again the difficulty of such analysis. In this study, we evaluated the use of the HPLC-ICP-MS coupling for the speciation analysis of mercury in food samples. The separation was achieved with the use of an organic solvent gradient to accelerate the separation. With the help of the RF-generator used in the 7700x system proposed by Agilent Technologies, the switch between aqueous and organic solvent is handled without affecting the plasma stability. Method validation was achieved thanks to the use of certified reference materials. In addition, the new ICP-MS MassHunter software authorizes the full control of the HPLC-ICP-MS coupling. Therefore, a complete and fully integrated solution on the Agilent systems was developed for the speciation analysis of mercury by HPLC-ICP-MS.

Keywords: Speciation, mercury, ICP-MS, HPLC

G-59
DETECTION OF GENETICALLY MODIFIED POTATO EH92-527-1 (BPS-25271-9) IN FOOD AND FEED PRODUCTS COMMERCIALIZED IN SARDINIA

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The Amflora EH92-527-1 (Unique identifier BPS-25271-9) is a potato, Solanum tuberosum L., genetically modified for enhanced content of the amylopectin component of starch. The Amfora is derived from the Cultivar Prevalent. Potato leaf discs were transformed by Agrobacterium-mediated gene transfer technology. The modification involves inhibition of the expression of granule bound starch synthase protein (GBSS), responsible for amylose biosynthesis. As a results, the starch produced has little or no amylose content and consists only of amylopectin (branched starch), which modifies the physical properties of the starch itself. A gene conferring kanamycin resistance (nptII) was used as a selectable marker. The Amflora has been developed for amylopectin (branched starch) production, and it is placed on the market for technical use, such as paper, but not for food products. By-products of the starch extraction process are used for animal feed (e.g. pulp) or for other conventional non-food purposes (e.g. potato juice used as soil fertilizer). In general, the GM potato tubers are not intended for direct human consumption, but it cannot be excluded the presence of GMO potato (and derived products) in food, as adventitious or technically unavoidable event. The aim of this study was the development of appropriate methods for detection of genetically modified Amflora in processed potatoes, allowing to monitor and verify the absence or the adventitious presence in Sardinia. The main steps of the work were the comparison and evaluation of DNA extraction protocols, aiming to obtain enough good-quality DNA for subsequent PCR real time amplification. The results obtained showed the ability of methods to isolate DNA from processed food and moreover we determined the quantity of Amflora to be less than 0.9% in all the analysed samples.

Keywords: Amflora, food and feed, real-time PCR

G-60
NEW TECHNOLOGICAL TOOLS FOR ISOLATING AND MEASURING GROWTH PROMOTING AGENTS IN EDIBLE TISSUES AND BIOLOGICAL FLUIDS

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According to the European Regulation, growth promoting agents are forbidden in animal breeding (Annex I of council directive 96/23/EC). Nowadays, the main known xenobiotics, (e.g. methyltestosterone or clenbuterol), are efficiently implemented in screening and/or confirmatory methods applied by the laboratories in charge of control. Conventional analytical strategies for these substances are based on relatively specific sample preparation procedures followed by liquid or gas chromatography coupled to tandem mass spectrometry (LC-MS/MS, GC-MS/MS). However, the constant evolutions of illegal practices as well as continuous technological improvements impose to revisit periodically such analytical approaches. For instance, it seems probable that either new b-agonist or natural steroid compounds can be used in meat producing animals for anabolic purposes, while remaining transparent to the current analyses. Indeed, the analytical signals are respectively not monitored because their structures are unknown or masked by their natural occurrence in the matrix of interest. The implementation of adequate strategies for fishing for such new compounds is then a matter of concern. Another important current challenge in the field of food safety is the more global 'horizon scanning' of potential new chemical hazards representing a significant threat to human health. The definition of new sample preparation and measurement approaches aiming to identify rapidly such emerging substances is thus of high interest for the authorities. The integration of ecological and sustainable development issues is also to be considered. In this context, the aim of this work is to describe several innovative analytical tools dealing with improved sample preparation techniques associated to specific detection modes, keeping in mind a decrease of solvent volumes and sample preparation time. After general considerations, we will base our discussion on several real case examples, including (1) the screening of b-agonist related drugs in animal feed using MIP technology for sample preparation associated to an LC-MS/MS measurement in ESI+ and neutral loss acquisition mode; (2) the analysis of estrogen steroid compounds at ultra-trace levels from reduced volume of serum combining an MIP extraction and a GC-MS/MS analysis after TMS derivatisation, and (3) the comparison of zeranol and Fusarium sp. metabolites analysis by LC-MS/MS (ESI-) after different sample preparations, i.e. immunoaffinity, MIP or successive SPE.

Keywords: Growth promoter, Sample preparation, Mass spectrometry, Specificity

G-61
MULTIVITAMIN CORN: TOXICITY AND ALLERGENICITY SAFETY ASSESSMENT

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Genetically engineered (GE) crops must be assessed to evaluate their effects on human and animal health and the environment. This is an integral part of the approval process before entering the market. The project is focus on a preliminary assessment assessing toxicity and allergenicity of a multivitamin corn line expressing four transgenes involved in β-carotene (as a vitamin A source), ascorbic acid (vitamin C) and folic acid (vitamin B9) biosynthesis. Bioinformatics analysis to compare the expressed recombinant protein sequences to known allergens was performed. No allergenicity or allergic cross-reactivity was identified. Potential allergenicity in vitro experiments have been initiated to assess the resistance of the recombinant proteins to human simulated fluid digestions and also to determine their heat stability. Preliminary acute and subchronic toxicity feeding trials were performed in vivo. Mice fed with diets enriched with GE corn and control diets (conventional corn counterpart and standard mice diet) were carried out to study potential toxicity. Based on a comparative approach, statistical methods were used to analyze data for food consumption, body weight, hematological and biochemical blood parameters, organ weight and histopathology. Our preliminary acute and subchronic studies indicate that the GE corn exhibited no toxicity and no potential for allergenicity.

Keywords: safety assessment, genetically engineered corn, toxicity, allergenicity

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G-62
INERTNESS PERFORMANCE OF CAPILLARY GC COLUMNS AND LINERS IN FOOD ANALYSIS

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As regulatory agencies drive limits of detection lower for increasingly active and more complex samples adsorption caused by flow path activity becomes a larger issue. The conversation flowing from GC column manufactures has shifted from discussions on low bleed to high inertness. This shift in focus has coincided with continuous improvements in the materials being used to manufacture and the manufacturing process in general for suppliers of capillary GC columns. Manufactures now have better tools at their disposal to meet demanding customer based inertness performance requirements. Contaminants analyzed in food matrices are present in trace level concentrations which makes analysis of these compounds very challenging. Chromatographically active compounds in food matrices can adsorb onto active sites in the sample flow path, particularly at trace levels, comprising an analytes' response. These active compounds tend to show peak tailing through interaction with active sites in a chromatographic system. Minimizing activity in the capillary GC column is essential to ensure accurate quantitation. Highly inert columns minimize column activity so difficult and active analytes can be consistently analyzed at trace levels. Another potential source of activity in the sample path is in the GC inlet. In residue analyses, repeated injections of matrix samples can lead to a gradual accumulation of nonvolatile matrix components in the inlet liner and column head, producing active sites and the need for maintenance. This heavy matrix-induced effect can impact peak shape, response, and retention. A highly inert liner with wool minimizes liner activity and helps prevent matrix component buildup at the inlet base and column head by trapping the non-volatiles on the deactivated wool. Unreliable results can have catastrophic implications in terms of environmental safety, the quality of the foods we eat, and inaccurate drugs of abuse accusations. Since identification and quantification are more difficult in complex matrices such as fruits, vegetables, soils, and biological fluids, extra vigilance is necessary. A clean flow is critical to make sure results are not compromised by analytes of interest adsorbing on active surfaces in the flow path This poster shows examples of highly inert capillary GC columns used in different food analyses showing performance on active compounds like pesticides.

Keywords: GC analysis, trace level analysis, inertness

G-63
A PROTEOMICS APPROACH TO LISTERIA IDENTIFICATION BY MALDI MASS SPECTROMETRY

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Listeria monocytogenes is the causative agent leading to listeriosis, an illness in humans and animals which can be fatal. There is a growing need to rapidly identify and characterize food-borne bacterial pathogens such as Listeria. MALDI mass spectrometry can be used as a sensitive, simple and rapid method to detect and identify bacterial strains present in samples. Proteomic approaches involving profiling of intact proteins or proteolytic peptides can reveal differences between Listeria strains. In this study, Listeria bacterial cultures were analyzed by MALDI MS and definitive identification of the bacterial species was performed by database searching of MS/MS data acquired on tryptic digests of the proteins. Bacterial cultures from 12 different Listeria monocytogenes isolates were ethanol-inactivated. Bacterial proteins were extracted from the cell pellets using several different extraction solvents. Samples were subsequently deposited onto stainless steel MALDI plates in replicate spots and allowed to dry. MALDI matrix was applied to one replicate of the spots and the intact proteins were analyzed in themass range 2–12 kDa on a MALDI TOF/TOF™ system. The second set of spots was subjected to tryptic digestion. MALDI matrix was applied to the spots and the resulting peptides were analyzed using high resolution MS (mass range 800–4000Da). For identification of the bacterial strains, the detected peptides were subjected to MS/MS fragmentation. The acquired spectra were used for database searching Intact protein profiling of the bacterial culture extracts by linear mode revealed unique species-specific marker peaks for each of the Listeria samples in the 7900–8000 Da mass range. These markers could subsequently be used to classify unknown samples as the peaks appeared to be consistent across different strains within the same species. Digestion of the bacterial protein extracts using trypsin generated peptides used for profiling at the peptide level. Unique peptide markers corresponding to the different species were identified. In addition, using an automated information-dependent acquisition workflow, a subset of these peptides was then subjected to MS/MS fragmentation yielding spectra which were used for database searching. This method for protein identification was optimized through the comparison of the protein profiles obtained using the different extraction solvents. Although some differences were observed between the various solvents, the identified proteins were found to be mostly ribosomal proteins and these could be used to distinguish the different Listeria species. Species-specific peptides from this protein class could be used to develop MRM targets for use in future rapid screening methods to identify bacteria derived from different sources.

Keywords: MALDI, Profiling, identification, Listeria species

G-64
QUANTUM DOTS AS NEW LABEL FOR RAPID TESTS

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Quantum dots (QDs) are inorganic luminescent semiconductor nanocrystals, exhibited size-dependent fluorescence emission spectra. For the last years QDs application in immunoassay could be mentioned as the most growing area of immunoassay labeling. QDs were used as fluorescent labels for microtiter plate assay (fluorescence-linked immunosorbent assay FLISA) for the detection of analytes of different nature in various matrixes. QDs application as labels for non-instrumental tests were not published till 2009 (according to Web of Science). And in 2010 application of QDs as labels for lateral flow immunoassay was described as for small molecule, as for proteins. As a rule QDs used as biolabels have core-shell structure. The shell of higher band gap semiconductor sufficiently improves brightness and stability of QDs. We prepared water-soluble CdSe-based core-shell QDs with two different colours of fluorescence: green and orange. Both types of QDs have CdSe core with zinc-blende structure synthesized by a rapid hot-injection method. The CdSe QD diameter was regulated by the reaction time. Both types of QDs were transferred to aqueous solution by the ligand exchange with mercaptopropionic acid (MPA). It is known that QDs covered with MPA are less bright and stable compared to QDs passivated with hydrophobic ligands. To improve both chemical stability and fluorescence QY we incubated MPA-coated QDs with denaturated bovine serum albumin (dBSA). The final QY are 36% (CdSe/ZnS) and 30% (CdSe/CdS/ZnS). The obtained water-soluble QDs are bright and stable enough to use them as biolabels in immunoassay. We have developed new column gel-based immunoassays for the model analyte benzo[a]pyrene and compared three different kinds of labels, i.e., horseradish peroxidase, colloidal gold and QDs with respect to rapid visual on-site testing. It was shown, that the best sensitivity and the shortest assay procedure were obtained with QD label. For the model analyte (benzo[a]pyrene) limit of detection was at 5 ng L^{-1}.

Keywords: gel-based immunoassays, quantum dots, benzo[a]pyrene

G-65
APPLICATION OF CHEMOMETRIC METHODS TO ASSESS THE IMPACT OF INTENSIVE HORTICULTURE PRACTICES ON GROUNDWATER CONTENT OF NITRATES, SODIUM, POTASSIUM AND PESTICIDES

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Groundwater is a valuable natural resource and as such should be protected from deterioration and chemical pollution however changes in agricultural practices during 20th century have significantly contributed to increase the concentrations of pollutant substances is this resource. A monitoring programmer of nitrate, nitrite, potassium, sodium and 28 pesticides was carried out in water samples from an intensive horticulture area in a vulnerable zone from North of Portugal. Eight collecting points were selected and water analyzed in five sampling campaigns, during one year. The multidimensional data analysis methods are very attractive in environmental studies dealing with measurements and monitoring, looking for possible grouping and sources of data variation. The application of such tools is expected to help rationalize confused intrinsic associations within real data and give an insight to identify the pollution sources for effective water resource management and pollution control. Chemometric techniques, such as cluster analysis (CA), principal component analysis (PCA) and discriminant analysis (DA) were used in order to understand the impact of intensive horticulture practices on dug and drilled wells groundwater and to study variations in the hydrochemistry of groundwater. PCA performed on pesticide data matrix yielded seven significant PCs explaining 77.67% of the data variance. Although, PCA rendered considerable data reduction, it could not clearly group and distinguish the sample types. However, a visible differentiation between the water samples was obtained. Cluster and discriminant analysis grouped the eight collecting points into three clusters of similar characteristics pertaining to water contamination, indicating that it is necessary to improve the use of water, fertilizers and pesticides. Inorganic fertilizers such as potassium nitrate were suspected to be the most important factors for nitrate contamination since highly significant Pearson Correlation (p=0.691) was obtained b etween groundwater nitrate and potassium contents. From a total of 28 pesticides (8 fungicides, 11 insecticides and 9 herbicides) 5 of them were not detected in any sample, acetamiprid, endrin, pendimethalin, phosmet and 2,4D. The most frequent fungicides were cyprodinil, metalaxyl and azoxystrobin. Whereas the most frequent insecticides were pirimicarb and thiamethoxam, followed by dimethoate, dieldrin, cyromazine, o,p'-DDT, methoxychlor and aldrin. The most frequent herbicides were atrazine and terbuthylazine and their desetyl-metabolites. It should be pointed out that some pesticides not in use nowadays were detected in some samples. Water from dug wells is especially prone to contamination from the grower and their closer neighbor's practices. Water from drilled wells is also contaminated from distant practices.

Keywords: Intensive horticulture, pesticides, nitrates, groundwater, chemometric analysis

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G-66
INVESTIGATION OF METAL-OMICS OF VEGETABLES GROWN IN CONTAMINATED AREAS BY PRINCIPAL COMPONENTS &CLASSIFICATION ANALYSIS

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The aim of this study is to apply the chemometric tools, especially Principal Component &Classification Analysis for better understanding of correlation between heavy metals contents from different vegetables grown in 2 contaminated areas comparatively with a reference area. The variables used in our investigation were heavy metals contents (Fe, Mn, Zn, Cu, Ni, Cd and Pb) identified in the edible part of common eating vegetables (parsley, carrot, onion, lettuce, cucumber and green beans) grown in farms located in contaminated mining areas or in reference area. The vegetable samples were collected from 2 contaminated areas from old mining activity in Banat County, Romania: Ruschita (R) area, where Pb is the principal heavy metal contaminant and Moldova Noua (M) area, where Cu is the principal heavy metal contaminant. The comparison was made with a non contaminated (Ref) area, located in the same geographically condition. For R area, 2 principal components are sufficient to explain more than 95% of model's variance and for M area 4 principal components are necessary for explain the same % of model's variance. This data reflects the complexity of heavy metal correlations in M area. The concentrations of Fe, Mn, Zn, Cu, Ni, Cd and Pb in the vegetable samples were determined after plant mineralization by using flame atomic absorption spectrophotometer with high resolution continuum source (Model ContrAA 300, Analytik Jena, Germany), fitted with a specific conditions of particular metal. For quality control purposes, blanks and duplicates samples were analyzed during the procedure and NCS Certified Reference Material was analyzed for quality assurance. The data were statistically analyzed using a statistical package Statistica.

Keywords: heavy metals, vegetables, chemometry, polluted areas, FAAS

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G-67
LEVELS OF BENZOIC AND SORBIC ACID PRESERVATIVES IN PROCESSED FOOD IN TURKEY (2008–2011)

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From 2008-2011 a total of 853 samples of a diverse range of food products were analyzed to check whether levels of benzoic and sorbic acid preservatives were in compliance with Turkish Codex limits. These limits depend on the specific food type and for example vary from 150 mg/kg for use of benzoic acid alone in non-alcoholic beverages, together with sorbic acid to a combined limit of 600 mg/kg in fruit concentrates to a combined limit of 2000 mg/kg in supplements and together with sorbic acid and p-hydroxybenzoate to a combined limit of 1500 mg/kg in confectionary. In some foods such as jams and molasses nether preservative is permitted to be used and in bakery products only sorbic, but not benzoic acid can be used. Over the 4 year period a total of 32 samples (4%) were not compliant with the regulations. Only 3 samples contained preservatives above the prescribed limits with the major problems occurring with detection of non-permitted benzoic acid in bakery products (11 samples) and non-permitted use of both benzoic and sorbic acid in jams and molasses(18 samples). Analysis employed the Nordik method 664.8.035:543.544 which involves methanol extraction of the foodstuff and direct injection of the extract into the HPLC with UV detection. Quality assurance measures were employed with each batch of samples and accuracy was ensured through annual participation in FAPAS rounds for preservatives in cola drinks achieving satisfactory Z-scores.

Keywords: Benzoic acid, sorbic acid, preservatives, Turkish food, processed food

G-68
MONITORING THE ILLEGAL USE OF DYES IN CHILLI POWDERS IN TURKEY (2008–2011)

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Over the period 2008–2011 monitoring of chilli powders produced in Turkey has continued with the aim of detecting the presence of illegal dyes. From the beginning of 2008 until April 2011 a total of 323 samples were analyzed, of which 32 were found to contain one or more of either Sudan I, or Sudan II or Sudan IV at levels above 0.5 mg/kg. Para-red was not detected in any of 20 samples monitored and Sudan III was not detected in any of the 323 samples. Analysis was conducted by solvent extraction of the powders using acetone, dichloromethane and methanol (3:2:1) with direct analysis by HPLC with UV detection. Recoveries ranged from 93 to 99% for the five illegal dyes and 0.5 mg/kg was used as the reporting limit. The majority of the positive samples contained only Sudan I at levels from 1 to 20 mg/kg but two samples from the same supplier in 2010 contained high levels of both Sudan I together with Sudan IV (676 & 466 mg/kg in one sample and 190 & 104 mg/kg in another sample for Sudan I and IV respectively). These results demonstrate that despite the widespread publicity concerning the use of illegal dyes and continued regulatory action this practice still continues to a worrying extent.

Keywords: sudan, illegal dye, HPLC, chili powder, Turkey

G-69
CALIBRATION OF LOW COST ON-LINE VISIBLE-NEAR INFRARED SENSOR FOR THE MONITORING OF THE FERMENTATION PROCESS AND THE QUALITY OF THE CIDER

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This paper describes the first step of the calibration process of a low-cost on-line Visible-Near Infrared sensor for the monitoring of the parameters relating with the fermentation process and the quality of the cider. The spontaneous fermentation of apple juice is the common procedure for making cider for many years in The Basque Country (Northern Spain). This method depends on the presence of indigenous yeasts and lactic acid bacteria in the must to perform alcoholic and malolactic fermentation, respectively. The aim of the sensor's development is to monitor the main parameters that affect to the quality of the final product during the fermentation process. A total of 17 cider samples were analyzed in the Basque Country University. These samples were obtained from different barrel in different stages of fermentation from October 2010 to March 2011. The alcoholic proof, glucose + fructose were analyzed to control alcoholic fermentation; total acidity, L-Malic and L-Lactic acid content to control malolactic fermentation; polyphenol index and methanol content. The optical, mechanical and electronic design of the sensor have been developed in Tekniker IK4 research center just as its manufacturing process which has been carried out combining different innovative manufacturing techniques. The measurement range of the sensor is 400–1100 nm thus it covers the visible range (400–700 nm) and the short wave near infrared (700–1100 nm). To fulfil with its objective the sensor system manages a halogen light source and a silicon detector which receives the transmitted light. Furthermore, a fluidic cell has been designed and manufactured where the cider is measured in. An ad-hoc electronic system has been built to manage the information and for the implementation the calibration algorithm. Chemometric techniques were applied with the purpose of obtaining a calibration model for each parameter correlating the spectra obtained by the sensor with the parameters analyzed in the laboratory. The calibration models were developed by Partial Least Squares Regression (PLSR) obtaining one model for each parameter. In order to obtain the best outcome different pre-processing strategies such as 1st derivative, smoothing by Savitzky-Golay and Multivariate Scatter Correction (MSC) were applied to the spectra with the aim of choosing the optimum pre-processing strategy. The validation of the model was made by "leave one out" cross validation which is a validation technique based only the calibration data. Finally the calibration models were choosing taking into account the statistical values obtained from the model and the variation of the Root Mean Square of Cross Validation (RMSECV) with the number of Principal Components (PCs) of the model. These kinds of sensors belong not to the future but to the present as means to reduce the costs in the control of the manufacturing process in the food industry and the costs due to laboratory analysis.

Keywords: cider, low-cost sensor, Vis-NIR, calibration, chemometrics

G-70
LEAST MEDIAN OF SQUARES CALIBRATION USING EXCEL

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In analytical chemistry, calibration involves establishing an equation that relates the signal to the concentration. Most commonly, calibration is accomplished through fitting a straight line to empirical data by minimizing the sum of the squared deviations between observed and modelled response values. This technique, well-known as least-squares regression (LS), supposes a normally distributed response while it considers the explanatory variable to be nonstochastic. However, real data sets often contain outliers, sometimes with serious consequences on the quality of the statistical analysis. Robust regression offers a reasonable alternative that is less sensitive to outliers. A highly robust regression technique is the least median of squares method (LMS) which minimizes the median of the squared residuals, rather than their sum. Although its usefulness for calibration purpose has been demonstrated in numerous studies, least median of squares fitting is hardly applied in routine laboratories. One reason might be that the LMS method does not provide specific equations for the direct calculation of the coefficients (i.e., a specialized computer program is necessary). Unfortunately, the typical LC-MS/HPLC/GC software packages for quantitative analysis do not include robust calibration. This contribution presents an excel VBA macro to perform LMS calibration and gives an illustrative example.

Keywords: Calibration, Least median of squares, Excel

G-71
DEVELOPMENT, VALIDATION AND APPLICATION OF A METHODOLOGY FOR THE DETERMINATION OF A-, B – UNSATURATED HYDROXY ALDEHYDES IN SAMPLES OF EDIBLE SOYBEAN OIL

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During the heating process, edible vegetable oils undergo thermal and oxidative reactions which may imply in losses of nutritional quality and formation of toxic compounds, like the α-, β- unsaturated hydroxy aldehydes. Due to their high reactivity, these aldehydes are able to promote changes in proteins, nucleic acids and other types of biomolecules. The aim of this work was to develop, validate and apply to a real sample a method, based on LC-DAD, to determine 4-hydroxy-2- trans-hexenal (HHE) and 4-hydroxy-2- trans-nonenal (HNE) in samples of soybean oil, the most consumed in Brazilian homes. Oil samples (2 mL) were fortified with 500 µL of a standard mixture of the hydroxy aldehydes. Extractions were carried out with the addition of acetonitrile (ACN, 1 mL), centrifugation during 15 min at 4000 rpm and removal of the supernatant phases (ACN) which then reacted with 1 mL of an acid solution of 2,4 dinitrophenylhydrazine 0,4% w/v, staying for resting for 3 hours. The formed hydrazones were analyzed in a LC-DAD system and compounds were identified by comparing their DAD spectra and retention times with those of standards. For method validation, the following parameters were evaluated, at three different fortification levels (0.1; 1.2 and 3.7 µg g^{-1}), the results ranging between the following values: linearity: 0.1 to 3.7 µg g^{-1}; LOD: 0.04 to 0.05 µg g^{-1}; LOQ: 0.10 µg g^{-1}; recovery: 78 to 99%; interday precision (n=15): 2.3 to 3.5% and intraday precision (n=7): 1.3 to 2.0%. The correlation coefficients of the analytical curves were 0.9991 and 0.9990, for HHE and HNE, respectively. The developed method was then applied in the analysis of triplicate samples of soybean oil, before heating and after heating at 180°C for 60 minutes under a 1 mL min^{-1} flow rate of synthetic air. The concentrations for HNE ranged between < LOD and 2.80 ± 0.05 µg g^{-1}, the lowest value corresponding to the samples without previous heating and the highest corresponding to the samples heated at 180°C. Regarding HHE, although a peak has been observed in the chromatograms, with a tR value very close to that of compound, the UV-Vis spectra could not confirm its identity, being possible this hydroxyaldehyde has been degraded during the heating. Finally, results reported in the literature regarding determination of HNE in samples of soybean oil [1] have shown concentrations of about 1.00 µg g^{-1} after 1 hour of continuous heating at 185°C. This concentration, when compared to this work, was about three times lower, due probably to the lower surface-to-volume ratio utilized, as well as to the more strict conditions in which samples were exposed to the air during the experiments, both of them important factors which affect the reaction rate of lipid oxidation in vegetable oils.

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Keywords: soybean oil, thermal oxidation, 4-hydroxy-2-trans-hexenal, 4-hydroxy-2- trans-nonenal, LC-DAD

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G-72
TWO NEW MODIFIED ACTIVATED CABONS BY HISTIDINE AND ARGININE FOR THE SOLID PHASE EXTRACTION OF TRACE LEAD IN WATER SAMPLES AND SOME OF FOOD SAMPLES

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Two new methods that utilizes histidine-modified activated carbon (AC-His) and arginine-modified activated carbon (AC-Arg) as solid-phase extractants have been developed for simultaneous preconcentration of trace Pb(Π) prior to the measurement by flame atomic absorption spectrometry (FAAS).The separation/preconcentration conditions of analyte were investigated, including effects of pH, the stirring time, the sample volume, the elution condition and the interfering ions.At pH 3.8 and 5.8, maximum absorption capacity of Pb(Π) onto the AC-His and AC-Arg were 0.5 and 0.8 mgg^{-1}, respectively. The adsorbed metal ions were quantitatively eluted by 5 mL of 3 $molL^{-1}$ HNO_3 for both adsorbent. Common coexisting ions did not interfere with the separation. According to the definition of IUPAC, the detection limits (3σ) of these methods for AC-His and AC-Arg were 3.7 and 3.3 $ngmL^{-1}$; respectively. The relative standard deviations under optimum conditions are less than 2% (n=6). The methods have been applied for the determination of Pb(Π) in water samples and some of food samples such as tea, honey and mushroom with satisfactory results.

Keywords: Histidine; Arginine; Lead; Activated carbon; Solid phase extraction.

Acknowledgement: Firoozabad Branch, Islamic Azad University

G-73
THE APPLICATION OF HYPHENATED SEPARATION TECHNIQUES FOR RESEARCHING OF LUNG CANCER BIOMARKERS

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Lung cancer is one of the most often diagnosed types of cancer. It is very common in men and women. Lung cancer is detected mainly in IV stage of disease when prognoses are very week and tumour gives metastasis. Analysis of substances present in exhaled breath seems to be easy, cheap, fast and noninvasive method which can be used for cancer detection. It is possible because compounds produced inside the body are released to the blood and then might be detected in breath. The aim of the investigation was determination the group of compounds that can be recognized as lung cancer biomarkers. For this propose, breath samples collected from patients with biopsy confirmed lung cancer were analyzed. As a reference group, expired air of healthy volunteers with different smoking status (i.e. nonsmokers, active and passive smokers) was applied. Volatile organic compounds (VOCs) present in breath were analyzed by gas chromatography and mass spectrometry (GC/MS) and gas chromatography time of flight mass spectrometry (GC/TOF-MS). Solid phase microextraction (SPME) technique was used for analytes preconcentration. Statistical methods such as discriminant analysis (DA) and CHAID model tree were used for obtained data processing and evaluation. Chromatographic analyzes of expired air collected from patients with lung cancer showed increased concentration of ethanol, acetone, butane, dimethyl sulfide, isoprene, propanal, 1-propanol, 2-pentanone, furan, o-xylene and ethylbenzene in comparison of healthy nonsmokers. Furthermore, pentanal, hexanal and nonane were identified only in breath of people suffered from cancer. DA confirmed the importance of these compounds in distinguishing patients from healthy volunteers and pointed out additionally pentane, butyrolactone, 2-methylbutane, 1-(methylthio)-propane, carbon disulfide and others as interesting compounds according to breath analysis. In breath of healthy smokers (passive and active), increased concentration of acetonitrile, benzene and furan derivatives was observed.

Keywords: breath analysis, lung cancer biomarkers, gas chromatography and mass spectrometry (GC/MS), volatile organic compounds (VOCs)

G-74
QUANTITATIVE LATERAL FLOW STRIPS FOR MULTI-ANALYTE ASSAYS OF FOOD CONTAMINANTS

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In recent years, immunochromatographic tests are being elaborated intensively for “on site” control of chemical contaminants in agricultural products and foodstuffs. The traditional format of these assays results in qualitative conclusion about whether the concentration of target compound exceeds some permissible level or not. However, the possibilities of these analyses expand significantly after their transformation into multiparametric assays with quantitative estimation of results. In the presentation the immunochromatographic tests for determination of antibiotics, mycotoxins, pesticides in agricultural products and foodstuffs in conjunction with a developed portable reflectometric registering device are discussed. The tests with colloidal gold nanoparticles as labels have been realized for ampicillin, streptomycin, chloramphenicol, ochratoxin A, aflatoxin B1, zearalenone, simazine, and atrazine. Advantages of videodigital data processing relatively to measurement of total staining values in selected zones are demonstrated. Efficiencies of different approximations for calibration curves have been compared. The reproducibility of the assays results has been characterized, and recommendations about procedures of test lines formation for better accuracy are proposed. Under optimized conditions content of target contaminants can be measured within 10-15% accuracy after standard 7-10 min protocols of lateral flow assays. The obtained information expands possibilities of operate decisions in the processing of agricultural raw materials and the production of feeds and foodstuffs. Quantitative estimation of immunochromatographic data was considered also as a tool for directed changes of detection limit and working range for the tests in the accordance with practical demands. The possibility to adjust the threshold for distinguishing between positive and negative samples in the 10-20 fold range by varying the compositions of antibody-colloidal gold and hapten-protein conjugates is demonstrated. Quantitative image processing in immunochromatography allowed to carry out simultaneous control of different compounds with significantly reduced binding zones of test strips. The proposed in our laboratory technique of multipoint reactants application allows to combine advantages of lateral flow strips and array technologies. Under selected conditions, the tests provide information about dozens of zones on standard test strip without interference between different binding processes. This assay format is applicable for quantitative control of multiple contaminants using reflectometric registration. The comparison between novel and traditional formats of the strips has been carried out on the examples of antibiotics detection in milk. Reliability of the obtained quantitative results has been confirmed by comparison with commercial tests. The proposed approaches provide possibility of quick high-throughput screening of chemical contaminants in food.

Keywords: Immunochromatography, test strips, antibiotics, mycotoxins, reflectometric detector

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G-75
COMPARISON OF DNA EXTRACTION METHODS TO DETECT TRACE AMOUNTS OF TREE NUT ALLERGENS IN CHOCOLATES

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Food-induced allergies represent an emerging problem of food safety. Thus, to safeguard the health of sensitised consumers, food ingredients that may cause allergic reactions should be properly labelled and possible cross-contamination should be avoided. Among food allergies, abnormal immunological responses towards tree nuts are pointed as a frequent source of serious atypical reactions, in which the hypersensitivities associated to almond and hazelnut ingestion are considered dangerous due to their incidence and severity [1,2]. Although immunological methods have been used for the direct detection of the almond and hazelnut allergens with high sensitivity, these assays are susceptible to cross-reactivity with other tree nuts. More recently, alternative approaches based on polymerase chain reaction (PCR) have been developed for the detection of almond and hazelnut allergens in foods [3-5]. However, highly processed food matrices such as chocolate, are very rich in polyphenols, carbohydrates and aromatic compounds that can interfere and inhibit DNA amplification. Since molecular methods are extremely dependent on the DNA extraction and purification procedures, adequate recovery of nucleic acids and removal of PCR inhibitors are required. Presently, several extraction methods are commercially available for the isolation of DNA from foods although only a few can provide extracts with suitable quality and purity for PCR amplifications. In these work we intend to compare different DNA extraction protocols (CTAB, Wizard, Wizard Magnetic and Nucleospin methods) with and without the addition of polyvinylpyrrolidone (PVP) and/or the presence of RNAse. For this purpose, model chocolates containing known amounts of almond or hazelnut (10-0.01%) were prepared and the DNA isolated with the selected protocols. DNA amplification was tested by qualitative PCR and real-time PCR with the use of specific fluorescent probes for almond and hazelnut. From the tested protocols, Nucleospin Food Kit evidenced the best results for almond and hazelnut isolation and amplification. This method showed the highest reproducibility of PCR fragments for almond and hazelnut chocolates until a relative limit of detection (LOD) of 0.01%, which was confirmed by real-time PCR. CTAB-PVP and Wizard methods exhibited the second best results for almond and hazelnut extraction, respectively. However, both methods were less reproducible since the DNA extracts presented major variations in PCR amplifications. To our knowledge this was the first attempt to compare different extraction methods for the specific detection of nut allergens in chocolates.

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Keywords: Food allergens, almond detection, hazelnut detection, PCR, chocolate

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MYCOTOXINS, MARINE AND PLANT TOXINS

(H-1 – H-52)

H-1
THE UPTAKE OF 14C-ATROPINE FROM SOIL BY WHEAT AND ITS TRANSLOCATION TO SHOOTS

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Plant uptake of toxic contaminants and their translocation to edible plant parts is one possible route for the transfer of toxins into the terrestrial food chain. The tropane alkaloids are toxins naturally produced by the family Solanaceae, comprising over 100 genera and 3000 plant species found worldwide. Variable amounts of alkaloids are produced, particularly in the seeds, which are potentially poisonous. Bulk commercial grains (wheat, rye, soybeans, linseed, corn and solanaceous crops) may be contaminated by alkaloid producing plants that coexist with the crop to be harvested, or possibly by uptake of alkaloid toxins from the soil. The absorption of the tropane alkaloid, atropine, from soil by wheat and its translocation to the shoots was investigated using both labelled (14C) and unlabelled atropine. Wheat plants were grown in soil spiked at either low level (13676 dpm/g soil) or high level (37203 dpm/g soil) with 14C-atropine and sampled at 15, 30, 60 and 90 days after sowing (DAS). Plants sampled at 15 DAS had taken up approximately 0.42% of the applied radioactivity for low-level spiking and 0.17% for high-level spiking, decreasing to approximately 0.17% (low level) and 0.04% (high level) in plants sampled at 90 DAS. The increase in the spiked concentration in the soil did not affect the absolute concentrations of 14C-atropine in the wheat. The highest accumulation of 14C-atropine was detected in the leaves (approximately 83% of the absorbed amount) while lower levels were detected in the roots (8.6%), stems (4.2%) and seeds (4.1%). 14C-atropine was detected in soil leachate at a level of 0.5% of the applied amount at 30 DAS, decreasing with time to 0.01% at 90 DAS. Soil analysis showed that 60% of the applied radioactivity was adsorbed at 30 DAS increasing to 70% at 60 DAS and remaining stable at this level at 90 DAS. Unlabelled atropine, applied to soil at a concentration of 250 ng/g, was detected at 30 DAS in whole plants at a concentration of 3.9 ng/g biomass. The absorption of atropine from soil and its translocation to the edible plant parts, and the observed bioconcentration factor (2.3±0.04) suggest a potential route of contamination that may have implications for food safety.

Keywords: Plant toxins, Atropine, Transfer, Translocation

H-2
DETERMINATION OF SELECTED MYCOTOXINS IN CEREAL PRODUCTS

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Mycotoxins are metabolic by-products of various species of fungus. Various groups of such compound are regarded as dangerous food chemical contaminants. Mycotoxins shows toxicity against humans and are also suspected human carcinogens. Currently, standing EU legislation for the maximum levels of mycotoxins in food calls for sophisticated analytical methods and techniques, that could coup with low levels of those compounds in cereal products. Low permitable levels of myxotoxins require implication of both sensitive and selective analytical methods. The aim of this work was to develop a screening multiresidue method for determination of selected myxotixins in cereal products. The folowing metabolites were investigated: deoxynivalenol (DON), HT-2 and T-2 toxin, ochratoxin A (OTA), fumonisin B1 and B2. Final determinations were performed by liquid chromatography and mass spectrometry. Ion trap mass spectrometer with electrospray ionization was used (LC-ESI-MS/MS Thermo Finigan LCQ). Separations were performed on a Kinetex 100×2.6 µm PFP. The folowing adduct ions were selected as colission induced disociation precursors: DON-355.0 $[M+Ac]^-$, OTA – 404.0 $[M+H]^+$, HT-2 – 447.4 $[M+Na]^+$, T-2 – 489.0 $[M+Na]^+$, fumonisin B1 – 722,5 $[M+H]^+$, and fumonisin B2 – 706.0 $[M+H]^+$. Sample preparation procedure involved liquid extraction with an acetonitrile: water (80:20) mixture and subsequent clean-up on a Strata Screen C 500 mg / 6 ml (Phenomenex) solid phase extraction cartridges. Sample was loaded on the pre-conditoned cartridge and washed with phosphate buffer. Mycotoxins were then eluted with methanol and ethyl acetate. After concentration on a rotary evaporator sample was re-dissolved in the mobile phase and transfered into a chromatographic vial. Limits of determinations for analysed toxins determined in valudation experiments conducted on some laboratory made spiked samples were: DON: 16 µg/kg, HT-2: 31 µg/kg, T-2: 8 µg/kg, OTA: 15 µg/kg, fumonisin B1: 10 µg/kg, fumonisin B2: 18 µg/kg. Recoveries from spiked samples were in 50–100% range.

Keywords: mycotoxins, cereal products, LC-ESI-MS/MS

H-3
ELISA AND SAMPLE CLEAN-UP METHODS FOR DETERMINATION OF OCHRATOXIN B

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Ochratoxins are a mycotoxin family of metabolites produced by certain ubiquitously occurring Aspergillus and Penicillium species. The toxins are common contaminants of human food and animal feed and are regularly found in human and animal serum samples. Ochratoxins have been showed to be nephrotoxic, teratogenic and carcinogenic in various laboratory animals. A direct, competitive enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody has been developed for the quantitative determination of ochratoxin B (OTB) in coffee and wine. The assay range is between 3.0 and 100 ng/mL. For the determination of OTB in coffee and wine a simple methylene chloride extraction is needed followed by dilution in PBS. Analyis of OTB in other matrices and other sample clean-up/concentration methodologies will be presented.

Keywords: Ochratoxin B, Mycotoxins, ELISA, IAC

H-4
EFFECT OF FUSARIUM CULMORUM ON QUALITY OF SIX WHEAT VARIETIES

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Fusarium head blight (FHB), caused by Fusarium spp., is a destructive disease of wheat and other small grain cereals, with negative consequences for grain quality with regard to mycotoxin contamination and reduced technological quality. The objective of this study was to investigate the effect of wheat infection by F. culmorum on quality parameters and deoxynivalenol (DON) levels in wheat grain, flour, and final product. Material used in this study consisted of six winter wheat varieties (Akteur – class E, Bakfis – class A, Cubus – class A, Ebi – class A, Eurofit – class A, and Ludwig – class E), grown in 2010 at one location, both with and without inoculation at the flowering stage. DON content was determined using an ELISA method, methods of quality parameters analysis (bulk density, crude protein and wet gluten content, gluten index, Zeleny test, falling number, farinograph, test baking) were based on relevant standards (CSN). Non-inoculated samples had rather low DON content (≤ 80 $\mu g \cdot kg^{-1}$) with small differences between varieties. Inoculated samples were generally high in DON (5000 $\mu g \cdot kg^{-1}$ to 12000 $\mu g \cdot kg^{-1}$) with exception of moderately resistant variety Bakfis (500 $\mu g \cdot kg^{-1}$). Our results indicate that high Fusarium infection accompanied with high DON contents deteriorate baking quality. Infection had significant influence on bulk density and protein quality of grain (Zeleny test, gluten index), but not on protein quantity (crude protein and wet gluten content) and falling number. In case of farinograph parameters small and insignificant changes were noted, especially in degree of softening. In baking test showed significantly lower volumes and flattened shape. Comparison of DON levels in grain, flour, and loaves of inoculated samples revealed generally higher levels in whole grain, while flour and loaves had DON lower and mutually comparable.

Keywords: Deoxynivalenol, Fusarium spp., wheat, breadmaking quality

Acknowledgement: This project is funded by the Ministry of Agriculture of the Czech Republic (RO0211) and Czech Science Foundation (525/09/P647).

H-5
CRITICAL ASSESSMENT OF DETERMINATION OF T-2 AND HT-2 TOXINS IN CEREALS: RESULTS OBTAINED BY UPLC–MS/MS AND ELISA METHODS

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The Fusarium microscopic fungi species belong to the most prevalent toxin-producing fungi in moderate climate conditions. HT-2 and T-2 toxins are the main representatives of trichothecenes A and their presence was mainly proved in oat, barley, wheat, other small grain cereals and products derived from. With regard to the health concerns associated with consumers´ dietary exposure to T-2 and HT-2 toxins, the demand for collection of more monitoring data was emphasized by Commission Regulation No. 1881/2006 as well as by European Food Safety Authority (EFSA). It should be noted, that maximum limits for both toxins still have not been established, especially because of lack of sufficient occurrence data. Therefore, our study was focused on development and validation of reliable analytical method based on UPLC-MS/MS principal. Within another part of experiments, a critical assessment of both immunochemical and instrumental strategies conceivable for determination of the major representatives of trichothecenes A group was carried out. Altogether, six types of commercially available ELISA kits dedicated for determination of T-2 toxin in cereals were investigated (Ridascreen: R-Biopharm Rhone Ltd., AgraQuant: Romer Labs, Veratox: Neogen, EIA: Eurodiagnostica, MaxSignal: Bioo Scientific and T2 Toxin Plate Kit: Abraxis). Worth to notice, that none of these immonoassays is specific only for target toxin, cross reactions to various type-A trichothecenes including HT-2 are declared by producers. Concentration levels of T-2 toxin largely varied among the kits used for sample examination. Kits were tested on previous oat FAPAS (Food Analysis Proficiency Testing Scheme) samples. The assigned values from FAPAS reports were taken as reference values of particular samples for ELISA results evaluation. In the next step, we compared results obtained by LC-MS-based procedure with different types of extractions and sample preparation. All together, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction principle, immunoaffinity clean-up and pure extract of cereals were tested in purpose to obtained sufficient performance characteristics of the analytical method. Isotope dilution technique employing 13C24-T-2 toxin and 13C22-HT-2 toxin was used to for high accuracy of generated results. Comparison of all results obtained by usage of all mentioned procedures will be presented.

Keywords: mycotoxins, oats, T-2 toxin, UPLC-MS/MS, ELISA

Acknowledgement: The results were obtained with the support of the Ministry of Agriculture of the Czech Republic, projects No. QH81060.

H-6
PHOMOPSIN A IN FOOD SAMPLES FROM RETAIL IN THE NETHERLANDS

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Phomopsin A is a toxic secondary metabolite produced by the fungus Phomopsis leptostromiformis in lupine (6). However, phomopsin A contamination of chestnuts and mango's cannot be excluded (1). Contamination of lupine debris with this fungus is reported in Germany, Denmark, Poland, South Africa and Australia (7). Infected stubble containing phomopsin A can cause lupinosis in grazing sheep (7). Primary target organ for phomopsin A in sheep is the liver (3). Australia and New Zealand have set a regulatory limit of 5 µg/kg product for phomopsin A (2). Lupine has comparable nutritional characteristics to soy and can be used to replace genetically modified soy in foods (5). Numerous products containing lupine are currently available on the European market (2; 5). EFSA will, therefore, carry out a risk assessment on phomopsins for the European situation (4). A straightforward LC-MS/MS method was developed for determination and confirmation of phomopsin A in lupine flour and lupine containing food. The method involved extraction by a mixture of acetonitrile/water (80%/20%) with 1% acetic acid added and direct injection of the crude extract after centrifugation. The method was validated at 5 and 25 µg/kg. The average recovery and RSD obtained were 79% and 9%, respectively. The limit of detection (LOD) was 1.22 µg phomopsin A/kg product. Twenty samples, containing various levels of lupine as ingredient, were bought from grocery and internet shops in The Netherlands. A total of three lupine flours; five bread samples; one flour for bread; four biscuit/cakes; three samples of frozen lupine flakes (retail); two lupine snacks; and two pasta products were analysed. The origin of lupine used for preparation of the food products was unknown. None of the samples in this first brief survey, contained phomopsin A in levels above the LOD.

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Keywords: Phomopsin, mycotoxin, lupine, The Netherlands, food

H-7
FT–NIR SPECTROSCOPY AS A SCREENING TOOL FOR DEOXYNIVALENOL CONTAMINATION IN WHEAT

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Deoxynivalenol (DON) is a type B trichothecene mycotoxin mainly produced by several Fusarium species occurring in cereals. Chromatographic methods are the most widely used for quantitative determination of DON in foodstuffs and feedstuffs. However, these methods are destructive, time-consuming, expensive, unsuitable for screening purposes, and require a preliminary cleanup of the extracts. A range of alternative methods have been published, including infrared spectroscopy. Some studies on the use of near infrared spectroscopy and mid-infrared spectroscopy to predict DON contamination in whole grain and flour of wheat, maize and other grain cereals have been reported. The feasibility of using Fourier-transform near infrared (FT-NIR) spectroscopy for rapid and non-invasive analysis of DON in unprocessed durum wheat at levels close to the EU regulatory level (1750 µg/kg) has been recently reported. A partial least-squares (PLS) regression model was developed using correlation data between FT-NIR and HPLC/FLD (confirming method). We have further implemented the PLS model in a larger study involving more calibration (n=230) and validation (n=230) samples from different cultivars of wheat naturally contaminated with DON at levels up to about 16000 µg/kg DON. Slope, coefficients of correlation (r) and root mean square errors (RMSE) were close to 0.73, 0.85 and 300 µg/kg, respectively, in both calibration and validation PLS models. Similar results were obtained when the PLS model was developed by using the cross validation approach on the entire set of data. The reliability of FT-NIR spectroscopy for qualitative discrimination of wheat samples based on DON content was also investigated. Linear discriminant analysis (LDA) was performed on the same calibration and validation sets of durum wheat samples. When a cut-off limit of 1500 µg/kg was used to distinguish the samples classes, the LDA analysis was able to correctly classify more than 85% of wheat samples. Performances of LDA and of PLS regression models suggest that FT-NIR analysis might be a promising screening tool to rapidly analyse durum wheat samples for DON content. Further activities will be carried out to improve the predictive ability of the FT-NIR calibration models in the tested range.

Keywords: FT-NIR spectroscopy; deoxynivalenol; wheat; PLS regression, discriminant analysis

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H-8
DETECTION OF PYRROLIZIDINE ALKALOIDS IN HONEY, MILK AND CHEESE USING LC–MS TECHNOLOGIES

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Pyrrolizidine Alkaloids (PAs) are known plant toxins that cause hepatotoxicity in humans and animals. Human ingestion of PAs can occur through contaminated grain (acute toxicity), herbal products and foodstuffs such as honey and milk. Along with being hepatotoxic, PAs are also recognised as carcinogenic, genotoxic, and pneumotoxic compounds. PAs which cause toxicity are esters of 1-hydroxymethyl-1,2-dehydro-pyrrolizidine. The potential for PA-contamination within the food chain, resulting in chronic toxicity, is likely. For this reason there is a need to develop reliable, sensitive and rapid detection methods for PAs and LC-MS/MS would be the preferred technology. It is our intention to emphasise the need to establish standardised validated detection methods and maximum residue limits (MRLs) for PAs in food since currently there are only tolerable daily intake (TDI) guidelines available. In this study, a large scale survey was undertaken to detect PAs in retail honey, milk and cheese. Over 500 commercially available honey, milk and cheese samples were tested. The developed and validated method utilised strong cation exchange (SCX) solid phase extraction (SPE) for matrix cleanup. SCX-SPE cartridges used in the study were selected based on the testing of four leading brand products for clean-up ability and high percentage recoveries. Sixteen reference PA compounds were used in the methodology. Samples were analysed on an Agilent 1200 series LC coupled to a 6340 Ion-trap Mass Spectrometer (MS) and on a Thermo Scientific Quantum Discovery Max Triple Quadrupole (QqQ) MS. All positive samples were further analysed on a Thermo Scientific LTQ XL Orbitrap MS for high mass accuracy data as a confirmatory measure.

Keywords: Pyrrolizidine Alkaloids; plant toxins; LC-MS/MS; food safety

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H-9
THE ADVANTAGE OF FULLY STABLE 13C-LABELED INTERNAL STANDARDS IN LC–MS/MS MYCOTOXIN ANALYSIS

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The LC-MS/MS technology popularity is constantly increasing. More and more laboratories are now using LC-MS/MS also for mycotoxin routine analysis in food. Nevertheless, a problem with LC-MS/MS can be interferences from matrix components leading to differences in analyte ionization. Ionization efficiencies can vary between matrix samples and pure standard calibrants. Hence, the mass spectrum shows different signal intensities and because of this the sample analyte peak cannot be compared to the calibration curve (made from pure standard calibrants) for concentration calculation. To overcome this ionization effect, internal standards have to be used. One possibility are isotope labeled standards, where one or a number of constituting atoms are replaced by a different stable isotope. A possible isotope can be 13C. 13C-labeled mycotoxins still have the same characteristics as their 12C analogues, eluting at the same retention time in chromatography. When the detection is performed with mass spectrometry, both eluted forms are found and can be separated because of the mass difference between 12C and 13C mycotoxins. The 13C peak, representing the known amount of 13C labeled mycotoxin added can be used to calculate the unknown amount of the 12C mycotoxin. Using fully stable 13C-labeled mycotoxins as internal standards has several advantages over deuterated (2H) internal standards. Replacing 12C by 13C changes the total mass of the atom only slightly, while using Deuterium, the mass doubles, thus, 2H labeled mycotoxins might show retention time shifts, resulting in less accurate LC-MS/MS results. This presentation will demonstrate how to use an internal standard in mycotoxin analysis of food samples. Furthermore, it will illustrate the importance of applying internal standards when running quantitative mycotoxin analyses on an LC-MS/MS system.

Keywords: LC-MS/MS, 13C-labeled standards, matrix effects, ion suppression, ion enhancement

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H-10
ANALYSIS OF MYCOTOXINS IN POPPY SEEDS

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Poppy (*Papaver somniferum* L.) grown in the Czech Republic is primarily intended for food purposes. Since 2004, the Czech Republic has become the biggest producer of this crop in the world. For its high quality, the Czech poppy seeds have gained popularity abroad, and are widely exported to many countries. However, during the poppy growing, affecting by microscopic filamentous fungi followed by mycotoxins production can occur. At present, no sufficient data about presence of mycotoxins in poppy seeds are available, that is why an extensive screening of mycotoxins in this commodity is needed. Within this study, poppy seeds purchased in the Czech retail market, both from organic, and from conventional farming, were analyzed (altogether 20 samples). For isolation of mycotoxins from the sample, the QuEChERS-based method was employed. For removing of fat from the sample extract, Bondesil-C18 sorbent was used. Recoveries of target analytes were not influenced. For identification and quantification of Fusarium mycotoxins (deoxynivalenol, deoxynivalenol-3-glucoside, nivalenol and 3-sum 15-deoxynivalenol, HT-2 toxin, T-2 toxin, and zearalenone), and Alternaria mycotoxins (alternariol, alternariolmonomethylether a altenuene), the method of ultra-performance liquid chromatography coupled with high resolution mass spectrometry (U-HPLC–orbitrapMS) was enabled. Fusarium and Alternaria mycotoxins were not detected in any tested sample. The thick protection layer of poppy head probably protects poppy seeds against mould pathogens.

Keywords: mycotoxins, poppy seeds, QuEChERS

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H-11
CO-OCCURENCE OF MYCOTOXINS IN FEEDS PRODUCED IN THE CZECH REPUBLIC

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EU legislation related to feeds is focused on production and distribution of feeds that will not cause any risks for humans, animals and/or environment. Directive 2002/32/ES and Recommendation 2006/576/ES indicate guidance values for the presence of some mycotoxins that shall not be exceeded. UKZUZ has been checking raw materials and feed samples from all steps of feedstuffs production since 2004 on a regular basis. Aflatoxins B1, B2, G1, G2, Fumonisins B1, B2, Ochratoxin A, Deoxynivalenol (DON), Zearalenon (ZON) and T-2 and HT-2 toxins were determined in maize, wheat, barley, silage, haylage, grass hay, and complementary and complete feedstuffs. Imported feeds such as soya extracted groats, cotton seeds or cocoa hulls were analyzed as well. Analyses were performed either by HPLC or LC-MS by validated and accredited methods. A basic set of data on the presence of mycotoxins in the Czech feeds is now available. The most frequent mycotoxins found in all types of studied feeds were DON, ZON and Fumonisins. Complete feeds, maize and silage represented the most contaminated feeds. Fusarium mycotoxins proved to be the most frequent contaminants found in feeds, but the levels were always lower than EU guidance values. For future needs of official controls a multiresidual method based on modified unbuffered QuEChERS sample preparation and UPLC-MS/MS determination is under development. This method will enable to increase number of samples and also number of mycotoxins present in feeds.

Keywords: Feed, Zearalenon, Fumonisins, Deoxynivalenol, LC/MS/MS

H-12
DETERMINATION OF DON IN CEREALS AND CEREAL–BASED PRODUCTS, COMPARISON OF RESULTS OBTAINED BY ELISA AND LC–MS

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Enzyme-linked immuno sorbent assay (ELISA) is a biochemical technique, which uses specific antigen-antibody binding to detect certain analytes like food contaminants (e.g. representatives of Fusarium mycotoxins). A significant disadvantage of immunoassays is an inability to distinguish minor differences in the structure of an antigen. It may lead to overestimated ELISA response, thus to incorrect results. In the case of deoxynivalenol (DON) dedicated ELISAs, strong cross-reactivity to DON conjugate DON-3-glucoside (DON-3-Glc) and considerable overestimation of obtained results was proven during our previous research. The aim of the up-coming research was to monitor this trend continuously during three years 2008-2010. Presented experiments were focused on comparison of DON levels in 67 cereal samples obtained by a reference LC-MS method and particular ELISAs. In the later case, four various ELISA kits (Ridascreen®, R-Biopharm; EIA, Euro-Diagnostica; Veratox®, Neogen®; AgraQuant®, Romer Labs®) were tested. Higher levels of DON obtained by enzyme immunoassay compared to LC-MS were expected and later confirmed. The highest increase of DON levels was observed in the case of AgraQuant® (52%) and closely followed by Ridascreen® (51%). Two remaining kits – EIA and Veratox®, showed mean increase up to 48% and 25%, respectively. In another part of the work, cross-reactivity of ELISAs in the case of barley-based malts was assessed. It was found that the ELISA response is higher in several cases due to the presence of Maillard reaction products.

Keywords: deoxynivalenol, ELISA, crossreactivity

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H-13
THE ANALYSIS OF TETRODOTOXINS IN FISH AND SHELLFISH USING UPLC–MS/MS

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Tetrodotoxins (TTXs) are natural toxins produced by symbiotic bacteria which can be present in marine animals such as frogs, gobies, newts, trumpet shells and puffer fish. Consumption of these toxin containing animals can cause severe intoxications. TTX intoxications are most frequently occurring in Japan and other Asian countries where puffer fish (fugu) is served as a delicatessen. The adverse effects observed after consumption of TTX contaminated fish or shellfish include nausea, vomiting, paralysis of muscles which results in breathlessness and in severe occasions eventually death can occur. With respect to Europe these toxins are only found occasionally in shellfish. Despite the fact that these toxins are not that often occurring within Europe methods for rapid testing are still needed in order to protect the consumers from exposure to this highly toxic substance. In recent years various methods such as immunological methods (SPR and ELISA), functional binding assays and analytic chemical methods have been developed for TTXs. The analytical chemical methods are based on liquid chromatography (LC) either coupled to UV detection or to tandem mass spectrometric (MS/MS) detection. Nowadays, preferably LC-MS/MS is used for its high sensitivity and selectivity compared to LC-UV. TTXs is a polar compound and therefore under reseversed phase LC conditions no retention is occurring. In order to have retention and separation of TTXs hydrophilic interaction liquid chromatography (HILIC) can be applied. With this retention mechanism it is possible to have retention of polar compounds such as TTXs and also other neurotoxins such as paralytic shellfish poisoning (PSP) toxins. With the conventional HILIC columns long equilibration times are needed in order to obtain stable retention times for the toxins. Therefore, to improve the speed of analysis a method using Ultra Performance Liquid Chromatography (UPLC) tandem mass spectrometry (MS/MS) has been developed. This fast method is capable to analyse TTXs within several minutes. The developed method was in-house validated and subsequently applied for the detection of TTXs in various species of puffer fish.

Keywords: tetrodotoxins, marine biotoxins, puffer fish, UPLC-HILIC-MS/MS

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H-14
FATE OF DEOXYNIVALENOL, T-2 AND HT-2 AND THEIR MODIFIED FORMS DURING BREAD-MAKING BY HPLC–ORBITRAP MS BASED METHOD

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Cereals represent a common substrate for mould grow then promoting the production of toxic secondary metabolites. In particular deoxynivalenol (DON), T-2 and HT-2 toxins belong to the family of Trichothecenes and are produced by several Fusarium species. They represent a relevant problem for their widespread occurrence in cereals and derived products due to their toxicity. To protect consumers European Commission has set official limits for mycotoxins in raw materials and foods. Currently, maximum levels for DON are 1250 µg/Kg in cereals, 750 µg/Kg in flour and 500 µg/Kg in bread. Conversely, maximum acceptable levels for T-2 and HT-2 toxins have not been set yet but due to the dietary risk associated with the trichothecene class A, T-2 and HT-2 have been evaluated by the European Food Safety Authority (EFSA) as candidates for European future regulations. Limits among raw and processed foods are generally different due to the change in mycotoxin concentration during processing. Cleaning, milling, baking can in fact influence the distribution of trichothecenes in the differently processed foods thus altering their concentration in the final products. The present work investigates the fate of DON, T-2 and HT-2 during bread-making employing for their simultaneous determination, a new sensitive method based on HPLC separation coupled to high resolution mass spectrometry. The high accuracy and resolution power offered by the single stage MS Orbitrap mass spectrometer enables the identification of the three mycotoxins and some modified forms at the highest confidence with an accuracy below 0.5 ppm. Naturally and artificially breads contaminated with the three mycotoxins were prepared in our laboratory according to a standard recipe and modified forms of DON were detected in bread samples. Trichothecenes levels were then determined in samples withdrawn at different times during bread-making by the LC-MS method. Findings showed a gradual reduction of DON concentration during bread-making probably due to a combined effect of fermentation and baking as already reported in the literature. As for T-2 and HT-2, it was found that T-2 levels deeply decreased during bread-making despite HT-2 whose level showed to increase markedly. Finally the study demonstrates that the final levels of DON, T-2 and HT-2 toxins are altered by the bread-making process confirming previously studies already obtained for DON while bringing new results on the behaviours of T-2 and HT-2 throughout the cereal food chain.

Keywords: Orbitrap-MS, mycotoxins, bread, masked toxins

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H-15
EXPLORING THE CAPABILITIES OF ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF MYCOTOXINS IN FOOD MATRICES

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Mycotoxins are a group of toxic contaminants produced by several fungi that grown under certain conditions of humidity and temperature in a great variety of foods. The International Agency for Research on Cancer (IARC) has classified some of these toxins as carcinogenic or potentially carcinogenic. In order to preserve the human health, some governments have established maximum permitted levels for some of these toxins in several foods, such as European Union in the Commission Regulation EC/1881/2006 with reporting levels of parts per billion, or even lower. Therefore, sensitive and reliable analytical methodology is required in order to determine mycotoxins at the lowest levels established. The aim of this work is to develop a rapid method for the simultaneous determination of 18 mycotoxins (including all regulated mycotoxins) at levels lower than the maximum permitted in 25 different matrices. For this purpose, ultra high pressure liquid chromatography/electrospray tandem mass spectrometry (UHPLC-MS/MS) has been selected as the preferred technique. Sample treatment has been minimized in order to decrease analysis time as well as solvents use, reducing undesired losses during sample treatment. Solid samples were extracted with 10 mL of a mixture acetonitrile:water (80:20) acidified at 0.1% with formic acid. After 90 minutes of mechanical extraction, samples were centrifuged or filtrated depending on the matrix, and the final extracts were diluted with HPLC water in order to minimize signal suppression or enhancement due to the matrix effect. On the other hand, liquid samples where only diluted. All diluted extracts were filtered before being injected (10 µL) into the chromatographic system. Selected compounds were determined in selected reaction monitoring (SRM) mode, using a triple quadrupole analyser (TQS, Waters) with a rapid chromatographic separation of only 4.5 minutes. The method was validated for all compounds in all studied matrices by means of recovery experiments at two different levels for each selected compound. Recoveries between 70-110% and relative standard deviations (RSDs) lower than 15% were obtained for the majority of matrix-compound combinations. Matrix-matched calibration was used for a correct quantification. Limits of quantification were lower than maximum permitted levels for every matrix-mycotoxin combination. Furthermore, the high sensitivity obtained, allowed their determination at the levels required for babyfood and food for infants, without using any kind of pre-concentration or purification techniques. The developed method was applied to the analysis of around 250 samples corresponding to 25 different matrices obtained from several markets from the Valencian Region. The acquisition of three SRM transitions for each compound allowed the unequivocal confirmation of positive samples, which was supported by the accomplishment of their intensity ratios and retention time when compared with reference standards.

Keywords: Mycotoxins, UHPLC, MS/MS

H-16
MYCOTOXINS IN FOOD SUPPLEMENTS

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Extracts from medical herbs are often used as a part of dietary supplements intended to enriching of the human diet and provide nutrients that may be missing or may not be consumed in sufficient quantities. For example, black cohosh, a perennial plant native to North America, is often used as a dietary supplement for reduction of menopause effects. Milk thistle is a medical plant native to southern Europe, which is commonly used to maintain the health of liver and treatment of liver diseases. Turmeric (curcuma), a rhizomatous herbaceous perennial plant of the ginger family, is often used for heartburn, stomach pain, diarrhea, stomach bloating, loss of appetite, and kidney problems. Further, genus of the Boswellia trees is widely known for its fragrant resin which has many pharmacological uses, particularly as anti-inflammatory agent or substance for asthma treatment. Last, but not least, the orange peel extract is also often used as a food supplement intended to e.g. relief from occasional heartburn, acid indigestion, and upset stomach. However, besides to these beneficial health effects, also some health troubles connected with co-presence of certain undesirable naturally occurring compounds may discover. From this point of view, mycotoxins, toxic secondary metabolites of microscopic filamentous fungi, may represent this problem. Within presented study, a set of food supplements samples (among which the above listed were present) were investigated for presence of 53 mycotoxins (Fusarium, Alternaria, Penicillium, Aspergillus, Claviceps, and others). To our surprise, some of the mycotoxins were present at levels up to 10 mg/kg. For mycotoxins isolation, QuEChERS-based method was employed, for identification and quantification, ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC–MS/MS) was enabled.

Keywords: mycotoxins, food supplements, liquid chromatography, mass spectrometry

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H-17
DETERMINATION OF OCHRATOXIN A IN ROASTED COFFEE AND COFFEE PRODUCTS IN SERBIAN MARKET BY HIGH PRESSURE LIQUID CHROMATOGRAPHY/ FLUORESCENCE DETECTION

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High pressure liquid chromatography/fluorescence detection (HPLC/FLD) method was used for determination of ochratoxin A in coffee and coffee products. Ochratoxin A (OTA) was extracted from 5 g samples by 40 mL mixture of methanol/3% sodium bicarbonate water solution (50/50, *v/v*) in blender jar, for 2 minutes. Methanol/water extract was treated with 2×10 mL of dichloromethane, filtrated, and an aliquot of 2mL was diluted with 48 mL PBS buffer and cleaned up with immunoaffinity column (OtaClean, LCTech). OTA was eluted with 2×1mL of methanol. Eluate was evaporated to dryness, reconstituted with 0.5 mL of mobile phase and the final extract was analyzed by HPLC/FLD. Zorbax Eclipse Plus C18 column was used for separation, with a mobile phase consisted of acetonitrile/2% acetic acid (50/50; *v/v*). The linearity of the standard calibration curve (r^2) was >0.999 for OTA levels of 0.3–100.0 ng/mL (0.5–160.0 µg/kg in coffee samples). Recovery rates from soluble coffee samples ranged from 76.2 to 100.8%, for flavored coffee beverages from 56.1–63.0%, and for roasted coffee samples from 63.0 to 99.1%. The intermediate precision (RSDr) was between 9.1 and 9.4% for soluble coffee and between 14.3 and 15.5% for roasted coffee analysis. The limit of quantification is equivalent to 0.50 µg/kg in coffee samples. This work also includes an application to samples obtained from retail markets and from border inspection. 27 samples of soluble coffee and 25 of roasted coffee and 12 samples of flavored coffee beverages were analyzed using the validated method. All soluble coffee samples contained OTA at levels that ranged from 0.5 to 6.0 µg/kg. Nine of the 25 roasted coffee samples analyzed (36%) contained OTA at levels above 5.0µg/kg which is maximum permitted level. In all 12 samples of flavored coffee beverages level of OTA was below MRL but in 9 samples OTA was detected above limit of quantification. This method is sensitive, accurate and selective, and can thus be applied as a confirmatory p rocedure in establishing non-compliance with National permitted maximum levels (5.0 µg/kg for roasted coffee and 10.0 µg/kg for soluble coffee).

Keywords: Ochratoxin A, HPLC/FLD, immunoaffinity column, coffee

H-18
VALIDATION OF AN UHPLC–MS/MS METHOD USING STABLE ISOTOPE DILUTION FOR THE DETERMINATION OF MYCOTOXINS REGULATED IN THE EUROPEAN UNIO

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All mycotoxins regulated in the European Union for solid food commodities can be analyzed in a multi-target method by LC-MS/MS. However, the accurate quantification in food samples is often constrained by matrix effects resulting in signal suppression or enhancement. Approaches to deal with matrix effects are matrix matched calibrations, standard addition or a sample clean-up procedure reducing the matrix. Another possibility is the addition of isotopically labelled internal standards which are available for all mycotoxins regulated by European Commission Regulation (EC) No 1881/2006 and its amendments. In this work we show the validation for an UHPLC-MS/MS method for the quantitation of 11 regulated mycotoxins including aflatoxins (AFB1, AFB2, AFG1, AFG2), fumonisins (FB1, FB2), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEN), as well as HT-2 and T-2 toxin in maize. Samples were extracted twice with different acidified water-acetonitrile mixtures at room temperature on a rotary shaker. After combining the extracts and centrifugation, an aliquot of the raw extract was transferred into an HPLC vial and was spiked with the corresponding 13C-labelled internal standards. Analysis was done using electrospray ionization and Dynamic Multiple Reaction Monitoring (DMRM) enabled for fast polarity switching. Method validation was performed by spiking blank maize samples with native mycotoxins before and after extraction on multiple levels in triplicate. In addition certified reference materials were analyzed. By using an UHPLC-MS/MS method chromatographic run time could be reduced with improved resolution and without losing sensitivity. The use of DMRM enabled for fast polarity switching allowed the determination of all mycotoxins in the most abundant ionization mode. The addition of internal standards to the raw extract compensated for all matrix effects at minimum costs. Resulting limits of quantification were in the low µg kg-1 range much below the maximum residue levels (MRL) of all toxins allowing the elimination of an enrichment step in the extraction protocol. The new extraction procedure resulted in apparent recoveries of 88 to 105% for all compounds for 6 different spiking levels with relative standard deviations (RSD) below 10%. This has been proven by analyzing certified reference materials for all groups of regulated mycotoxins, except for HT-2 and T-2 which are currently not regulated.

Keywords: Mycotoxins, Stable isotope dilution assay, Method validation, UHPLC-MS/MS

H-19
INTRA-LABORATORY VALIDATION OF A FAST AND SENSITIVE UHPLC–MS/MS METHOD WITH FAST POLARITY SWITCHING FOR THE ANALYSIS OF LIPOPHILIC SHELLFISH TOXINS

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Recently the mouse bioassay as the long-standing reference method for lipophilic shellfish toxins has been replaced by a LC-MS/MS method by EU Legislation. There have been several reasons for that, among others the lower false positive and negative rates as well as the better precision and accuracy of the chemical assay. This has been shown in the interlaboratory validation study coordinated by the EURLMB, as well as in some other independent interlaboratory validations carried out in the EU, using different LC-MS/MS methodologies. Whereas different HPLC conditions have been used, common for both methods is that the determination of all regulated lipophilic shellfish toxins requires acquisition in positive and negative electrospray ionization (ESI) to gain the best possible sensitivity. However, there is concern that throughput for the LC-MS/MS method is not sufficient for routine use. In this work we show the results of an intra-lab validation for a fast UHPLC-MS/MS method working under acidic conditions. 14 lipophilic marine toxins have been acquired in fast polarity switching and using Dynamic MRM for optimizing the duty cycle of the MS. Whereas azaspiracids, pectenotoxins, 13-desmethyl spirolide C, and gymnodimine have been analyzed in positive mode; yessotoxins have been analyzed in negative mode. The okadaic acid group compounds have been analyzed in both, positive and negative ionization and results for both ionization modes have been compared. The validation has been done using the experimental design and samples of the interlaboratory validation study for the EU harmonized SOP coordinated by the EURLMB over a 3-day period. When using Dynamic MRM in fast polarity switching limits of detection (LODs) have been lower and reproducibility and linearity have been better compared to static MRM. The UHPLC separation allows for higher sample throughput in routine use. Compared to the previously used HPLC-MS/MS method LODs have been improved up to a factor of 10 in mussel extract. For the OA group results acquired with negative ionization showed better sensitivity and lower matrix suppression. Matrix effects have been evaluated by comparing standards prepared in solvent with matrix matched calibrations prepared in blank mussel tissue. For accurate quantitation matrix matched calibrations have been used. When analyzing reference mussel materials apparent recoveries have been between 95 and 110% with RSDs below 5% over the 3 day validation procedure. For selected samples new triggered MRM acquisition mode has been applied for additional confirmation of AZA1 and PTX2 showing more than one peak for both, the quantifier and qualifier ions.

Keywords: Lipophilic Marine toxins, UHPLC-MS/MS, Method validation, Triggered MRM

H-20
METHOD VALIDATION OF MICROCYSTIN-LR IN WATER BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) method has been developed and validated to identify and quantify trace levels of cyanotoxins or microcystins (MCs) in lake waters. The method enables confirmation of three MCs (Microcystin-RR, YR and LR) with a single chromatographic run. Quantitative results were obtained only for microcystin-LR. By using LC-ESI–MS/MS in multiple reaction monitoring (MRM) mode, with specific ion transitions m/z (995.5 $\rightarrow$ 283 for quantification and 995.5 $\rightarrow$ 373.5 for qualification.) the limit of detection and quantitation for the microcystin-LR studied, was determined to be 0.01 and 0.10 µg/L, respectively. A sample preparation method involving solid-phase extraction (Oasis HLB, Waters) gave satisfactory recoveries of the analyte. Precision and accuracy of this method were determinated with samples prepared to contain MC-LR at 0.1 and 1.0 µg/L. Mean repeatability (RSDr) was 5.91%, and mean recovery was 118.7%. Water samples were collected from three different water reservoirs in Sava Lake in one month period, where MC-LR was not detected so far, although the presence of Cyanobacteria was detected in one reservoir.

Keywords: Microcystin-LR, LC-ESI–MS/MS, water, Oasis HLB

H-21
SELECTED FUSARIUM MYCOTOXINS IN BARLEY AND MALT

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Microscopic fungi of Fusarium spp are widespread plant pathogens. These “field fungi” invade grains and other plant tissues in a pre-harvest period but under favorite conditions they can also grow in the course of storage. They are producers of considerably different secondary metabolites – mycotoxins that affect health in humans and animals. The main classes of mycotoxins produced by Fusarium spp. include trichothecenes, fumonisins, and zearalenon. The occurrence of trichothecene mycotoxins: deoxynivalenol (DON), T-2 toxin, HT-2 toxin; fumonisins (FB1, FB2), and zearalenon (ZON) was studied in barley and malt samples. Barley samples were obtained from 3 different growing stations. Malts were prepared in the micromalting plant of the Malting Institute of the RIBM in Brno using the traditional EBC method. After extraction the homogenized barley and malt samples were cleaned on the immunoaffinity column DZT MS-PREP (DON, ZON, T-2, HT-2) and the immunoaffinity column FUMONIPREP (FB1, FB2). Subsequently, they were concentrated using a vacuum evaporator, transferred to aqueous methanol and analyzed with the LC-MS/MS. The methods were validated.

Keywords: fusarium mycotoxins, barley, LC-MS/MS

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H-22
DETECTION OF TYPE A TRICHOTHECENE MASKED MYCOTOXINS (MYCOTOXIN GLUCOSIDES) BY HIGH-RESOLUTION LC–ORBITRAP MS

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Fusarium fungi are plant pathogens infecting cereals known to cause disease called Fusarium head blight or scab. These pathogens infect ears and reduce grain yield and quality. Some of them also produce mycotoxins such as trichothecenes and zearalenone. Recently, the existence of masked mycotoxin (mycotoxin glucoside) was reported, and DON-3-glucoside (DON3Glc) is known as the typical one. Due to its higher polarity, masked mycotoxin is generally not detected by the conventional analytical methods. We formerly reported the detection of masked mycotoxins derived from type B trichothecenes, nivalenol-glucoside (NIVGlc) and fusarenon X-glucoside (FUXGlc) in wheat grain. In this study, we detected new masked mycotoxins derived from type A trichothecenes, T-2 toxin-glucoside (T-2Glc) and HT-2 toxin-glucoside (HT-2Glc). Maize sample naturally contaminated with T-2 and HT-2 toxins was extracted with acetonitrile/water (80:20, v/v), and centrifuged. The supernatant was loaded on a SPE column, and the eluent was taken and evaporated. The residue was dissolved in acetonitrile/water/acetic acid (5:94:1, *v/v/v*) and detection was performed with a high resolution LC Orbitrap mass spectrometer (LC-Orbitrap MS) "Exactive" (Thermo Fisher Scientific). Chromatographic separation was performed on a HyPurity C18 column (250 × 3 mm i.d., 5 µm particle size) at 40°C. The system was operated in the full spectral acquisition mode in the mass range of 70–1,000 *m/z* at an ultra-high resolving power of 100,000 FWHM (200 *m/z*). An accurate mass/high resolution (AM/HR) full scan (Scan event 1) and all ion MS/MS (HCD) spectrum acquisition with collision energy (Scan event 2) were concurrently performed in a single run. Although the absolute structures of T-2Glc and HT-2Glc were not clarified, 3-OH glucosylation appeared to be the most probable structure based on the fragment profile and concomitant detection of DON3Glc in the same sample. These masked mycotoxins are suggested to present an addit! ional po tential risk for mycotoxins, since they seemed to be converted to corresponding mycotoxins under certain conditions, for example during various food processing operations, and/or in the digestive tract of mammals after ingestion. Considering that the toxicity of T-2 or HT-2 toxin is more potent than that of DON (group PMTDI for T-2 and HT-2 toxins, alone or in combination of 60 ng/kg body weight/day was proposed by JECFA 2001), our finding of masked mycotoxin derived from both of them might be significant.

Keywords: LC-Orbitrap MS, masked mycotoxin, Fusarium mycotoxin, maize

H-23
NEW APPROACHES FOR SCREENING AND QUANTITATION OF MYCOTOXINS IN DIFFERENT BABYFOOD MATRIX POSSIBILITIES AND CHALLENGES

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Mycotoxins are known to harm the health of humans and animals. They are known either as carcinogenic or otherwise cytotoxic and impair the immune system. Therefore, different countries have set regulations on mycotoxins. In the EU mycotoxin limits are harmonized in the regulation for contaminants in foodstuffs EC 1881/2006 of 19 December 2006 and the amended regulation EC 1126/2007 of 28 September 2007. In July 2010 two new analytical methods for measuring Aflatoxin B1 and Zearalenone in infant food have been adopted as European benchmark (EN 15850, EN 15851). Both methods are based on a immunoaffinity column clean-up HPLC-FLD detection. In this paper we want to present the possibility to analyze Aflatoxin B1 (AFB1) and Zearalenone (ZON) at a comparable detection level implemented into a LC/MS/MS screening method. For our measurements the AB SCIEX Triple Quad™ 5500 LC/MS/MS System was used. In one single LC/MS/MS run of 13 minutes 18 compounds were detected; 12 of them in the positive ionization mode and 6 of them in the negative ionization mode. The crude extracts of different infant foods were just diluted and injected without any additional sample preparation. Detection limits of AFB1 and ZON were found to be comparable to the required values by EN standards. Reproducibility in matrix was found to be lower than 10%. New Analyst™ software allows us to perform a scheduled MRM for both positive and negative experiment in one injection. So, we can demonstrate that both reproducibility and Quantitation accuracy can be improved with no compromising on instrument sensitivity. Additionally we want to demonstrate the approach of screening for mycotoxins at babyfood concentration levels with a TripleTOF™ 5600 System. Due to its high resolution capabilities matrix effects can be further discriminated. Additionally quantitation and high resolution library confirmation can be achieved with just one single injection.

Keywords: mycotoxin, babyfood, EU regulation, screening, Quantitation, sensitivity

H-24
DETERMINATION OF ANATOXIN-A IN SPIRULINA BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Spirulina, a planktonic cyanobacterium featuring spiral filaments, becomes increasingly popular in recent years as human and animal dietary supplement due to its significantly high nutritional values and therapeutical potentials. Spirulina platensis (*Arthrospira platensis*), Spirulina maxima (*Arthrospira maxima*) and Spirulina fusiformis (*Arthrospira fusiformis*), which are naturally present in tropical and subtropical alkaline waters, are the three most commonly used species in the production of Spirulina supplements. As Spirulina is cultivated in and directly harvested from natural water bodies, there is possibility of contamination with other cyanobacteria, some of which are able to produce toxins such as anatoxin-a. Anatoxin-a (2-acetyl-9-aza bicyclo[4.2.1]non-2-ene), is a potent alkaloid neurotoxin produced by various freshwater cyanobacteria species including *Anabaena, Aphanizomenon, Cylindrospermum, Oscillatoria, Microcystis, Planktothrix* and *Raphidiopsis*. It functions as a nicotinic agonist, which depolarizes muscular cells via binding to acetylcholine receptors of the nervous system, resulting in muscle fasciculation, convulsion, tremor, paralysis and even death due to suffocation. A number of methods have been developed to analyze anatoxin-a in cyanobacteria- contaminated water. However, there are few publications that focus on the anatoxin-a content in commercial products of Spirulina. In this study, a simple, fast and sensitive method using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been developed for the determination of anatoxin-a in Spirulina tablets and capsules. The use of internal standard phenylalanine-d5 minimized matrix effect, hence, eliminated the necessity of performing standard addition for quantitation of anatoxin-a. As no pre-concentration of the analyte was required, the sample preparation time was greatly shortened. A detection limit of 0.06 mg/kg was achieved for this method. Single laboratory method validation was performed in terms of linearity, precision and accuracy.

Keywords: Spirulina, Anatoxin-a, LC-MS/MS

H-25
EFFECT OF STORAGE CONDITION OF TRICHOTHECENES A AND B CONCENTRATION IN CEREALS

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Mycotoxins are highly toxic compounds that have a very negative impact on human health, contributing many diseases such as cancer. Due to the variety of toxic effects and resistance to high temperatures, the presence of mycotoxins in foods carries a risk to human health. Numerous scientific studies have shown the contamination of cereals and their products by mycotoxins produced by fungi strains from the Fusarium family. Trichothecenes, fumonisins, moniliformina, zearalenone and its derivatives can be distinguished among these mycotoxins. Trichothecenes include approximately 150 compounds with similar molecular structure. Among them the most important are in group A: T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), in group B: deoxynivalenol (DON), nivalenol, fusarenon. Mycotoxins produced by Fusarium fungi are frequently found in cereals and their products in Central Europe. The species commonly occurring in regions of Northern Europe is a Fusarium culmorum, which is insulated from all parts of agricultural crops. They can be find in various type of cereals such as wheat, barley, rye, oats and corn. The content of fungi metabolites in the final product is affected not only the processing of raw material during post-harvest (transport, storage, cleanliness), but also various types of treatments field (forecrops, fungicides, crop type). The presence of mycotoxins in foodstuffs may be a result of primary infection, or also due to improper storage. The aim of this study was to assess the impact of storage conditions of cereals and cereal products on trichothecenes A and B concentration. Research materials were cereals (oats and wheat) from different Polish regions, ie Małopolskie, Podkarpackie, Świętokrzyskie, Lubelskie, Mazowieckie, and wheat bran and oat purchased in retail outlets. Mycotoxin content was determined using two-dimensional gas chromatography coupled with mass spectrometer GC×GC-TOF/MS Pegasus 4D. The investigated samples showed the presence of large quantity of HT-2 toxin. An increase of the toxin content for oats and wheat after first period storage in the incubator was observed. These levels remained constant even after the second period of storage. Significant increase of HT-2 toxin level for samples of wheat bran and oat had after the first storage period was found. These levels after the second period of storage were increased as well. After storage under refrigerated conditions increase of HT-2 toxin level was observed for wheat samples after the first storage period. These levels after the second period of storage have not changed. No influence of storage period on toxins concentration in samples of oats was found. In case of bran upward trend was not observed.

Keywords: mycotoxins, trichothecenes, cereals, GC×GC-TOF/MS

H-26
EVALUATION OF ATOXIGENIC STRAIN OF ASPERGILLUS FLAVUS TH 97 AS BIOCONTROL AGENTS FOR AFLATOXIN IN RICE

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Aflatoxins are highly toxic, cancer-causing chemicals produced by fungi belonging to the genus Aspergillus. Aspergillus flavus is the most important causal agent of crop aflatoxin contamination. As one strategy to reduce this toxicant, several non aflatoxin-producing (atoxingenic) strains of A. flavus are currently being applied to agricultural fields to competitively exclude aflatoxin-producing Aspergillus flavus species. Of 99 fungal strains isolated from rice and soil in the North of Vietnam, strain TH 97 shown to lack the four gens (aflJ, pksA, ver-I and ordA), coding for key enzymes and proteins in aflatoxin biosynthesis, suggesting it is an atoxingenic mold. The abilities of this strain to reduce aflatoxin contamination were evaluated in grain competition experiments in the labotary conditions. Treatment consisted of inoculation of grain with the toxigenic isolate or atoxigenic isolate alone and co-inoculation of atoxigenic isolate and toxigenic isolate. Aflatoxin contents were significantly lower in the co-inoculation treatments (inoculum rate 1:1) compared with the treatment in which aflatoxin-producing isolate was inoculated alone. In field plot experiments, strain TH97 was applied to soil during the 2009 and 2010 growing seasons. One year after treatment, a relative level of aflatoxin reduction observed is 99.08% in soil and 95.25% in rice. Incidence of the atoxigenic strain is increased and incidence of the aflatoxin-producing strain is decreased without increasing the overall quantity of Aspergillus flavus in the soil.

Keywords: Aflatoxin, Aspergillus flavus, Competitive exclusion, Gene cluster, Rice

H-27
DETERMINATION OF ZEARALENONE BY THE CAPILLARY ZONE ELECTROPHORESIS–UV DETECTION AND ITS APPLICATION TO POULTRY FEED AND CEREALS

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A capillary zone electrophoretic method for the determination of zearalenone is described in this study. A run buffer consisted of 20 mM of sodium tetraborate at pH 9.0 with 15 % aqueous acetonitrile and applied voltage of 15 kV (normal polarity) and injection time of 10 s at 50 mbar were utilized. Phenobarbital was a suitable internal standard and they were recorded at 254 nm. Zearalanon and internal standard appeared at 5.46 min (RSD% 0.20) and 6.35 min (RSD% 0.18), respectively. The repeatability results as represented with RSD% values are in the range of 0.01–1.60 for intra-day and 0.01–1.58 for inter-day. The relationship between detector response and concentration in the range of 5.20x10-7 M to 7.86x10-5 M ZEA was completely linear [rPN = 244803.8 C(M) – 0.0348] in the mentioned range with a good correlation coefficient (r=0.9999).The LOD and LOQ were 2.70×10^{-8} M (8.25 µg L^{-1}) and 8.17×10^{-8} M (25 µg L^{-1}), respectively. Then, the method was successfully applied to the analysis of ZEA in poultry feeds, flour of maize, grain, certain cereal samples such as fibrous biscuit and popcorn and rice crisp.

Keywords: zearalenone, capillary electrophoresis, mycotoxin, poultry feed, cereal

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H-28
SUM-ANALYTICAL DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN SWISS HONEY BY GC–MS

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Introduction Pyrrolizidine alkaloids (PAs) are secondary plant constituents showing acute toxic, genotoxic and carcinogenic properties after metabolic activation in the liver. They are produced by plants of the families Asteraceae, Boraginaceae, Apocynaceae and the genus *Crotalaria* within the Fabaceae. About 350 potentially toxic structures are described in the literature. The primary health hazard for humans is the consumption of plant parts containing PAs in herbal teas, seasoning herbs or via contaminated food such as salad or grains. There may also be a health risk from consumption of secondary contaminated foods such as honey, milk or eggs, with honey being regarded as the most critical.
The current presentation shows a method for quantitation of PAs in honey and in possible relevant source plants.
Method Honey is dissolved in diluted sulfuric acid and the N-Oxides are reduced using zinc dust. After concentration of the basic compounds on a strong cation exchange phase, the residue is dissolved (alkaline aqueous methanol) and extracted with dichloromethane. The extract is dissolved in a solution of tetramethylammonium hydroxide (TMAH) in methanol. TMAH cleaves the esters in a flash methylation and methylates the resultant necine bases (retronecine, heliotridine, supinidine) into their corresponding dimethylethers, which are then analyzed by GC-MS.
Plant samples are pulverized, homogenized and extracted by methanol. Aliquots of this extract are processed similarly to the honey samples.
In comparison to other procedures, this method is sensitive (LOD 1 ng/g for honey samples) and the reproducibility is acceptable. The recovery for different alkaloids in honey samples varies up to a factor of 3. Analysis of the same samples by LC-MS-MS showed higher concentrations. Therefore, the PA content of honeys containing Echium alkaloids may be underestimated using this analytical procedure.
Results and Discussion 40 Swiss honeys (production years 2009, 2010), six commercial, globally traded honeys as well as four Echium honeys from Spain and New Zealand (blue borage honey) were analyzed for their PA contamination. 30% of the Swiss honeys (average PAs in positive honeys 4 ng/g; maximum 16 ng/g), 83% of the commercial honeys (average PAs in positive honeys 8 ng/g; maximum 21 ng/g) and all four Echium honeys (average PAs in positive honeys 324 ng/g; maximum 495 ng/g) contained PAs. Swiss honeys showed relatively low concentrations of PAs in comparison to globally traded honeys.
Floral samples of *Adenostyles* (12 mg/g), *Eupatorium* (14 mg/g), *Senecio* (2–8 mg/g) and *Echium* (8 mg/g) showed the expected high concentration of PAs. *Myosotis* (0.2–3 mg/g) and *Borago* also contain relevant concentrations. Usually the flowers (blossoms) contain higher concentrations than the leaves.

Keywords: GC-MS, sum-analytical, tetramethylammonium hydroxide (TMAH), pyrrolizidine alkaloids, honey

H-29
IMPROVING LC–MS/MS METHODOLOGY FOR LIPOPHILIC MARINE BIOTOXINS ANALYSIS USING UPLC AND NOVEL MS TECHNIQUES

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Although long-anticipated, recent changes in EU shellfish safety regulations have raised questions about the applicability of many LC and MS techniques to the analysis of marine biotoxins in real matrices. Based on recommendations from various sources as well as in-house experimentation, CEFAS have developed a fast analytical method which meets the demand for low limits of quantitation for multiple analytes in a variety of matrices. Extensive single-site validation has been carried out and has proven the method to be robust, sensitive and accurate. It is now in routine use in the UK biotoxins monitoring programme. Such methods are often subject to scrutiny due to the inherently changeable nature of the matrix. A novel feature of this instrumentation allows real-time monitoring of the chemical background by acquiring qualitative full-spectral data concurrently with standard MRM data for quantitation and confirmation. This not only facilitated development of the method by increasing understanding of the various tissue types, it is also used routinely so as to enable scientists to make informed decisions about the accuracy of questionable results. The method described here allows a good margin of error (LOD of approximately 10 x less than the required level on the least responsive analyte). However, with a universal move to LC-MS/MS techniques in all EU testing facilities, further review of the legislation surrounding these compounds is expected to continue. In anticipation of more stringent legislation, the method has also been transferred to a instrument with a novel ion source, designed to increase the signal associated with targeted analytes whilst minimising background noise, thereby enabling much lower LODs to be achieved.

Keywords: marine, biotoxins, LC-MS/MS, basic, lipophillic

H-30
ELISAS FOR DETECTION OF THE SHELLFISH TOXINS DOMOIC ACID, OKADAIC ACID AND SAXITOXIN

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Shellfish toxins are naturally occurring compounds produced by algae and phytoplankton. Under normal conditions these compounds do not cause any problem. However, filter feeders such as clams, mussels, oysters and crustaceans can consume large quantities of these algae when environmental conditions result in harmful algal blooms. High concentrations of shellfish toxins then accumulate in these animals causing illness amongst people who eat them. There are four syndromes called shellfish poisoning, i.e. paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP). PSP is caused by saxitoxin (SAX) and its analogs. DSP is primarily caused by okadaic acid (OA) and several analogues of OA, whereas ASP is caused by domoic acid (DA). In the European Union, Regulation (EC) no 853/2004 stipulates that live bivalve mollusks should not contain shellfish toxins exceeding the following limits: 800 µg/kg for the PSP toxins, 20 mg/kg DA equivalents for the ASP toxins, and 160 µg/kg OA equivalents for OA, dinophysistoxins, and pectenotoxins together. Using polyclonal rabbit antibodies, three competitive ELISAs were developed for the detection of SAX, DA and OA in shellfish [1,2]. In a validation study, the detection capability (CCβ) of each of these ELISAs was determined as well as the cross-reactivity (specificity), precision (inter- and intra-assay variation), recovery and stability. These validation studies were performed in order to commercialize the kits. CCβ(ng/ml) Mussels Oysters Scallops DA 60 60 150 OA 40 40 ND SAX 4 3 ND Cross-reactivity (%) DA OA SAX DA 100 <0.1 <0.1 OA <0.1 100 <0.1 SAX <0.1 <0.1 100 DTX-1 <0.1 78 <0.1 DTX-2 <0.1 2.6 <0.1 DC-SAX <0.1 <0.1 19.2 GTX5 <0.1 <0.1 26.2 GTX2/3 <0.1 <0.1 5.6 CVinter (%) CVintra (%) Mussels Oysters Scallops DA 5.5 2.8 1.7 4.4 OA 7.1 1.9 2.8 ND SAX 12.2 10.5 8.5 ND The obtained % recovery of DA, OA and SAX mussels, scallops and oysters all fell into the range of 70-120%. When stored at +4°C, the ELISAs were stable for at least 12 months.

[1] Dubois M., Demoulin L., Charlier C., Singh G., Godefroy S.B., Campbell K., Elliott C.T., Delahaut P. (2010). Development of ELISAs for detecting domoic acid, okadaic acid, and saxitoxin and their applicability for the detection of marine toxins in samples collected in Belgium. Food Additives and Contaminants, June 27(6), 859-868.
[2] Campbell K., Huet A.-C., Charlier C., Higgins C., Delahaut P., Elliott T. C. (2009). Comparison of ELISA and SPR biosensor technology for the detection of paralytic shellfish poisoning toxins. Journal of Chromatography B, 877, 4079-4089.

Keywords: biotoxins, domoic acid, okadaic acid, saxitoxin, ELISA

H-31
MICRO-BIOAFFINITY NANO–LIQUID CHROMATOGRAPHY MASS SPECTROMETRY OF MYCOTOXINS

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Functional binding assays with antibodies, transport proteins and molecular receptors are important tools in the future detection of known and unknown chemical contaminants in food. However, mass spectrometric identification of both known and unknown bioactive compounds in positive (non-compliant) samples will be crucial. Therefore, bioaffinity-based isolations and detections, based on the same bioreagents, are being developed in this project. Since biomaterials (e.g. IgGs, receptors) are costly, microscale isolations are combined with nano-LC-Q-TOF-MS. The detection of ochratoxins, a group of the most abundant food-contaminating mycotoxins in the world, with monoclonal antibodies (Mabs) on superparamagnetic colour-encoded microspheres is used as a first model. A flow cytometric immunoassay (Luminex FM3D) was developed using functionalised superparamagnetic microspheres coated with Mabs directed against ochratoxin A (OTA) in combination with a fluorescent label (OTA-phycoerythrin) and an easy extraction method for wheat and cereal samples (1 g + 10 mL buffer). By means of this easy and quick inhibition screening assay using 10 “blank” and 10 spiked wheat samples, the screening of OTA and a non-chlorinated analogue (ochratoxin B (OTB)) was achieved at relevant levels (1 ng/g and 5 ng/g). The blank wheat samples were

Keywords: bioaffinity isolation, mass spectrometry, mycotoxins, wheat, cereal

H-32
SIMULTANEOUS DETERMINATION OF FOUR FUSARIUM MYCOTOXINS IN BABY FOOD USING DZT MS–PREP

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Simultaneous Determination Of Four Fusarium Mycotoxins In Baby Food Using DZT MS-PREP® D. Leeman & C. Milligan, R-Biopharm Rhône Ltd Fusarium mycotoxins can be found in a variety of commodities when foods are stored under adverse conditions including temperature and humidity. Legislation for mycotoxins has recently increased to incorporate additional matrices and toxins, therefore resulting in an increased demand for faster and less labour intensive tests. Immunoaffinity columns are rapidly becoming the routine standard method of choice for complying with regulatory mycotoxin analysis however there is a growing need for multi-mycotoxin analysis using a single extraction method. In response, R-Biopharm Rhone has produced a multi-toxin immunoaffinity column, DZT MS-PREP®, which enables the isolation and concentration of four commonly occurring Fusarium mycotoxins; deoxynivalenol, zearalenone, T-2 and HT-2. The advantages of this new immunoaffinity column are that only one sample preparation method and one single LC-MS/Ms run is required for quantifying all four mycotoxins therefore having greater sample throughput and a reduction in the use of solvents and consumables. The method was validated in-house using DZT MS-PREP® to test various baby food samples. Recovery data ranged from 90–109% (RSD 0.3–3.1%) for column repeatability, while recovery data ranged from 84–106% (RSD 2.7–5.6%) for method repeatability. DZT MS-PREP® was shown to perform in line with current EU recommendations whilst providing a rapid and robust method of analysis enabling accurate, low level quantification of all 4 mycotoxins in baby food samples.

Keywords: LCMS, Immunoaffinity Column, Baby Food, Mycotoxin

H-33
ACHIEVING HIGH SENSITIVITY IN ANALYZING TRICOTHECENES IN GRAINS BY APPLYING LC–MS/MS TECHNIQUE–AN MRM TRANSITION AND MS^3 QUANTITATION APPROACH

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A highly sensitive LC-MS/MS method was developed to analyze tricothecenes in grains. The tricothecenes are Nivalenol (NIV), Deoxynivalenol (DON), 3-acetyl-Deoxynivalenol (3ADon), 15-acetyl-Deoxynivalenol (3ADon), Deoxynivalenol-3-glucocide (Don3G), T-2 and HT-2 toxins, Fusarenon X (FusX) and Diacetoxyscirpenol (DAS). Acetic acid and ammonium acetate were used to convert the analytes into their respective acetate adducts and ammonium adducts under negative and positive MS polarity conditions, respectively. The mycotoxins were separated by reversed-phase LC and detected using an electrospray ionization interface (ESI) in a 15-min run. Analyte specific mass/charge ratios were used to perform quantitation under MRM transition and MS3 modes. FAPAS materials for Deoxynivalenol, HT-2 and T-2 toxins were used as internal quality control (IQC) materials. Spike recovery was performed at 50 ng/kg, 100 ng/kg, 200 ng/kg, 300 ng/kg, 400 ng/kg and 600 ng/kg (n=3) in 10 g matrix. Detection limit of 0.5 ppb (in grains) was achieved for all analytes (in matrix) by applying surface response optimization strategies on the MS conditions and solvent system compositions. The gradients obtained from the calibration curve in pure standards and in matrix were matched by applying (13C-isotopes) internal standards. A recovery of about 95–105% was reported.

Keywords: Grains, MRM, MS3, Tricothecenes.

Acknowledgement: AbSciex Singapore

H-34
INTENSE FORMATION OF "MASKED MYCOTOXINS" DURING FOOD PROCESSING

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So called "masked mycotoxins" are glycosilated, sulfated or acetylated derivatives of well known toxins like deoxynivalenol (DON) or zearalenone (ZON) that often co-occur with the unconjugated forms in food and feed. Unlike the precursors DON and ZON that are predominantly formed by Fusarium species, the conjugates originate from the secondary metabolism of plants or microorganisms. But to date, official regulations with respect to maximum levels are focused only on mycotoxins formed as secondary metabolites of fungi, e.g. DON or ZON. As the steps of analytical procedures are mostly specific for the regulated target mycotoxins (e.g. immunoaffinity clean-up or LC-MS/MS mass transitions) masked mycotoxins are often not included in common LC-MS methods and therefore prone to be overlooked in residue analyses. However, the few existing studies provide evidence that DON-3-glycoside (D3G) is present in several foods (e.g. beer) to the same amount as the free DON. To date, neither toxicology nor bioavailability of the mycotoxin conjugates have been investigated. However, it can be assumed that during their passage through the gastro-intestinal tract significant amounts of the glycosilated toxins will be cleaved from their conjugates. In order to answer the question whether the process of germination has an influence on the enzymatic transformation of DON to D3G two approaches were chosen. First, various grain types were spiked with DON and germinated for three days in laboratory scale. Subsequently, the mycotoxin pattern of the grains was analyzed by LC-MS/MS using isotope dilution analysis. It was shown that various germinating grains (wheat, barley, spelt, and rye) are capable of transforming up to 50% of the spiked DON amount into D3G, while millet and oats did not catalyze any glycosilation. In a second experiment, the DON conjugation was investigated under technologically authentic conditions in a model malting plant. About 35% of the spiked DON amount was glycosilated by germinating barley under common malting conditions. These experiments show that in beer or food and feed obtained from germinated grains significant amounts of the DON content can be masked, leading to an altered yet unknown toxicological potential. Additionally, fermentation processes may impact the formation of mycotoxin conjugates in various foods. While in LC-MS analysis it is possible to include D3G and other toxin conjugates as additional analytes for a detailed measurement of the mycotoxin content, immunological analysis is dependent on specific antibodies. The latter can have highly variable cross reactivities that require thorough testing. Consequently, an improvement of analytical methods and the generation of pure reference substances are necessary and urgently needed for future challenges. This also enables the accomplishment of studies evaluating the formation, bioavailability and toxicology of DON and ZON conjugates in order to assess potential health risks.

Keywords: masked mycotoxins, deoxynivalenol, LC-MS/MS, malting, DON-3-glucoside

H-35
NEW SELECTIVE SPE SORBENTS BASED ON MOLECULARLY IMPRINTED POLYMERS FOR SAMPLE PREPARATION OF COMPLEX FOOD MATRICES SUCH AS SPICES (OCHRATOXIN A), CLASSIC AND MULTIFRUIT APPLE PUREES (PATULIN), CORN AND BABY FOOD SAMPLES (ZEARALENONE)

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Molecularly Imprinted Polymer (MIP) represents a new alternative as a synthetic polymer sorbent that mimics antibodies recognition properties. MIP is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. MIP has the advantages to be not only highly selective and specific but also chemically and thermally stable, compatible with all solvents and cost-effective. This polymer is used as a powerful technique for clean-up and pre concentration applications. Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom, commonly known as molds. Mycotoxins can appear in the food chain as a result of fungal infection. To be detected at a trace level (range of µg/Kg), food analysis requires an efficient clean-up before analysis. To do so, very selective solid phase extraction (SPE) sorbents are often used. Despite low stability and high price, ImmunoAffinity SPE Columns (IAC) are the gold standard method for mycotoxin analysis. The efficiency of a method using MIP as selective sorbents for solid-phase extraction will be shown in respect to the clean-up and pre-concentration in different complexes matrices. New selective MIP sorbents will be presented, particularly for Ochratoxin A, Zearalenone and Patulin.

Keywords: Solid Phase Extraction, Molecularly Imprinted Polymers, Ochratoxin A, Patulin, Zearalenone

H-36
COMBINING LAB-ON-A-CHIP AND PLANAR WAVEGUIDE TECHNOLOGY FOR ON-SITE MULTIPLEX MYCOTOXIN TESTING

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Multiplex mycotoxin testing is gaining increasing interest in the global food and feed industry as more and more data on multi mycotoxin occurrence and synergistic effects between different mycotoxins are becoming available. Subsequently, the demand for rapid precise and easy-to-use on-site testing systems increases. The innovative analytical method presented here relies on planar wave guide (PWG) technology. Being a biochip technology, PWG allows for the simultaneous detection of multiple analytes. Due to the surface confinement of the biochip illumination, the development of easy-to-use assays obviating the need for washing steps is possible. A disposable cartridge (Bayer Micro-Lab, BML) was developed, applying state-of-the art microfluidics principles. The BML enables the reader (Bayer Quality Analyzer, BQA) to move the diluted sample extract from the sample compartment to the mycotoxin antibody compartment and further to the biochip and reaction chamber. All reagents necessary for analysis are stored within the BML in dry form, ensuring a long shelf life. The BQA has been constructed as a touch-screen guided plug and play instrument, containing a laser, incoupling unit, CCD camera, computer, temperature control and fluidics handling unit. BML and BQA together form an easy-to-use solution for multiplex quantitative mycotoxin measurement at the point of interest. For any mycotoxin measurement, follow a classical extraction protocol, fill the diluted extract into the BML, and introduce the BML into the BQA. Using its internal fluidics handling unit, the BQA automatically agitates the liquid inside the BML. After 12 minutes, when the test is completed, the BQA activates the laser, takes images of the biochip and calculates the quantitative mycotoxin values by using the calibration data stored on the Bayer Calibration Card (BCC). BCC guarantees the accurate calibration of the BML and is specific for the respective crop. Unlike other technologies the BQA does not require the use of mycotoxin standards for calibration. The whole process from sample to result takes less than 20 minutes. Currently, BML Wheat and Barley comprise DON, ZEA, T-2 and OTA. BML Corn comprises DON, ZEA, OTA, AFL and Fumonisin. Detection ranges for the individual mycotoxins have been developed to meet European food regulations. We will present most recent data on internal and external validations based on spiked and naturally contaminated wheat, barley and corn samples as well as processed materials. An outlook on potential future applications of PWG platform technology will also be discussed.

Keywords: Mycotoxins, Multiplex, Biochip, PWG

H-37
QUANTITATIVE DETERMINATION OF AFLATOXIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN WHEAT SILOS IN GOLESTAN PROVINCE, NORTH OF IRAN, IN 2009

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Introduction Aflatoxins are the most common mycotoxins that contaminate crops. They are produced by Aspergillus flavus and Aspergillus parasiticus. Fungal invasion may occur in the field or during post harvest and storage, particularly in favorable environmental conditions such as high humidity and high temperature. The aflatoxin exposure may lead to acute and chronic toxicity in human. Wheat (Tricitum aestivum) is the main food used in Iran, and the environmental conditions in north of Iran are favorable to fungal growth. Therefore, this study was designed in order to determine aflatoxin contamination in wheat samples of silos in Golestan province, located the south-east of the Caspian Sea and north of Iran, in 2009. Methods Samples were collected from three silos of Gorgan, Gonband and Galikesh in Golestan province in 2009. First, aflatoxins were isolated using immunoaffinity chromatography. Then the aflatoxin concentrations were determined by High performance liquid chromatography (HPLC) method and fluorescence detector. Result Ten out 34 samples (29.4% of samples) were contaminated by aflatoxins. No concentration was found above permissible aflatoxin levels in Iran (15ng/g). In one sample (2.9%), aflatoxin B1 was seen over the recommended limits in Iran. The highest level found in samples for total aflatoxin, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2 were 7.08 ng/g, 6.91 ng/g, 0.29 ng/g, 1.37 ng/g and 0.23 ng/g, respectively. Conclusion: Despite the total aflatoxins determined in samples were below the recommended limits in Iran, the 29% aflatoxin contamination rate can negatively affect health factors and it should not be neglected. Moreover, in the year that the study was done, the wheat reserves were lower compared to previous years. Therefore, it is predictable if the storage duration of samples increase, the aflatoxin contamination levels increase, too.

Keywords: Aflatoxin, wheat, HPLC, Immunoaffinity Chromatography

H-38
NEW CRM FOR T-2 AND HT-2 TOXIN: A CRUCIAL TOOL FOR QUALITY ASSURANCE AND CONTROL IN FOOD ANALYSIS

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Contaminations with moulds and mycotoxins may occur during the whole production chain of a food product (i.e. “from the field to the fork”). Due to serious toxic effects caused by mycotoxins, the determination and reduction of these compounds in food and feed is subject to the work of regulators, food business operators and researchers. Fungi of the genus Fusarium are the predominant mycotoxin producers in moderate climate zones. Fusarium toxins occur worldwide in a wide variety of foods, particularly in highly consumed cereal based products. The toxicologically - and hence also economically - most important Fusarium mycotoxins are zearalenone (ZEN) as well as the type A (T-2 and HT-2 toxin) and type B trichothecenes (deoxynivalenol (DON), nivalenol (NIV)). Driven by regulatory authorities extensive consumer protection efforts were made by establishing fast and reliable analytical methods for the determination of the most common Fusarium toxins in cereals and derived products. At the same time legally binding maximum levels were introduced for these matrices [1,2]. While for DON and ZON EU maximum levels are already in effect, new levels for T-2 and HT-2 toxin are currently under discussion. To enforce the maximum levels and thus reduce consumer risks, strict controls of food and feed are of prime importance. For the sum of these reasons, matrix matched certified reference materials (CRMs) for Fusarium toxins trichothecenes are required. CRMs can contribute to aid in method validation and increase comparability and traceability in trichothecene analysis. In the frame of the ERM® (European Reference Materials) initiative BAM has completed 3 CRM projects on the field of mycotoxins and initialised a new one for the Fusarium mycotoxins T-2 and HT-2 toxin in oat flakes (candidate reference material ERM®-BC720), to close the current gap of available CRMs for mycotoxins in food. The preparation and characterisation of the wheat material including homogeneity and stability studies based on ISO Guide 35 [3] will be outlined and discussed. First results of the interlaboratory comparison study for certification of the mass fractions of T-2 and HT-2 toxin are presented.

[1] Commission Regulation (EC) No 1881/2006.
[2] Commission Regulation (EC) No 1126/2007.
[3] ISO Guide 35 Reference materials: General and statistical principles for certification; ISO/REMCO, 2006.

Keywords: Fusarium mycotoxins, Trichothecenes, European Reference Material, HPLC-MS/MS

H-39
DETERMINATION OF MYCOTOXINS IN DRINKING WATER MATRICES BY SPE–HPLC–MS/MS

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Mycotoxins of B-type Trichothecenes, Aflatoxins and Zearalenone are found in food and feed as a result of fungal infection by Fusarium and Aspergillus. These mycotoxins are responsible for serious acute or chronic adverse health effects. Lately studies have been performed on food matrices [1–4] but very few were conducted on water matrices. The low concentration of these compounds in real water samples requires the use of selective and sensitive techniques. In this study solid phase extraction (SPE) followed by liquid chromatography coupled with tandem mass spectrometry an electrospray source operating in negative mode and a triple quadrupole as analyser were used in the identification and quantification of these mycotoxins in drinking waters matrices samples. Chromatographic and mass spectrometry conditions were optimized in order to achieve low limits of detection. The method was validated in terms of specificity, linearity, repeatability, precision and accuracy and LOD (Limit of detection) and LOQ (limit of quantification). Linearity was observed over a concentration range of 1.0–100 ng/mL for most mycotoxins. Limit of detection and quantification of 0.3 ng/mL and 1.0 ng/mL for Zearalenone and metabolites, 3.0 ng/mL and 10.0 ng/mL for B-type Trichothecenes. For repeatability study RSD<13% were obtained and for intermediate RSD<16%. Accuracy values were higher than 72% for Zearalenone mycotoxins family. B-type Trichothecenes exhibited lower recovery. Further studies are currently being conducted to improve the solid phase extraction conditions for B-type Trichothecenes and achieve lower detection limits. Real water samples were analysed.

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Keywords: Mycotoxins, HPLC-MS/MS, SPE, Water Samples, Validation

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H-40
A SYSTEMATIC ASSESSMENT OF THE VARIABILITY OF MATRIX EFFECTS IN LC–MS/MS ANALYSIS OF ERGOT ALKALOIDS IN CEREALS

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Liquid chromatography mass spectrometry (LC-MS) has become the preferred method for ergot alkaloids determination in various matrices. However, matrix effects are a major drawback of this technique as they can lead to inaccurate quantification. The currently described LC-MS methods for ergot alkaloids imply the use matrix-matched calibration curves to compensate for these effects [1,2]. This approach raises questions about the variability in matrix effects between different samples of a given matrix. In this study, the signal suppression/enhancement (SSE) for the six most prominent ergot alkaloids (ergometrine, ergosine, ergotamine, ergocornine, ergokryptine and ergocristine) as well as their corresponding epimers was assessed in a variety of wheat, rye and triticale samples, including different genotypes and different batches. A conspicuous variability in the matrix effects was observed between the different cereal types investigated. Signal suppression was higher in wheat than in rye or triticale samples. No significant difference in matrix effect could be demonstrated between different batches, nor between different varieties of a given cereal, though some variations were observed. Overall, the highest tendency towards signal suppression was observed for ergometrine. It was shown that signal suppression could be reduced through a careful selection of the sample preparation procedure or of the LC-MS/MS equipment.

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Keywords: Ergot alkaloids, Liquid chromatography mass spectrometry, Matrix effects, Cereal varieties

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H-41
OCHRATOXIN A IN TISSUE SAMPLES FROM SWINE AND CHICKEN: OCCURRENCE AND EXPOSURE ASSESSEMENT IN SERBIA

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Samples of pigs blood, kidney, liver and chicken gizzard were randomly selected from slaughtered animals (n=90) and analyzed for the presence of ochratoxin A by HPLC-FL. In porcine tissue samples, of the 90 liver samples, 26.6% contained OTA in the range of 0.22–14.5 ng/g. The incidence of OTA in serum and kidneys were very similar (31% and 33.3%, respectively), with a maximum concentration of 220.8 ng/mL, and 52.5 ng/g, respectively. Majority of chickens' tissues samples, were not found to contain measurable amounts of OTA. Moreover, the OTA levels found in analysed tissues were low in general. The results of this preliminary research indicate that the content of OTA in the examined tissues are far below the values that represent hazard to the health of consumers. However, the results of this study show that in Serbia, consumers are nevertheless frequently exposed to ochratoxins.

Keywords: ochratoxin A, swine and chicken tissues, exposure assessement

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H-42
AFLASENSOR: RAPID TEST FOR AFLATOXIN M1 IN MILK

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Aflatoxin M1 (AFM1) is one of the major Aflatoxin B1 (AFB1) metabolites excreted in mammalian systems in cow's milk, urine and animal serum. This toxin could also be at a less extent directly produced by fungi such as Aspergillus flavus and Aspergillus parasiticus. Like its parent compound, AFM1 keeps its carcinogenic, mutagenic and toxic effects. Its appearance in dairy products is commonly associated to a metabolic hydroxylation of AFB1 present in contaminated feed. In lactating cows, AFM1 appears in milk at levels proportional to the contamination level of the feed. The consumption of high levels of AFM1 can lead to a disease called Aflatoxicosis. To prevent the development of its hazardous effects on cows and consequently on humans, the European commission has established MRL ((EU) No 165/2010) for raw and heat-treated milk at 50 ppt. Face to the lack of rapid tests availability enabling to detect this low concentration, Unisensor, has developed the Aflasensor kit, a rapid lateral flow immunochromatographic test allowing to detect Aflatoxin M1 at 50 ppt in milk. This rapid semi-quantitative test uses freeze-dried gold-labeled antibodies that are in competition between AFM1 present in the milk sample and the toxin immobilized on the dipstick at the capture lines. When the sample is free of contaminant, the antibodies will be free to bind the immobilized toxin at the test line and gold particles will agglutinate to generate red color at the line. In opposition, when the sample is contaminated with AFM1, the antibodies bind the free toxin and no color will be generated at the test line. This new rapid test does not require any pre-treatment or cleaning of the milk. With the help of an optical reader the test gives a semi-quantitative response after 20 minutes of incubation like: 0-50 ppt (Negative); 50 ppt (Low Positive); 50-100 ppt (Positive) and > 100 ppt (Full Positive).

Keywords: Aflatoxin M1, lateral flow immunoassay, milk

H-43
MULTIPLEX LATERAL FLOW IMMUNOASSAYS FOR THE DETECTION OF PYRROLIZIDINE, TROPANE AND ERGOT ALKALOIDS

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Alkaloids are secondary metabolites produced by plants that contain one or more basic nitrogen atoms, usually located in a heterocyclic ring. They have important biological effects on the human health and can cause severe health problems. Most alkaloids exhibit physiological effects to humans and animals. The role of alkaloids in plants is notably to defend the plants against predators such as insects and animals. Indeed, many of those alkaloids are hepatotoxic, carcinogenic, teratogenic and mutagenic compounds. Alkaloids can be found in food of plant origin (e.g. cereals, herbs), animal origin (e.g. honey, eggs, and milk) and in animal feed. They can contaminate feed and grains through botanical impurities, mostly with alkaloid-containing weeds, but also honey with contaminated pollens transferred by bees into honey. Face to this contamination risk, the European commission wants to establish new regulations, but the lack of rapid detection methods for evaluating the real health risk for human acts as a brake. In the framework of the European project "CONffIDENCE", rapid lateral flow immunoassays for pyrrolizidine (PA), tropane (TA) and ergot alkaloids (EA) have been developed. The targeted toxins are jacobine and lycopsamine at 50 µg/kg in honey and feed for PA, atropine and scopolamine at 100 µg/kg in feed for TA and, ergotamine and ergocristine at 200 µg/kg in cereal and feed for EA. In this poster, the syntheses of immunogens and the characteristics of polyclonal antibodies raised against the different alkaloids of interest will be presented. Cross-reactivity studies will be shown and based on the specificity or on the generic character of these antibodies, single or double test lines dipsticks have been developed for each alkaloid family. The performance of those dipsticks in spiked extracts and in incurred matrices has been also evaluated. The total time of each dipstick assay is 15 minutes and results can be interpreted visually or with an optical instrument like the Readsensor.

Keywords: pyrrolizidine alkaloids, tropane alkaloids, ergot alkaloids, lateral flow device

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H-44
SIMULTANEOUS DETECTION OF TRICHOTHECENES, ZERALENONE AND OCHRATOXIN A IN CEREALS, FEED AND MEAT BY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTOR

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Mycotoxins analysis is usually performed through single compound also certain classes of mycotoxins determination by HPLC method coupled with UV- or fluorescence detectors, after immunoaffinity column clean-up, or else after derivatization [1,2]; otherwise chromatography electrospray ionization tandem mass spectrometry [3]. It has been developed quick and simple method for the simultaneous determination of deoxynivalenol, T-2 toxin, ochratoxin A, zearalenone for the mycotoxins analysis in cereal (corn, rice, wheat, oats, rye, barley, soya), feed and meat (pig, cow and chicken meat) after derivatization with threefluorineacetic anhydride witch were analyzed by gas chromatography with electron capture detector. The extraction procedures considered were QuEChERS (acronym of Quick, Easy, Cheap, Effective, Rugged and Safe) [4, 5]. Extraction procedure showed high-quality for mycotoxins recovery (>80%). Quantitation limits were 0.05–5 mg/kg (0.01–2 for ochratoxin A, 0.03–3 for T-2 toxin). Analysis duration is 1–1.5 h, the relative standard deviation of result does not exceed 0.08.

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Keywords: Mycotoxins, simultaneous detection, method of QuEChERS, cereal, meat

H-45
INTRAVALIDATION OF MULTIRESIDUAL METHODS FOR MICOTOXINES IN CEREALS AT PPB LEVEL USING ASCENTIS EXPRESS RP AMIDE AND F5, COUPLE WITH UHPLC/MS/MS

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The determination of multiresidual Micotoxine on cereals has become nowadays a routine analysis. The optimization of analysis time, the limit of detection and the robustness of the method are important parameters for the validation and certification of an optimum method. This Presentation describes the pairing of innovative UHPLC, Fused Core HPLC column technology and LC / MS / MS optimization for the validation of a fast, efficient and reproducible method, considering the issues of separation and detection of some mycotoxins.

Keywords: Mycotoxines, UHPLC/MS/MS, Fused core columns

H-46
PATULIN STATUES OF SEMIROM APPLE

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According the FAO estimate, Iran with 2 660 000 (Tonnes) apple production after the China and United States is among top ten apple producers in 2008. Semirom in Isfahan province produce 13–14% of total apple production in Iran. The dominant apple varieties in this area are Golden and Red apples. A part of this product was exported to other countries. This study was done to detection of patulin in the fruit for the fresh fruit market. Patulin is a secondary metabolite produced by a number of fungal species in the genera Penicillium, Aspergillus and Byssochlamys of which Penicillium expansum is probably the most commonly encountered species. Patulin has been found as a contaminant in many mouldy fruits, vegetables, cereals and other foods, however, the major sources of contamination are apples and apple products. Patulin, is acutely toxic, carcinogenic, teratogenic and mutagenic. Several countries have instituted patulin restrictions in apple products. The World Health Organization recommends a maximum concentration of 50 µg/L in apple juice. As the Horticulture in Semirom is under restrict supervision and the Recommended practices based on Good Agricultural Practice (GAP), the patulin content of apple was selected as one factor for the evalution of these practice. For this aim three different locations in Semirom were detected and from 3 different apple garden and in every garden from different trees about 4–5 kilogram apple was gathered. The samples from every garden were absolutely mixed and after blending, the apple juice send to laboaratory for patulin detection. Samples stored below 4°C before analysis. A total of 30 sample were send to laboratory and patulin content was detected by HPLC.The result of this study show that the patulin content of samples aren't detectable in fresh fruit ($p<0.01$) and we can call them patulin free in this stage. The control of patulin in fruit juice and fruit products could be achieved by using healthy fruit, hygienic storage, sorting damaged and rotten fruits, trimming off rotten tissue, filtration through activated charcoal and pasteurization. The result of this study show that for controlling the patulin, the efforts should be focused to other stage such as, post –storage grading of fruit for the fresh market of juice manufacture, transportation, checking, and pressing of fruit,packing and final processing of juice and fruit for the fresh fruit market have no patulin content.

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Keywords: Patulin, Semirom, fresh, apple

H-47
RIDA®QUICK TESTS PLUS RIDA®QUICK SCAN: A NEW APPROACH FOR MYCOTOXIN ANALYSIS

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Mycotoxins are secondary metabolites formed in agricultural products, such as cereals. Mycotoxin contamination of food and feed product impose a risk to human and animal health and have serious economic impact. To meet international regulations and guidelines products are tested for the amount of mycotoxins. During the production process of food and feed critical steps can be identified where it is possible to minimise the risk of unacceptable mycotoxin concentrations in the end product. The RIDA®QUICK lateral flow tests for mycotoxin analysis in combination with the RIDA®QUICK SCAN, enabling on-site quantitative analysis for Aflatoxin, DON and Fumonisin, have proven to be a very valuable tool. With minimal laboratory equipment samples can be analysed within 10–20 minutes. Results show an excellent correlation with HPLC for many commodities in a wide measurement range.

Keywords: Mycotoxins, Analysis, Lateral Flow

H-48
PROFICIENCY TESTING FOR DETERMINATION OF AFLATOXIN IN PEANUTS

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Proficiency tests are used to determine the performance of individual laboratories for specific measurements. However, there are few proficiency tests providers mainly to mycotoxin in foods in Brazil. The aim of this work was to prepare a reference material for aflatoxins in peanut and to provide a proficiency test in order to improve the quality of measurements performed on food in the country. The organization of this proficiency test was conducted through a partnership between the Inmetro and the National Institute of Health and Quality Control (INCQS/Fiocruz). The reference material was prepared by the INCQS from a batch of peanuts without skin and uncontaminated by aflatoxins. An adequate amount of peanut was ground in a mill with the same amount of water, which was fortified with aflatoxins B1, B2, G1 and G2. The prepared slurry was dried at 110°C in a stove and then powdered. The resulting flour was passed through a 1.68 mm pore sized sieve. After homogenization, 50 g of the material was packaged in sachets and then vacuum sealed. The method used to determine the amount of aflatoxins in peanuts was High Performance Liquid Chromatography with post column derivatization. For the homogeneity and stability studies, the samples were chosen by using a random stratified sample and were analyzed for their aflatoxins content in duplicate. The isochronous method was applied for stability studies. No significant slope at 95% of confidence level was detected for the material, demonstrating that the material was stable in the transport temperature and storage temperature (-20°C). The homogeneity results were evaluated based on ISO 13528. The material was adequately homogeneous and stable and for this reason suitable to be used in proficiency testing. The laboratories participating in the study used high-performance liquid chromatography with fluorescence or tandem mass spectrometry detectors, thin-layer chromatography, enzyme-linked immunosorbent assay and fluorometry. The assigned value w! as deter mined through the robust averages of the participant's results. The standard deviations were determinate through a modified Horwitz equation. The performance of each participating laboratory was designated by a z-score that was calculated using robust statistics, in which a satisfactory performance rating required |z-scores| ≤2 for the target aflatoxins. Of the 16 laboratories that reported results, 44% are accredited by ISO/IEC 17025. In Brazil, the regulation for aflatoxins in peanuts only sets the sum of aflatoxin (B1 + B2 + G1 + G2) in peanut and other products. Satisfactory performance was obtained by 50% of the laboratories that reported results for the sum of aflatoxins. When all the reported results are evaluated (n=61), 63% are considered satisfactory. From the results obtained in this study, it is concluded that the preparing method of the referred material developed in INCQS is suitable for the organization of proficiency tests.

Keywords: Proficiency testing, Aflatoxins, peanut.

H-49
MULTIPLEX LATERAL FLOW IMMUNOASSAY FOR FUSARIUM TOXINS IN CEREALS

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Fusarium species are plant pathogens commonly associated with cereals that, under favourable environmental conditions, can produce several secondary toxic metabolites. Fusarium toxins are widely distributed in the food chain in the EU and the major sources for their dietary intake are cereal products, mainly based on wheat and maize. The major Fusarium toxins found in cereals and cereal-based products that can be harmful to both human and animal health are deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZEA) and fumonisins (FB1, FB2). To protect human health from exposure to these mycotoxins, the European Commission has recently established regulatory limits for DON, ZEA and fumonisins (sum of FB1 and FB2) in cereals and cereal-based foods and feeds, while permissible levels of T-2 and HT-2 are under discussion (EC Regulations No 1881/2006 and 1126/2007). In the framework of the European project "CONffIDENCE" (FP7), rapid multiplex dipsticks allowing the detection of six Fusarium mycotoxins (DON, T-2, HT-2, ZEA, FB1 and FB2) were developed for maize, wheat and oat. The efficiency of one single extraction protocol for the different cereals of interest and the performances of this multiplex dipstick in detecting simultaneously not less than six toxins in spiked and in naturally contaminated matrices will be demonstrated. The originality of this new rapid test is the possibility to detect 6 mycotoxins in one single experiment of 20 minutes at 80% of their MRL (extraction time excluded) in raw cereals. This semi-quantitative test gives a negative or positive type-response compare to a cutoff of 1400, 280, 400 and 3200 µg/kg respectively for DON, ZEA, T-2/HT-2 and FB1/FB2 in maize and 1400, 80 and 400 µg/kg for DON, ZEA, T-2/HT-2 in wheat and oats. Good agreements between dipstick results and LC-MS/MS measurements have been established. Finally, the multiplex dipstick validation results for maize and wheat collected by the ANOVA method will be presented. Information on the share of the variability sources such as day, matrix and repeatability on the total variability will be given. The rate of false positive results for blank matrices has been calculated and finally (test line/control line) cutoff ratios have been determined.

Keywords: Fusarium toxins, multiplex, lateral flow immunoassay

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H-50
A PHOSPHATASE INIHIBITION ASSAY - OKATEST– AS A COMPLEMENTARY TEST TO THE REFERENCE METHOD (EC. NO. 15/2011) FOR DETECTION OF LIPOPHILIC TOXINS IN MOLLUSCS

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Okadaic acid (OA) and its analogues DTX1 and DTX2, together with their ester forms are known as the OA-group toxins. The toxicity of these toxins is directly related to their inhibitory activity against a family of structurally related serine/threonine protein phosphatase (PP) present in the cells, in particular PP1 and PP2A. OkaTest is based on this strong inhibitory property for determination of toxin content in shellfish using the enzyme PP2A and a specific colorimetric substrate. The enzyme hydrolyses the substrate and the product obtained can be determined by an absorbance measurement at 405 nm. As the ability of the PP to hydrolyse the substrate depends on the presence of OA and analogues in the samples, the toxin concentration can be calculated by using a standard curve. OkaTest is able to quantify the OA-group toxins in shellfish species such as mussels, clams, oysters and scallops. The assay is performed in a 96-wells microtiter plate and includes five ready-to-use standards. A total of 43 samples can be tested in about 2.5 hours. The performance characteristics of the kit were evaluated by the manufacturer in a Single Laboratory Validation and according to AOAC and Eurachem guidelines. A limit of detection and quantification of 44 and 56 µg/kg were calculated. Within-laboratory reproducibility values below 10% for mussel and scallop and recoveries between 78 and 114% were obtained. The kit was also evaluated by the EURLMB (European Reference Laboratory for Marine Toxins) and a small scale intralaboratory study was carried out prior to a full validation study. An international collaborative study was recently carried out with successful results. A total of 8 materials, in blind duplicates, including mussels, scallops, clams and cockles were analysed by 16 laboratories from 9 different countries. The overall mean values assigned for OA-toxins group for the test materials were 98.8, 175.4, 242.8, 255.0 and 275.0 µg total equivalents OA/kg. The estimated reproducibility standard deviation (SR) was from 10.7 to 23.2 µg/Kg, with reproducibility relative standard deviation (RSDR) values between 7.6 % and 13.2 % (mean 9.9%). The HORRAT values, the ratio between RSDR and a theoretically calculated RSDR, obtained were between 0.4 and 0.6. The results obtained in this validation study indicate that the colorimetric phosphatase inhibition assay, OkaTest, is suitable for determination of the OA-toxins group. OkaTest can be used as an alternative or complementary test to the reference method for monitoring the OA-toxin group.

Keywords: okadaic acid, phosphatase inhibition assay, lipophilic toxins, collaborative

H-51
MULTIPLEX DETECTION OF MARINE BIOTOXINS USING SPR TECHNOLOGY

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The measurement of the presence of a wide range of algal derived biotoxins in shellfish is required due to the need to protect the consumer and comply with legislation. While a number of different techniques are available all of these present difficulties such as ethical concerns (animal based tests) and expensive and complex instrumental methods (LC-MSMS). Furthermore the analytical based procedures are as yet unable to measure both lipophilic and hydrophilic toxins simultaneously. Within the EU project Conffidence the use of a multiplex optical biosensor system (SPR) was ultilsed to simultaneously detect and quantity toxins from four important classes of marine biotoxins, The Amnesiac Shellfish Poisons (ASP), The Diarrhoeic Shellfish Poisons (DSPs), The Paralytic Shellfish Poisons (PSPs) and the emerging biotoxins (palytoxin used as an example). A rapid sample preparation procedure was developed prior to the simultaneous analysis of the biotoxins present in the shellfish extracts by the SPR device. This procedure and the SPR assay was subjected to a rigorous validation study. The procedures that were developed and the results achieved will be presented.

Keywords: Biotoxins, detection, biosensors, multiplex

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H-52
NMR-BASED FOOD QUALITY SCREENING

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Based on the metabolomics approach, 1H-Nuclear Magnetic Resonance (1H-NMR) screening has rapidly expanded in recent years in the area of food quality control. Indeed, 1H-NMR screening is a fast, multiparametric method, requiring only a small amount of sample and minimal sample preparation. 1H-NMR is a global, non-targeted approach allowing the acquisition of spectral fingerprints. The reliability and reproducibility of 1H-NMR, makes it a technique of choice for profiling of samples, by allowing the creation of statistical models based on authentic reference samples. This non-targeted method allows the evaluation of numerous parameters linked to quality and authenticity, in only one measurement. Quantification of multiple relevant compounds, as well as classification and verification of the samples is done within minutes. This allows not only to assess the authenticity of the samples but also to detect unknown frauds that would not be detected by conventional targeted approaches. The full automation of the measurement, including automated data analysis report generation, allows high-throughput analysis and consequently low costs per measurement. A further advantage is that the direct quantification with NMR does not require the use of internal standards. The achievements of NMR-based screening of fruit juices and the different parameters evaluated will be discussed in detail. Validation results of the method will also be shown. In particular, comparison of NMR quantification results to official methods as well as results of proficiency testing with FAPAS® will be discussed. Based on the experience on fruit juices, similar screening methods have been developed and applied to other food products like wine, edible oil and honey. Application examples of the screening of these matrices will be presented. In conclusion, NMR is a very cost and time effective method for the simultaneous evaluation of many quality parameters in food.

Keywords: NMR, fingerprints, authenticity, fraud,

NANOPARTICLES

(I-1 – I-8)

I-1
COHERENCE CONTROLLED HOLOGRAPHIC MICROSCOPE (CCHM) A PROMISING PROGRESS FOR IN VITRO BIOLOGICAL TESTS OF FOOD SAFETY

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Coherence Controlled Holographic Microscopy (CCHM) is a novel technique of wide-field light microscopy. The difference from classic Digital Holographic Microscopy (DHM) lies in a possibility to illuminate with arbitrarily low coherence of light. This means that holographic observation is possible without laser light using an illumination system of standard wide-field microscopes. Owing to the ordinary illumination source the CCHM images are of low noise, deprived of coherence noise (speckles) and the lateral resolution is improved by a factor of 2 compared to classic DHM. These achievements further improved Quantitative Phase Contrast (QPC). QPC from cell biology point of view represents the main methodical contribution of DHM. It ascribes numerical value of object beam phase shift in nm compared to reference beam for every pixel. For phase shift is proportional to equivalent of dry mass the QPC allows detection of both cell translocation as well as intracellular motion. Based on QPC we elaborated a method of Dynamic Phase Differences (DPD). Typical results of QPC and DPD can be shown on videos of raw and processed data or in static images of only 2D (x,y) format. This is a new option of how to print/publish evidence obtained from the dynamic video 3D information (x,y images in time). In this way an equivalent of intracellular dry mass translocation can be evaluated and documented, which in turn opens an approach to detect so far unattainable disturbances in patterns of live cell behaviour. Exploitation of this CCHM potential for examining interactions of living cells in vitro with engineered nanoparticles (ENM) is expected to contribute to "the safety assessment of applications involving the application of nanoscience and nanotechnology to food and feed"(EFSA Journal 2011;9(5):2140).

Keywords: safety assessment, engineered nanoparticles, cell motility, holographic microscopy, quantitative phase contrast

Acknowledgement: The work is supported by the Ministry of Education of CR (grant MSM0021630508).

I-2
DETERMINATION OF ORGANIC ENGINEERED NANOPARTICLES IN FOOD USING UPLC–TOF MS

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Nanotechnology is developing rapidly and the number of products containing nanomaterials has increased also in food and pharmaceutical industry. In food sector, application of nanotechnologies - derived food ingredients, additives, supplements, and contact the material is expected to grow continuously. The behaviour of nanoparticles using in organisms is affeced by a wide range of factors including particle number and mass concentration, elemental composition, as well as structure and shape. To date, little is known about the occurrence, fate and toxicity of nanoparticles. The limitations in our knowledge are partly due to the lack of methodology for the detection and characterisation of organic engineered nanoparticles in foodstuffs and nutraceuticals. Some methods have been developed for anorganic nanoparticles in simple matrices. Methods for separation and detection of organic engineered nanoparticles are under development. Within the EU NanoLyse project (www.nanolyse.eu) the analytical methods for detection and characterisation of engineered nanoparticles in food are investigated. In presented study various approaches for organic engineered nanoparticles (ENPs) based on Polysorbate 20 (containing vitamin E or medium chain triglycerides) and Polysorbate 80 (containing glucose) in beverages (orange juice) were tested. Application potential of ultra-performance liquid chromatography coupled to a time-of-flight mass spectrometer (UPLC–TOF MS) system for determination of these organic ENPs in real matrix using detection of polysorbate in-source fragment (LOQ~5 µg/ml) and/or active compound included in ENPs will be demonstrated.

Keywords: organic engineered nanoparticles, polysorbate, UPLC-TOF MS

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I-3
SCREENING FOR ENGINEERED NANOPARTICLES IN FOOD USING SURFACE PLASMON RESONANCE-BASED BIOSENSOR

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Recent advances in nanotechnology have introduced novel engineered nanomaterials (ENPs) into our food and the environment. The most perspective and widespread nanomaterials include nanosilver (AgNPs) and encapsulating ENPs. Silver based compounds are recognized as effective antimicrobial agents and have been implemented in various consumer products: washing machines, refrigerators, clothing, medical devices and food packaging. Whereas organic nanostructures are used in the food industry to introduce valuable ingredients to the food matrix via encapsulation mechanism. A wide range of analytical techniques is available for the detection and characterization of the ENPs, however an efficient screening method for detection of intact ENPs has not been reported yet. Here we present the development of a Surface Plasmon Resonance biosensor for the detection of AgNPs and protein-based encapsulating NPs in food. Utilization of ENPs binding proteins on the sensor chip surface in combination with SPR-based label-free detection allowed rapid detection of ENPs with µg/L sensitivity. The proposed biosensor has been characterized for its applicability to measurements in real life samples, stability and selectivity. This approach can be applied to a routine screening method for ENPs in food and environmental samples, providing rapid and automated analysis needed for efficient environmental and food safety monitoring.

Keywords: engineered nanoparticles, surface plasmon resonance, biosensor, food screening

Acknowledgement: The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245162.

I-4
DART–MS A POTENTIAL TOOL FOR DETECTION OF ORGANIC ENGINEERED NANOPARTICLES (ENPS) IN FOODSTUFFS

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Nanotechnologies are increasingly used in the food and beverages industries, including the addition of organic engineered nanoparticles (ENPs) to food and food contact materials. The toxicological relevance of organic ENPs in food has not been yet fully evaluated. In addition, risk assessors and managers as the well as regulators have no tools available to determine the presence and levels of organic ENP in food. EU NanoLyse project (www.nanolyse.eu) aims to develop analytical methods for sample preparation, separation, detection, and characterisation of nanoparticles in food. In presented study determination of organic ENPs based on Polysorbate 20 (containing vitamin E or medium chain triglycerides) and Polysorbate 80 (containing glucose) in beverages, water and real food matrix – orange juice, was examined. High resolution ambient mass spectrometry, enabling analysis of ordinary objects in the open atmosphere without or with only small sample preparation, was employed for rapid fingerprinting / profiling of polysorbate nanoparticles. Application of DART (Direct Analysis in Real Time) ion source coupled with MS detection (TOF MS resolution 1 – 6000 FWHM and Orbitrap MS resolution 10 k – 100 k FWHM) was tested. It will be demonstrated that by DART – MS technique, in positive ionisation mode, it is possible to detect organic ENP fragment ion of polysorbate and active compound included in ENP. DART – MS thus provides the fast determination of different polysorbate type organic nanoparticles in food and beverages.

Keywords: organic engineered nanoparticles, polysorbate, DART - MS

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I-5
DETERMINATION OF SiO_2-NANOPARTICLES IN FOOD SUPPLEMENTS USING ASYMMETRICAL FLOW–FIELD–FLOW–FRACTIONATION

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In several food supplements nanoparticles are claimed either as essential trace elements such as colloidal minerals or as nano-scale carriers (liposomes) for active ingredients. Furthermore, consumer organisations point out the possibility that nanoparticles might be used by the food industry, since several approved food additives could also be manufactured with particle sizes in the nanometre range (e.g. SiO_2, TiO_2). In order to evaluate the possible exposition of humans to synthetic nanoparticles and the distribution of nanoparticles in the environment analytical methods are required to identify a wide range of chemically different nanoparticles in various matrices. In this study asymmetrical flow-field-flow-fractionation (AFFFF, A4F) was applied for the separation and size measurement of SiO_2-particles in food supplements. The detection of the fractionated particles was achieved by UV-VIS-spectroscopy and multi-angel-light-scattering. In order to find a suitable analytical method the effect of various homogenisation methods (ultraturax, ultrasonic bath and ultrasonic probe) was evaluated for deagglomeration of available reference particles and solid food supplements. For stabilisation of the nanoparticles different carrier solutions (0.9% NaCl, 15% methanol, and a mixture of surfactants) were used to find the optimal conditions for separation of the particles. Furthermore cross flow rates were varied to achieve optimised fractionation of the nanoparticles. The various methods were applied to reference SiO_2-nanoparticles with a diameter of 10 nm (Aerosil 200 and Levasil 300) and three food supplements which are advertised with the term “nano”. For comparison, a series of food supplements containing SiO_2, which are not explicitly advertised with the term “nano” were also analysed. The size range of particles in the “non-nano” food supplements lies between 100 and 600 nm showing a bimodal size distribution with maxima at 200 and 500 nm. Only one of the “nano” food supplements contained actually nanoparticles in the size range below 100 nm. In this one case up to 20% of the total particle number concentration has a diameter between 20 and 100 nm.

Keywords: nanoparticles, food supplement, silica, homogenisation, asymmetric flow-field-flow-fractionation

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I-6
ANTIMICROBIAL PACKAGING FILMS WITH NANOPARTICLES OF SILVER AND TITANIUM DIOXIDE

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Nanotechnology has potential to greatly influence the food packaging. Nanoscale innovations in the forms of antimicrobial packaging could potentially introduce many amazing new improvements in this area. It was discovered that nanoparticles may exert more powerful antimicrobial activity than particles in conventional scale. The aim of this work is to design, prepare and study the efficacy of new types of antimicrobial films with incorporated nanoparticles of titanium dioxide and silver. The inhibitory mode of action of TiO_2 is due to the photocatalytic generation of free radicals after illumination of TiO_2 particles with near UV light, whereas the antimicrobial activity of silver is related to the active silver ion released from silver-containing materials or its catalytic action. Coextruded olyamide/polyethylene film was coated by commercially available polyvinyldichloride (PVdC) lacquer with 5% addition of titanium dioxide in mineral form anatase (~21 nm particle size) and/or silver nanoparticles (< 100 nm particle size) in the laboratory. X-ray fluorescence spectroscopy confirmed the presence of fixed nanoparticles of titanium dioxide and silver at levels up to 0.65% in the prepared films. Migration of titanium dioxide into 3% acetic acid was not found by atomic absorption spectrometry, whereas migration of silver into simulants of 3% acetic acid and 0.1% L-cystein aqueous solution was at the levels of 0.04–0.3 mg/dm2 of films. Generation of free radicals by TiO_2 incorporated in lacquer was not suprisingly proven by Free Radicals Kit, however photocatalytic activity of the film containing TiO_2 was demonstrated by degradation of methylene blue dye after illumination with sunlight or UV light with maximal intensity at 365 nm. The evaluation of antimicrobial activity of the films on the growth of selected indicator bacteria Escherichia coli, Bacillus subtilis, Psedumonas fluorescens, Lactobacillus helveticus, Listeria ivanovii and innocua is discussed in the poster presentation.

Keywords: antimicrobial packaging, nanoparticles, silver, titanium dioxide

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I-7
APPLICATION OF TITANIUM DIOXIDE NANOPARTICLES FOR PHOTOCATALYTIC DISCOLORATION OF DATE SYRUP IN FOOD INDUSTRY

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Date syrup is the main by product of date which could be valuable to be consumed in food industry but its consumption has been limited due to the presence of some coloring and turbidity agents. Thus in order to consume this by product as a source of sugar in other products such as drinks, it is necessary to clarify and decolorize it. Most of the traditional or common approaches for date syrup discoloration would suffer of different disadvantages e.g. low rates or process, high energy cost, low capacity and environmental pollution. According to the severe extending capabilities of nanostrcutures and nanoparticles in different field of science and trechnology, titanium dioxide nanoparticle was proposed to be capable of presenting new consequences in field of date syrup discoloration besides common industrial discoloring techniques like using activated carbon. This nano structure was used as a photocatalyst in the presence of ultraviolet radiation in order to investigate the decrement in high color and turbidity of date syrup. Photocatalytic discoloration process was optimized using statistical design of experiment. The effect of TiO_2 catalyst portion, date syrup concentration, UV power and processing time on the qualitative characterization of the date syrup including color, turbidity, ash and reduced sugars content was investigated to realize the optimum condition.

Keywords: nano TiO_2, date syrup, discoloration, DOE, photocatalyst

I-8
SEPARATION AND CHARACTERIZATION OF ORGANIC NANOPARTICLES USING HYDRODYNAMIC CHROMATOGRAPHY AND MALDI–TOF ANALYSES

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The feasibility of using MALDI-TOF analyses for characterization of organic nanoparticles was investigated. Four classes of organic nanoparticles can be distinguished on the basis of the chemical nature of their constituents: lipid-based, protein-based, carbohydrate-based and carbon-based (e.g. C_{60} and C_{70}-fullerenes). Preparation of nanoparticles was accomplished using protocols from the scientific literature and commercial suppliers. MALDI-TOF analyses of a few examples of these nanoparticle classes show the mass fingerprints of the monomeric composing elements, but not the intact nanoparticles. In case of protein-derived nanoparticles also aggregates, up to pentamers, were observed. To ascertain that intact nanoparticles are characterized we tested a concept where nanoparticles are first separated on the basis of (nano)size and subsequently characterized by MALDI-TOF. Separation can be achieved by HydroDynamic Chromatography (HDC), Field Flow Fractionation (FFF) or Gas-Phase Electrophoretic Mobility Molecular Analysis (GEMMA). Using HDC we were able to distinguish three commercially available liposomes on the basis of their size: 150 nm, 185, and 211 nm, respectively. Using MALDI-TOF these nanoparticles showed distinguishable mass fingerprints with phosphatidylcholine as the major, characteristic phospholipid component in the positive mode and phosphatidylglycerol as the major, characteristic phospholipid component in the negative mode. This strategy is now explored with the other classes of organic nanoparticles.

Keywords: characterization, hydrodynamic chromatogarphy, MALDI-TOF, organic nanoparticles

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NOVEL FOODS & SUPPLEMENTS

(J-1 – J-9)

J-1
DEVELOPMENT OF WHITE LUPIN (LUPINUS ALBUS) BASED MILK SUBSTITUTES

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Milk allergy is a food allergy, an adverse immune reaction to one or more of the proteins in cow's milk and/or the milk of other animals and can cause anaphylaxis. Milk protein intolerance (MPI) is delayed reaction to a food protein that is normally harmless to the non-allergic, non-intolerant individual. Milk allergy is the most common food allergy in early childhood. Currently the only treatment for milk allergies is total avoidance of milk proteins. Products in addition to milk itself to be avoided by those with milk allergy include yogurt, butter, cheese, and cream. Goats' milk products may also need to be avoided. Casein (from Latin caseus "cheese") is the predominant phosphoprotein that accounts for nearly 20% of proteins in cow milk and cheese. Casein has a molecular structure that is quite similar to that of gluten. Thus, some gluten-free diets are combined with casein-free diets and referred to as a gluten-free, casein-free diet. There are many commercially available replacements for milk for children and adults - Rice milk, soy milk, oat milk, coconut milk and almond milk are also sometimes used as milk substitutes, but are not suitable nutrition for infants. However, special infant formula based on soy, rice, almonds or carob seeds are commercially available. Lupin seeds have high 40–45% protein content and have a good amino acid composition. Lupin proteins can be an excellent choice for improving the nutritional value of food products. Lupin is gluten-free, which makes possible to make products safe to eat for people with wheat allergies or coeliac disease. In our work we produced healthy lupin drinks, which can be made from either lupin protein extracts or directly from lupin seeds. The protein emulsions were stabilized with different emulsifying agents. The Lupin drinks can be a good choice for those who are on gluten-free, casein-free diet.

Keywords: white lupin, protein, milk substitute, functionalized food

J-2
ANALYSIS OF VITAMINS SUPPLEMENTS BY MICROWAVE ASSISTED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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High Performance Liquid Chromatography (HPLC) is a well established technique to carry out the separation of a wide range of chemical compounds. However, the importance of rapid analytical methods for the food industry has promoted the appearance of new analytical techniques. Thus High Temperature Liquid Chromatography (HTLC) has emerged as a fast separation technique due to the fact that the use of temperatures higher than ambient reduce the retention times, improves the diffusivity of the analytes and, hence the separation efficiency. However, this separation technique suffers from severe drawbacks such as the stationary phase degradation, the need for expensive instrumentation and the difficulty to couple it to some conventional detectors. The purpose of the present work was to develop a liquid chromatographic method based on the use of microwave radiation (MWLC) to overcome the drawbacks of HTLC and to improve the chromatographic separation both in terms of retention time and resolution for the analysis of vitamins supplements. The reason for this first attempt to use microwave in HPLC is basically to check the improvement of sample heating as a result of the direct interaction of the microwave field and the sample components. The results indicated that the use of column radiation is a promising and innovative method for the determination of vitamins in supplements analysis of organic compounds.

Keywords: HPLC, MW, vitamins supplements

J-3
THE APPLICATION OF PROBIOTIC CULTURES IN SAUERKRAUT

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Functional food with declared probiotic activities are permanently growing, in addition to the traditional dairy probiotic products probiotic cultures are used also in other food commodities, such as meat and vegetable products. Preliminary fermentation experiments were carried out: The growing ability of probiotic cultures Lactobacillus casei L-26, Lactobacillus acidophillus LA5 and Bifidobacterium animalis ssp. lactis Bb12. in cabbage juice was tested. All bacteria displayed a usual growth curve. These growth experiments were also conducted in the juice of sauerkraut, but there none increasing of microorganisms' number was observed because of the reduced content of nutrient. Only negligible reduction of the cell number was observed. After the preliminary fermentations the inoculates of the probiotic bacteria were used for the production of sauerkraut, cut cabbage was inoculated with probiotic strains, two parallel fermentation experiments were done with Bb12 and LA5. During the fermentation the principle analyses were followed (lactic and acetic acids, sugars), the courses of fermentation probiotic and traditional were comparable. It was found, that the sauerkraut with probiotic cultures have increased content of lactic acid and lower content of acetic acid than the control sample. Also, the colour of these samples was darker than that one of the control sample and measured power required for cutting through of these samples were lower. The results of sensorial evaluation show, that the sauerkraut with probiotic cultures has more intensive taste and it is juicier.

Keywords: Probiotic culture, Lactobacillus, Bifidobacterium, sauerkraut

Acknowledgement: Financial Support from Specific University Research (MSMT No. 21/2011) and from MŠMT 6046137305.

J-4
NOVEL SNACK FOOD WITH GRAPE POMACE ADDITIVE: OVERALL QUALITY AND PHENOLICS

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Grape pomaces contain high level of phenolics that are reported to possess antioxidant, anti-inflammatory, anti-cancerogenic properties and can be prevent cardiovascular,cancer diseases. In this research, grape pomace powder (GPP) obtained from winemaking by-product of Alicante Bouschet was added to extrude snack mixture as 2%, 7% and 12% proportion at 130 and 150°C by Kraft Food cooperation. Total phenolic matter was found as 470.09±2.23 mg GAE/ g in GPP whereas 22.44–39.04 mg GAE/g in manufactured chips (MCs). Total anthocyanin (TA) was 584.02±1.68 mg/kg in GPP and 48.68–287.42 mg TA/kg in MCs. DPPH antioxidant activity was founds as 503.65±1.21 IC50 µg /g in GPP while 0.75–45.95 IC50 µg /g in MCs. Procyanidin B1 (Pro B1) and Catechin (C) were 90.09±37.63 and 83.07±36.16, respectively in GPP while 0.00–5.21 mg/kg Pro B1 and 0.00–5.26 mg/kg C in novel extruder products. It was determined that using the GPP additional proportion, L (lightness) and b(yellowness) values decreased and a(redness) values increased. In performed sensory evaluations of novel chips, 7% GPP proportion content at 150°C extrusion came into prominence. It was stated that TA can be expressed as 97.1% level positively by Hunter a™ values. It was confirmed that the correlations between TFM and AA values was statistically very significance (r=0.976) in novel chips (p<0.01). With the abovementioned correlations, it has been revealed that AA can be explained as 97.6% of TFM levels. It was put forwarded the applicability of GPP in novel chips manufacturing which developing as new extruder snack product. It was also established that quality parameters of chips contents can be enriched with GPP additive which includes healthy antioxidants.

Keywords: Grape Pomace, extrude chips, phenolic, antioxidant, analytical.

Acknowledgement: Celal Bayar University Research Fund and Kraft Food Europe

J-5
RAPID DETERMINATION OF SELENIUM IN FOOD PRODUCTS BY TXRF

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This presentation will highlight the physiological role of selenium (Se) and its quantitative detection in different food products by total reflection X-ray fluorescence (TXRF) spectrometry. Se represents one of the few essential elements for humans. It is the only trace element for which the position within proteins is defined precisely by the genetic code. The individual Se status, dietary Se intake and SePP expression are interrelated and become increasingly recognized as an important pathophysiological parameter for disease risk and prognosis [1]. The physiological importance of selenium triggers the development of selenium-enriched food products, particularly in many European countries, where an average daily consumption of 25 to 50 µg is significantly below the recommended value of 70 µg/day (German Society of Nutrition) or below 95 µg/day for full expression of selenoproteins as shown in recent studies [2]. For practical solutions to sort food material during processing, e.g. meat at the slaughterhouse or grain before milling, a rapid method for identifying Se-rich food has to be developed. In this paper we describe the preparation of food suspensions followed by direct trace element analysis with a portable TXRF spectrometer. With this method Se values with 95% confidence were achieved within 30 min. We conclude that TXRF spectrometry opens new doors for a fast and sensitive multi-element analysis of a variety of food products.

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Keywords: Selenium, essential, element, analysis, grain

J-6
ANALYTICAL COMPOSITION OF WHITE LUPIN SEEDS AND DEVELOPMENT OF WHITE LUPIN BASED FUNCTIONALIZED FOOD PRODUCTS

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White lupin (*Lupinus Albus*) is a promising foodstuff with its high protein content and an economically and agriculturally valuable plant which is able to grow in different soils and climates. Interest in lupin production is increasing, due to its potential as a source of protein. Lupin seeds have high 40-45% protein content. Lupin proteins can be an excellent choice for improving the nutritional value of food products. Lupin proteins have high lysine content and they are one of the best sources of arginine, an amino acid which is thought to improve blood vessel performance. Lupin proteins are low in sulphur containing aminoacids, but this can be compensated by mixing lupin flour with wheat flour, which deficient in lysine, but have adequate amounts of sulfur-containing amino acids. Therefore, legume proteins can be successfully used in baked products, to obtain a protein-enriched product with improved amino acid balance. Lupin has the lowest Glycemic Index (GI) of any commonly used grain. The fat content in Lupin Flour is around 8-9% and a large part of it is polyunsaturated and contains significant amounts of omega 3 and omega 6 plus has high antioxidant capacities. In addition, lectins and protease inhibitors, which can reduce protein digestibility, are found at lower levels in lupins than in many other legumes. Lupin is gluten-free, which makes possible to make products safe to eat for people with wheat allergies or coeliac disease. Protein extracts of white lupin could be utilized to make healthy lupin-milk and lupin protein drinks as well as enrich the nutritional value of other food products In our work we examined the analytical composition of Lupinus Albus cv. Nelly seeds. By mixing white lupin flour with wheat flour we produced sweet biscuits, salty crackers, breads and pastas, with improved aminoacid balance. We examined the heat stability of white lupin proteins during baking by SDS-PAGE, and baking conditions were optimized based on the SDS – PAGE results. We also managed to produce gluten free lupin based biscuits and crackers by completely omitting wheat flour from our recipes. Beside bakery products we also develop white lupin based protein drinks.

Keywords: white lupin, functionalized food, gluten-free

J-7
USE OF ENZYMES AND EMULSIFIERS TO IMPROVE BREAD AND ANTI-STALING PROPERTIES

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In this study we investigated the effect of bakery enzymes and emulsifiers on the shelf-life prolonging, bread properties and sensorial evaluation of final products. A standard bread was made using emulsifiers (E 471 and E 472e) with the addition of ascorbic acid (for better volume) and with the addition of bakery enzymes (Novamyl 10000 BG and Lipopan Xtra BG). Bread was stored in plastic bags for 3 days at room temperature (21–24°C). Every day a breadcrumb penetration test was performed to evaluate the shelf-life improvement. Sensorial evaluation was performed in the first day after the bread was cooled down. The water activity of bread at the beginning and at the end of our test was also tested. The enzymes and emulsifier addition caused significant changes in specific bread volume, sensorial properties and shel-life. The best properties were reached if the emulsifier E 472e was used and the enzyme combination Lipopan Xtra BG and Novamyl 10000 BG, which achieved sensorial evaluation of 37.2 points to standard with 34.2 points. Also bread volume has increased from standard 300 cm^3 to 387 cm^3 in modified bread and according to breadcrumb penetration this enzyme combination prolonged bread shelf-life over 1 day.

Keywords: enzymes, emulsifiers, Novamyl, Lipopan, shelf-life

J-8
SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN B6 IN SUPPLEMENTARY PREPARATIONS USING MULTIVARIATE CURVE RESOLUTION ALTERNATING LEAST SQUARES

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There are several analytical methods developed for qualitative or quantitative analysis of vitamins which are applied in food and pharmaceutical industries. HPLC is one of the most employed analysis strategies for vitamins determination, in spite of the fact that the sample preparation usually requires laborious and time consuming steps. Quantitative analysis of vitamin B6 in supplementary products was conducted by UV-Vis spectrophotometry using multivariate curve resolution - alternating least square (MCR-ALS) chemometric approach to investigate the effect of pH on estimations' quality. The spectrophotometric data were obtained at different pH conditions, being analyzed by a factor analysis based technique (MCR) coupled with the optimization procedure (ALS). The performance of final model was evaluated according to statistical parameters. Standard deviation of residuals for experimental data and the variance explained at the optimum condition (r^2) were 0.008 and 99.94% respectively.

Keywords: vitamin B6; chemometrics; quantitative analysis; MCR-ALS

J-9
UPLC®–MS/MS–BASED DETERMINATION OF FOLATES IN TRANSGENIC RICE LINES AND WILD TYPE POTATOES

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Introduction: Folate deficiency is a widespread health problem and several precautions have already been taken in developed countries. However, for the population in poor, rural regions, it is not always feasible to meet an adequate dietary folate intake, nor to obtain it by supplementation. In these cases, the biofortification (the enhancement of folate content by means of genetic modification) of staple crops may become a worthy alternative. Since these food groups represent a large part of daily meals, cultivation will not require additional costs after having invested in their development. Recently, an estimated DALY (disability-adjusted life years) approach supported folate biofortification of rice as a valuable strategy to reduce folate deficiency in the poor, rural Shanxi Province in China: folate enhancement was achieved in Nippon Bare Japonica rice by overexpressing two enzymes (GTPCH I and ADCS) involved in the in situ condensation of folate monoglutamates. Transgenic rice lines (grown under controlled condition) with folate content up to 1700 µg/100g (dry weight) were obtained. As potatoes are a major food source in a large part of the world, this crop also represents a good candidate for biofortification. Given the need for extensive sample treatment and the labile nature of folates, a high-throughput sampling for the rapid determination of folates in potato tubers imposes itself when an efficient and accurate assessment of the biofortification is necessary.
Aim: Investigation of folate monoglutamate stability (in function of time and storage conditions) in transgenic rice lines and establishment of a proof of concept for a successful cultivation in China. Furthermore, the development of a method for the reproducible, reliable and simultaneous determination (UPLC™-MS/MS) of 6 folate monoglutamates in wild type potatoes has been started.
Results: After 6 months, an overall decrease of folates was found for the different storage conditions, except for seeds that were kept at -80°C. The first batch of transgenic rice lines grown in China had folate monoglutamate levels up to 400 µg/100 g. Preliminary results show that comparison between wild-type rice and potato samples reveal a 2-fold higher content of total folate in potatoes (>30 µg/100 g).
Conclusions: Since Folate stability in biological matrices is mostly achieved by polyglutamylation, the emphasis of genetic enhancement will have to lay with overexpressing polyglutamylation. This way, folate levels will increase in transgenic lines grown in China, and they will also remain higher when after storage. The developed UPLC™-MS/MS method will be suitable for the quantification of folate monoglutamates in both wild type and transgenic potatoes.

Keywords: Folates, UPLC-MS/MS, staple crops

ORGANIC FOODS

(K-1 – K-6)

K-1
ANALYTICAL METHODS APPLIED ON A COMPARISON OF NUTRITIONAL QUALITY BETWEEN CONVENTIONAL AND ORGANIC DAIRY PRODUCTS

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Various analytical methods were applied on organic food authentication. After a long debate on the comparison of nutritional quality between conventional versus organic products, the present study contributes on dairy products by integrating the last three year studies using a meta-analysis approach with Hedges'd effect size method. Organic dairy products contain significantly higher protein (d++, ±95%CI: 0.56, ±0.24), α-linolenic acid (ALA) (1.74, ±0.16), omega-3 fatty acid (0.84, ±0.14), cis-9,trans-11 conjugated linoleic acid (0.68, ±0.13), trans-11 vaccenic acid (0.51, ±0.16), EPA (0.42, ±0.23), and DPA (0.71, ±0.3) than those of the conventional. It is also observed that organic dairy products have significantly ($p<0.001$) higher omega-3 to -6 ratio (0.42 against 0.23) and Δ9-desaturase index (0.28 against 0.27) than the conventional one. The current regulation on organic farming indeed enables to drive the organic farm to produce organic dairy products with better nutritional quality than the conventional products. This can be detected by various analytical tools. The differences of feeding regime between conventional and organic dairy production is suspected as the reason behind this evidence.

Keywords: Organic milk, nutritional quality, comparison

Acknowledgement: JK wants to thank the German Bundesanstalt für Landwirtschaft und Ernährung for financial support (Wissenstandanalyse)

K-2
EVALUATION OF A METHOD BASED ON LC–ESI–MS/MS FOR THE CHARACTERIZATION OF THE POLYPHENOL PROFILE OF ORGANIC AND CONVENTIONAL TOMATOES

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Fundamental differences between organic and conventional production systems may affect the nutritive composition of plants, including secondary plant metabolites. The phenolic content of plants may be influenced by manipulating the agronomic environment in which they are grown. The level of nitrogen influences the level of phenol. Even if the levels of nitrogen, as well as potassium and phosphorous, are higher in organic conditions, the bioavailability of nitrogen from organic fertilization would remain lower than that achieved by synthetic fertilization; the levels of secondary metabolites with carbon, such as phenolic compounds, are higher in organic plants. It is necessary to develop analytical tools in order to monitor the quality of these products. In this work, the polyphenol profile of organic and conventional tomatoes was studied. The method was optimized and validated. For the quantification and identification of individual polyphenols, liquid chromatography coupled to mass spectrometry in tandem mode (LC/MS/MS) with negative ion detection was carried out. Positive identification of the compounds was based on their retention times in different MS/MS modes (product ion scan, precursor ion scan and neutral loss scan), and the multiple reaction monitoring (MRM) acquisition mode was used to quantify flavonols, flavanones and cinnamic acid derivatives. This multifaceted approach has revealed that the agronomic environment in which tomatoes are grown induces alterations in the phenolic content of tomatoes. Organic cultivation was found to provide tomatoes with a significantly higher content of functional microconstituents such as hydroxycinnamates, flavonols and flavanones. Defense-related secondary metabolites are generally considered to be the most important determinant of the nutritional value of fruits and vegetables, and thus organically grown products are more health-promoting than those grown using conventional methods.

Keywords: organic food, conventional food, HPLC-ESI-MS/MS, polyphenols

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K-3
SEMI-QUANTITATIVE ANALYSIS OF BREAD EMULSIFIERS BY U(H)PLC–HRMS

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An emulsifier is a natural or artificial chemical which enables good mixing of water and fat. Emulsifiers are widely used in the food industry to improve food product quality or the production process. As an example, in bread emulsifiers are used to improve the bread volume, crumb structure and the softness of bread. Furthermore, emulsifiers improve the dough stability, hence they are important processing aids. There are some natural emulsifiers such as lecithin, but mostly artificial compounds, such as E472e, E481/482 are used and often in combination. In organic food production however, the use of artificial additives is not allowed and organic foods are often tested for compliance with these rules. For the purpose of determination of presence or absence of artificial emulsifiers E472e, E481/E482 and E471 in organic bread production, a method was needed to determine main components of these emulsifiers in a rapid and cost effective analysis. Analytical methods used to measure food emulsifiers are derived from lipid analysis. They often involve soxhlet extraction, separation by chromatography and detection by ELSD. For the purpose of the detection of the presence or absence of the above mentioned emulsifiers in organic bread and organic bread ingredients, we developed a fast lipid method that provides us with semi-quantitative results. The emulsifiers are extracted from bread ingredient mixes with isopropanol. The lipid compounds are separated using UPLC and detected in full scan mode with a high resolution mass spectrometer, the LTQ-Orbitrap. Sensitivity and specificity is achieved by combination of retention time and accurate mass. Quantification is performed by comparing the detected concentrations with several dilutions of the emulsifiers themselves. In this way, it is possible to analyse E472e, E481/E482 and E471 emulsifiers in a single analysis, thus limiting the cost and the time of the analysis. The method was applied in two testing rounds in which organic bread ingredient mixes from several producers were tested for the presence of artificial emulsifiers. As this was the first compliance testing for these compounds to be performed in the Netherlands, the results were discussed with the ingredient manufacturers in order to help them improve their production. This poster will present a summary of the method and show examples of the application.

Keywords: liquid chromatography, mass spectrometry, emulsifiers, bread, organic

K-4
INVESTIGATING THE ORGANIC AND CONVENTIONAL ORIGIN OF SOME SLOVAKIAN WINES ACCORDING TO ANIONIC COMPOSITION

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In recent years, research has demonstrated the effects of organic systems on different qualitative and quantitative attributes of organic products. Seventeen organic and twenty nine conventional Slovakian varietal dry wines of five varieties (Chardonnay, Pinot blanc, Traminer red, Pinot noir and Cabernet Sauvignon) were collected during the vintage period 2007-2009 for comparison study. Relevant anionic profile through sulphate, oxalate, phosphate, tartrate, citrate, malate, lactate, aspartate, succinate, acetate, maleinate, glycolate, gluconate and malonate concentrations were analysed by capillary isotachophoresis. Capillary isotachophoretic separations were performed using the electrophoretic analyser EA 202M (Villa Labeco, Spišská Nová Ves, Slovakia) with ITP Pro 32 (v. 1.0.5) software package for data processing. Multiple comparisons of anionic composition between organic and conventional wines were performed by Kruskal-Wallis One-Way ANOVA with "t" distribution and by multisampling median test. Significant differences between the mean contents of tartaric, citric, lactic, malic, succinic, glycolic, sulphuric and phosphoric acids were observed in wines compared according to their terroir and wine-making procedure. The present study revealed that organic wines and grapes displayed a higher total anionic strength than conventional counterparts. Results of principal component and factor analysis provided evident clustering of wine samples according to examined attributes. Canonical discriminant analysis of wines performed according to viticulture and wine-making systems resulted in 100% of correctly classified samples in the case of separately analysed red and white wines and grape musts. Recognition ability of this classification was reduced only by 10% when the organic and conventional red and white wines were analysed simultaneously. Influence of grape maturity on wines´ anionic profile is discussed in the relation to vineyard soil nutrient composition.

Keywords: wine, organic, conventional, anionic composition, capillary isotachophoresis, PCA, CDA

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K-5
STUDY OF ESSENTIAL OILS AS NATURAL ANTIOXIDANTS ON STABILIZATION OF FLAXSEED OIL AGAINST HEATING, USING DATA ANALYSIS

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Flaxseed oil contains high level of unsaturated essential fatty acids such as α-linolenic acid (ALA). Therefore; the oil is more susceptible to oxidation. The oxidation process can be prevented by adding natural and synthetic antioxidants. In this paper, we have studied the effect of three natural essential oils including Marjoram (*Origanum vulgare L.*), Black cumin (Bunium persicum) and Thyme (*Thymus vulgarisms L.*) extracts, on oxidation stability of flaxseed oil. Then results were compared with common synthetic antioxidants such as Butylated hydroxytoluene (BHT). The essential oils were extracted via hydrodistillation from dried aerial parts of their plants and then we have prepared 50 and 500 ppm solutions from each of them. Then chemical compositions of essential oils were monitored by using Gas chromatography–mass spectrometry (GC-MS). Flaxseed oil oxidation was followed by measuring thiobarbitoric acid (TBA) value and peroxide value (PV). Also chemical composition changes of flaxseed oil were studied by GC/MS. The analysis is based on two factor analysis of variance (ANOVA).The results have shown that, oxidation stability of flaxseed oil throughout heating was developed by using Thyme, Marjoram and Black Cumin extracts. And antioxidant activities improved by increasing the concentration of essential oil ($P<0.05$). The chemical analysis essential oils by GC/MS showed that the main compounds were Carvacrol, p-cymene, thymol, caryophyllene, γ-terpinen, cuminyl acetate and linalool. Also results showed the antioxidant activity of Marjoram was better than BHT in two different concentrations (50 and 500 ppm) of both antioxidants. Consequently, selection of appropriate essential oils especially with phenolic base, can improve the stability of flaxseed oil against oxidative deterioration.

Keywords: flaxseed oil, thyme, Black Cumin, Marjoram, essential oil

K-6
APPLICATION OF PRINCIPAL COMPONENTS & CLASSIFICATION ANALYSIS IN LIPID-OMICS OF PLANTS FROM PERMANENT GRASSLAND

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The aim of this study is to apply the chemometric tools, especially Principal Component & Classification Analysis for better understanding of correlation between fatty acids composition and different plants families present in a complex matrix of forages from permanent grassland. Plants samples were taken in June, at complete maturity stage, from permanent grassland located in a hill area, 180 m altitude (Gradinari, Caras Severin District, Romania). Floristic composition was made gravimetrically. The dominant family is Poaceae which represent 56% from total species and are formed especially from: Festuca rupicola which is the dominant specie (42%) followed by Calamagrostis epigejos (8%) and 6 % from others (Alopecurus pratensis, Anthoxanthum odoratum, Arrhenatherum elatius, Briza media, Poa pratensis). Fabaceae family represents only 12% and is formed from Trifolium repens, Trifolium medium, Trifolium arvensis, Medicago falcata, Lathyrus pratensis, Lathyrus nissolia. 32% from total plants species are formed from other botanical families represented especially by Rosa canina (18%), Filipendula vulgaris (9%) and Inula britanica (3%). In these plants lipids are formed especially from bounded and unbounded fatty acids, which represent the raw material for animals (herbivore) lipids synthesis. The follow common 9 fatty acids (6 saturated and 3 unsaturated), predominantly found in forages were analyzed: Lauric, Myristic, Palmitic, Stearic, Oleic, Linoleic, Linolenic, Arahidic and Behenic. The contents of these fatty acids are the variables of the statistical model. Two principal components are sufficient to explain more than 95% of model's variance and a model with 3 principal components is capable to explain 99% of the variance. First principal component is formed in principal from Palmitic, Stearic, Oleic, Linoleic, Linolenic, Arahidic and Behenic fatty acids grouped in two clusters and second and third principal component are formed especially from Lauric and Myristic fatty acids. These two fatty acids are the key component of the Lipid-omics model; they can discriminate between Poaceae and Fabaceae family and other wild plants families. For fatty acids analysis from dry plants matrix (forages) a one-step extraction and derivatization method has been used, essentially based on a dispersive solid-liquid/liquid microextraction and transmethylation with BF3/methanol as derivatization reagent followed by GC MS (GC MS QP 2010 Shimadzu) analysis. The data were statistically analyzed using a statistical package Statistica.

Keywords: fatty acids composition, forages, chemometry, GCMS

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PACKAGING CONTAMINANTS

(M-1 – M-21)

M-1
OCCURRENCE OF DIPROPYLENE AND TRIPROPYLENE GLYCOL DIACRYLATES IN PACKAGING MATERIALS AND PACKAGED SUGAR

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The one portion packages of crystalline sugar providing unpleasant smell for organic compounds were analysed with regard to the volatiles content. Isolation and identification of volatile substances in the packaging material (film consisting from printed paper layer laminated with LDPE) were performed using solid phase micro extraction (SPME) technique coupled with GC-MS method. The presence of dipropylene glycol diacrylate (DPGDA) as well as tripropylene glycol diacrylate (TPGDA) in rather high level was identified as the cause of unacceptable odour of the product. Later on DPGDA and TPGDA were also found in packaged sugar. The diacrylates probably originated from the printing, as both chemicals are commonly used as UV/EB curable monomers in printing inks used for food packages. The poster will present (i) the results of quantification of DPDGA and TPGDA in several packaging materials used for sugar packaging as well as in the packaged sugar and (ii) the health risk assessment for consumers of food containing DPDGA and TPGDA.

Keywords: diakrylates, laminated paper packaging, packaging contaminants, off odour

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M-2
MONITORING OF MONOMER MIGRATION FROM PLASTIC UTENSILS IN KOREA MARKET

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In recent years, a lot of articles concerning the migration of specific substances from utensils and containers made from polymers to foods have been reported. For examples, articles on DEHP from bottle stopper ± and Bisphenol A from baby bottle ± were reported in 2006 and 2007, respectively. Under the influence of the reported articles, consumers have been anxious about the safety of the utensils and containers. In this regard, it was need to monitor the specific substances such as formaldehyde in the distributed utensils and containers. In the present work, we have monitored the migrated monomers in the utensil products. Total 300 samples including domestic and imported products were analyzed. As a result, most of the samples were not detected or did not excess the residual limits established in the Korean Food Code. However, formaldehyde (in melamine products) and acrylonitrile (in ABS/AS products) were more detected than the others specification substance respectively. Moreover, the detected level of the formaldehyde in melamine product was 4 times higher than maximum residual limits. In conclusion, we expect that the monitoring results will be helpful to establish a new policy and secure the safety of the distributed utensils and containers.

Keywords: Migration, Utensil

M-3
QUANTIFICATION OF COWS' MILK PERCENTAGE IN DAIRY PRODUCTS – A MYTH?

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Dairy products made from ewes' and goats' milk are of considerable economic importance. However, the substitution of these milks for cheaper cows' milk is a fraudulent practice in the dairy industry. As a consequence, an adequate methodology is required to control authenticity of dairy products. Moreover, soybean dairy-like products have to be checked to prevent potential adulterations resulting from the addition of casein and/or whey proteins to these products and their adverse effects on allergic people. The objectives of this study were the qualitative detection and the quantitative determination of cows' milk percentage in dairy and soybean products. Standard mixtures of milk from different species as well as model cheeses of different ages were used as references. Species identification was performed using different electrophoretic methods, and by conventional polymerase chain reaction (PCR) and quantitative real-time PCR using species-specific primers. Applied methods were evaluated regarding their applicability for the detection and quantification of cows' milk in mixed cheeses and in soybean products. Urea-polyacrylamide gel electrophoresis of caseins was restricted to the adulteration control of milk only. The official EU reference method (No 273/2008), which is based on the IEF of γ-casein fractions, was a reliable tool to detect cows' milk even in matured cheeses made from milk of other species. Moreover, after densitometric evaluation of γ-caseins, a quantitative estimation of cows' milk percentage was obtained in mixed cheeses. Conventional PCR was shown to be a qualitative method, although a semi-quantitative estimation could be achieved in some cases. Real-time PCR proved to be a highly sophisticated technique, which enables a semi-quantitative estimation of cow's milk percentage in mixed cheeses manufactured from milk of different species, but turned out to have an unexpected high error probability. This was probably due to the fact that DNA-based methods are to be applied for quantitative adulteration control of mixed cheeses with extreme care, only! Thus, analytical procedures used were appropriate for the qualitative detection of cows' milk in dairy and soybean products. However, quantitative results in adulteration control have to be understood as approximate values, and authentication of mixed cheeses still remains a challenge for food analysts.

Keywords: Cows´ milk percentage, quantification, mixed cheeses, dairy products

M-4
TARGETED SCREENING OF 35 VOLATILE INK PHOTOINITIATOR RESIDUES IN FOODSTUFFS AND RELATED PACKAGING MATERIALS BY GC–MS/MS

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Photoinitiators are used during the UV curing process to promote fixation of printing inks onto packaging materials. Due to their volatility, they may migrate either from the protecting packaging into the corresponding foodstuff or by a sett-off transfer phenomenon during manufacturing. Some of these potential contaminants are suspected to posses carcinogenic activity. Consequently, Swiss regulations have recently set two lists of authorized ink photoinitiators: those that have been toxicologically evaluated and for which a specific migration limit has been attributed as well as non-evaluated substances for which the migration limit has been established at 10 µg per kg of food. For the application of this ordinance, a targeted screening method has been developed allowing determination of 35 photoinitiators in foodstuffs and their packaging. Samples were extracted by accelerated solvent extraction (ASE) with acetonitrile, possibly defatted if necessary, and finally analyzed by GC-MS/MS in the multiple reaction monitoring mode using electron impact ionization. Method validation was based on an accuracy profile approach determining confidence interval over the whole concentrations range studied. Weighted linear regression was found suitable for all targeted compounds ranging from 10, 15 or 30 µg/kg (depending on compound analysed) to 3600 µg/kg. A survey including various foodstuffs (eg. flour, mueslis, cakes, cocktail biscuits, pasta, rice, cornmeal) packaged in cartonboard available on the Swiss market has shown several contamination cases with unacceptable residue levels of photoinitiators. In most of these cases, the presence of the contaminants was confirmed in the packaging material. These results proved the growing importance of performing analysis of ink additives used in food contact materials and the need of pursuing regulation issues for printing inks.

Keywords: Food packaging, migration, photoinitiators, contaminants

M-5
SCREENING PROCEDURE FOR THE ANALYSIS OF UV INK INGREDIENTS IN PACKAGED FOODS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Food packaging is printed not only to inform consumer (food ingredients, nutrition facts, etc) but also to ensure brand identification and ultimately differentiation. Among the printing techniques currently employed, those making use of UV-curable inks are gaining importance since environmentally friendly. Indeed, they do not contain any traditional organic solvents and require less energy to dry, when compared to solvent and water based inks formulations. UV inks typically contain acrylated monomers and oligomers, pigments, additives and photoinitiators (PIs). While no residue of organic solvents from such printed materials can contaminate the food, reported results in the literature conclude that ingredients in UV printing inks are not always fully integrated into the polymer matrix and may as well migrate into foodstuffs. The ITX case in 2005 and more recently benzophenones in breakfast cereals are the most recent and striking issues. Moreover, migration of other PIs is regularly pointed out in the European Rapid Alert System for Food and Feed (RASFF). Inks suppliers carefully formulate inks dedicated to food packaging by choosing low migration ingredients and by conducting migration experiments of printed materials into food simulants. Despite this, lack of good manufacturing practices, e.g. decrease in UV lamp efficiency during the ink drying process or extended storage time of printed packaging materials in stack before filling (which favours migration of ink ingredients by set off onto the unprinted side in contact with food) may be reasons for the presence of such contaminants in packaged foods. Finally, food simulants may also underestimate values of ink ingredients migrating into the food products. Consequently, a monitoring of these chemicals in packaged foods is required. Analytical methods devoted to the analysis of PIs in food are scarce and usually limited to few PIs analyzed simultaneously, while ca. 100 are listed in the Swiss list of permitted PIs for printing inks (Swiss Ordinance RS 817.023.21, May 2011). GC and LC coupled to mass spectrometry detectors are the preferred techniques for the confirmatory detection/quantification of this class of compounds. The present study describes the simultaneous analysis of 37 PIs (including 5 polymeric species) and 8 acrylates in food. The method entails an extraction of the analytes according to the QuEChERS (Quick Easy, Cheap, Effective, Rugged and Safe) protocol, followed by detection by LC-ESI-MS/MS using the selected reaction monitoring (SRM) mode. The method was validated in a dairy food product (yoghurt packed in polystyrene cup with UV printed lid) at the 10 µg/kg fortification level using a basic 1-point standard addition quantification method. Its applicability was further demonstrated in other packaged food categories i.e., ice cream, confectionary, cereals, culinary products.

Keywords: UV INK INGREDIENTS / PACKAGED FOODS / LC-MSMS

M-6
DEVELOPMENT OF A CHEMICAL SENSOR TO MONITOR THE MIGRATION OF BENZOPHENONES FROM FOOD PACKAGING INTO FOODSTUFF

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Recently, the Rapid Alert System for Food and Feed of the European Community has been alerted about the presence of high amount of benzophenone (BP) and 4-methylbenzophenone (4MBP) into cereal products [1]. In fact, 4MBP and BP, are used in the area of food packaging as a photo-initiator of UV-cured printing inks and, due to their volatility, they can migrate through the packaging into the food, since internal plastic bags do not always act as a functional barrier.
BP and 4MBP, possesses high proliferation activity (estrogenic like) and are capable to increase renal and hepatic tumours in rodents.
Printing inks are not covered by specific European legislation, however, the Scientific Committee on Food (SCF) [2] established a maximum of 0.6 mg per kilogram food as specific migration limit for BPs (Directive 2002/72/EC) and a tolerable daily intake of 0.01 mg per kg body weight.
Simple methods to monitor the amount of BP and 4MBP in food are required, even though the overall intake of benzophenones for consumers would still be underestimated, since other BP derivatives are used as constituents of synthetic perfumes, sunscreen agents and drugs.
The aim of the present work is the development of a chemical sensor to determine BP and 4MBP at trace levels by using Surface-Enhanced Raman Scattering (SERS) spectroscopy. This is a very sensitive technique successfully employed to enhance by a factor of 104-1015 the weak Raman signal of molecules adsorbed onto specific metallic nanostructures such as silver, gold and copper. Due to the low affinity of BP and 4MBP to the metal surface, the SERS spectrum of BP is relatively weak [3]. We were able to increase the SERS signal of BP and 4MBP by two orders of magnitude, increasing their affinity for the metal surface using a host molecule. Among the various possible host molecules, lucigenin (N,N′-dimethyl 9,9-biacridinium dinitrate) is capable to bind the metal and interact with the analytes bringing them closer to the metal surface. The reproducible substrate, thus obtained, allows us to detect BP and 4MBP at trace level (9 ppm) using colloidal nanoparticles in solution. The comparison of the SERS and Raman spectra allow us to study the interaction mechanism between the analytes with the host molecule and /or with the Ag nanoparticles.
Recently the SERS spectra of 4MBP at trace concentration immobilized on films of Ag nanoparticles has also been obtained.

[1] RASFF Information notification 2009.0118. Migration of 5-methyl benzophenone in chocolate crunch muesli from Belgium. 9.02.2009.
[2] First report of the Scientific Committee for Food on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs.
[3] http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_33.pdf
[4] Fleger et al., Surface Science 603 (2009) 788-793

Keywords: benzophenone, SERS, packaging contaminants, Raman

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M-7
DEVELOPMENT AND VALIDATION OF AN "OFFLINE" ANALYTICAL METHOD FOR THE DETERMINATION OF MOSH AND MOAH IN FOOD AND PAPER-BASED PACKAGING MATERIAL

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Recently high values (ppm) of MOSH and MOAH with alkyl chain lengths between C10 to C25 were determined in dry food in direct contact to printed paper-based packaging or packaging made of recycling paper. This contamination is caused via vapour space migration from mineral oil containing inks. For these compounds JECFA established a tolerable daily intake of 0.01 mg/kg body weight. Based on standard assumptions the Swiss SML for MOSH was set to 0.6 ppm in food. Actually the SML of MOAH has not been fixed; a value of 0.15 ppm in food is under discussion in Germany. Mineral oil is a complex mixture containing amongst others different hydrocarbon fractions. One fraction is defined as mineral oil saturated hydrocarbons (MOSH) characterized by both paraffin-like open-chained generally branched hydrocarbons and naphthenic compounds with at least one saturated ring structure. Often a quantitative maximum of carbon length of C18 to C20 is observed. Another fraction contains mineral oil aromatic hydrocarbons (MOAH) comprising systems of often high alkylated one to four aromatic rings. The nowadays commonly used analytical methods for MOSH/MOAH determination in food and paper-based packaging material published by Koni Grob and coworkers [1, 2] implicates the use of an online HPLC-GC-FID system for the separation of the MOSH and MOAH fraction prior to GC-FID detection. Often analytical laboratories lack such kind of instrumentation. The aim of this project was the development of a new method for the MOSH/MOAH analysis in food and paper-based packaging displaying the same quality properties like the methods mentioned above but without online HPLC instrumentation. The method should display a high sensitivity, accuracy and high robustness. The methodological steps of the new analytical method are as follows: after addition of the respective marker compounds and internal standards the compounds of interest were extracted by hexane and hexane/ethanol. The separation of the MOSH and MOAH fraction was carried out manually on cartridges with highly activated silica gel. Olefin-containing sample extracts were submitted to an epoxidation step, endogenous plant-derived n-alkanes were removed by a basic alumina cleanup step. The validation according to DIN ISO 32645 gave recoveries between 80 and 90% in food and paper-based packaging samples for MOSH and MOAH. The LOQs were 0.1 ppm for MOAH and 0.3 ppm for MOSH in food and 1 ppm for MOAH and 2 ppm for MOSH in paper-based packaging material, respectively. The very robust and reliable manual separation step of MOSH and MOAH fractions ensures a high safety for this kind of analysis also for labs without online HPLC-GC-FID systems. This method meets the requirements of actual and future SMLs by national and EU authorities.

[1] Biedermann, M., J. Agric. Food Chem.: 8711 - 8721 (2009)
[2] Biedermann, M., Eur. Food Res. Technol.: 785 - 796 (2010)

Keywords: MOSH, MOAH, Food, Packaging Material

M-8
ANALYSIS OF PHTHALATES IN BEVERAGES AND MILK USING AN AUTOMATED SYSTEM BASED ON TURBULENT–FLOW CHROMATOGRAPHY COUPLED TO LC–MS/MS

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Phthalates and other plasticizers occur as ubiquitous contaminants in food and beverages and have also been recently reported to have been used for deliberate adulteration of Sports drinks. Avoidance of contamination in the laboratory is critical in this analysis and procedures with minimal manual manipulation can be helpful in reducing background levels. In this poster we report an automated procedure using on-line extraction and clean-up with direct determination by LC-MS/MS using deuterated internal standards. Identification of phthalates was based on matching ion-ratios with standards and quantification employed multiple-reaction monitoring. Various beverage samples and milk were spiked at three levels with a mixture of eleven plasticizers (dimethyl-, diethyl-, dipheny-, butylbenzyl-, di-isobutyl-, dicyclohexyl-, dihexyl-, di-2-ethylhexyl-, di-isononyl and didodecyl phthalates together with di-ethylhexyl adipate) and aliquots were directly injected into a TurboFlow TLX system. In-house method validation was carried out which indicated method recoveries in the range of 67–124%, relative standard deviations in the range of 1–18% and limits of quantification from 4–100 µg/L. Samples could be analyzed in a fully automated mode with 22 min turnaround, demonstrating this method to be very suitable for routine high-throughput analysis.

Keywords: Phthalates, turbulent flow chromatography, beverages, milk, on-line sample preparation

Acknowledgement: The authors would like to thank Dr. Hamide Senyuva (FoodLife Int.) for her scientific – and Vincent Paez (Thermo Fisher Scientific) for his financial - support.

M-9
INCIDENCES OF ENDOCRINE DISRUPTING PHTHALATE ESTERS IN SELECTED FOODS AND FOOD WRAPPERS AROUND TSHWANE METROPOLIS, SOUTH AFRICA

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Oral ingestion is one of the pathways by which xenobiotic and toxic compounds enter the physiology of man and wildlife. This entry can be facilitated through chemico-dynamics of these toxic compounds from materials such as food wrappers (plastics, papers, tins, aluminium etc) into human. This article reports on the incidences of Phthalate Esters (PEs) in selected foods and food wrappers from some commercial stores in Tshwane Metropolis in South Africa. Three food samples (Vienna, Polony and Cheese) were purchased from selected commercial stores and processed. Phthalate Esters in these food samples were extracted using soxhlet extraction, cleaned-up via the column chromatography and quantification was by capillary Gas Chromatography equipped with Flame Ionization Detector (GC-FID). Quality assurance experiment through the use of Standard reference Materials as well as standard addition procedure were carried out. Good and applicable recoveries were obtained from the quality assurance experiment for all the analysed phthalate esters (DMP; DBP; DnBP; BBP and DEHA). Results showed differential pattern of contamination with about 40% detection in all analysed samples. Some of the detected levels of Phthalate Esters in the food samples were above permissible levels. Continual consumption has dire health implications.

Keywords: Incidence, Phthalate Esters, Foods, Food wrappers, Tshwane

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M-10
EVALUATION OF DIFFERENT CONDITIONS OF CONTACT FOR CAPROLACTAM MIGRATION FROM MULTILAYER POLYAMIDE FILMS INTO FOOD SIMULANTS

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The conditions of time/temperature of contact between packaging and food should be considered when the migration is evaluated. High temperatures used during cooking could accelerate migration and, from some plastics during microwave cooking the migration has been found to occur to a considerable extent. It is also expected that volatile compounds evaporate from the plastic and migrate into food at oven temperatures. Also, plastic components could be degrading at cooking temperatures giving off low molecular mass compounds, which have potential to migrate into food. Caprolactam, the monomer of polyamide 6 (PA-6), is one of these components. The cooking of foodstuffs inside the packaging, known as cook-in, is widely used to manufacture cooked products. The cook-in system currently presents PA in its structure. Multilayer film based on PA-6 is used as packaging intended for meat foodstuffs which are submitted to heat treatment after filling. Packaging containing PA-6 type microwave and roasting bags, oven roasting bag and boil-in-the-bag, in which the food can be heated, boiled or roasted in microwave or conventional ovens, are also used. The PA is one of the few polymers that can store food directly for cooking because it is heat resistant and has a capacity to retain exudates. The conditions of temperature and time for contact between food and packaging are standardized according to legislation. They simulate the actual use of packaging material and food. For the migration tests 40°C during 10 days simulates the real use of food packaging at up to 40°C during up to 24 hours, while 100°C during 30 min simulates food processing between 80-100°C. In addition to the storage conditions, it is also important to observe the conditions applied during the food processing and by the consumer. In this work multilayer films containing PA-6 used for meat foodstuffs (brands 1–8) and cheese (brands 9–13) were evaluated. For the migration test, films of 6 cm2 were placed in vials with 10 mL of distilled water, 3% acetic acid or olive oil food simulants and, the samples were stored at 40±1°C during 10 days and at 100±1°C during 30 min. The migration tests were carried out by total immersion, in triplicate. Caprolactam was analyzed by using a gas chromatograph with a flame ionization detector. The caprolactam migration into water and 3% acetic acid at high temperatures and short contact time could replace the need to apply the tests at 40°C/10 days, since similar results were obtained under both conditions. In the case of the packaging intended for meat foodstuffs, caprolactam migration into olive oil was highly affected by the different conditions of contact, showing values 2-3 times higher at 40°C during 10 days than at 100°C during 30min.

Keywords: caprolactam, migration, conditions of contact, food simulants

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M-11
CAPROLACTAM MIGRATION FROM MULTILAYER POLYAMIDE FILMS SUBMITTED TO GAMMA RADIATION INTO OLIVE OIL

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The irradiation is used to sterilize and to increase the shelf life of food. The use of polyamide-6 (PA-6) is approved for contact with prepackaged food during irradiation. The PA-6 is widely used in food packaging, especially for mono and multilayer films intended for meat foodstuffs and cheese. Caprolactam, the PA-6 monomer, remains in the resin after polymerization and can migrate into food in contact. The aim of this study was to evaluate the influence of irradiation of multilayer films containing PA-6 in the migration of caprolactam into olive oil food simulant. Multilayer films containing PA-6, commercially available, used for meat foodstuffs (brands 1-4) and cheese (brands 5–9) were evaluated. The irradiation was performed at the Center of Radiation Technology (CTR/IPEN), using Gammacell 60 cobalt irradiator of 12 KCi. The films (6 cm^2) were submitted to gamma radiation at doses of 3, 7 (meat foodstuffs) and 12 kGy (cheese), with dose rate of 3 kGy/h, in the presence of oxygen, at room temperature. Caprolactam was analyzed by using a Shimadzu 17-A gas chromatograph with a flame ionization detector. The DB-1701 column (30 m × 0.25 mm and 0.25 µm) was heated at 130°C for 1 min, programmed at 10°C/min to 170°C and maintained for 1 min, then heated to 10°C/min to 200°C and held for 2 min. The carrier gas was hydrogen (1 mL/min). Injections (1 µL) were made at 240°C (split, 1:20). The detection was at 250°C. For the migration test non-irradiated and irradiated films (6 cm^2) were placed in vials (20 mL) with 10 mL of simulant, which were hermetically closed. The samples were stored at 40°C/10 days. The migration tests were carried out by total immersion, in triplicate. The results showed that the highest level of caprolactam (11.9 mg/kg) migrated from non-irradiated films of the brand 3, with no difference ($p>0.05$) from brand 2. There was a reduction of 4–38% in the migration of caprolactam between 0 and 3 kGy and of 37-62% between 0 and 7 kGy. The highest level of caprolactam migration from the films used for cheese was 7.5 mg/kg (irradiated films, brand 5), with no difference ($p>0.05$) from brand 6. There was difference ($p\leq0.05$) in the caprolactam migration among non-irradiated and irradiated films of all the brands. For irradiated films of brand 8, caprolactam levels were below the LOQ of the method (1.06 mg/mL) and, for the non-irradiated and irradiated films of brand 9, caprolactam migration was not detected (LOD: 0.10 mg/mL). All the samples attended the specific migration limit established for caprolactam (15 mg/kg) and, therefore, the studied films were in accordance with legislation. The irradiation of the multilayer films containing PA-6 promoted a reduction in the caprolactam migration levels into olive oil with the increasing of the dose of irradiation for films intended for meat foodstuffs, while for films used as cheese packaging, the opposite behavior was observed.

Keywords: caprolactam, migration, gamma radiation, olive oil

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M-12
DEVELOPMENT OF A MOLECULARLY IMPRINTED POLYMER FOR THE ANALYSIS OF BISPHENOL A

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Rational design was used to tailor an imprinted polymer selective for the endocrine disruptor bisphenol A, for use as a solid phase extraction sorbent. X-ray crystallography and molecular dynamics were used to probe non-covalent interactions in the pre-polymerisation system. Binding properties of the polymers were assessed by solid-phase extraction. Bisphenol A itself was deemed unsuitable as a template, as leaching could affect trace analyses. Hydroquinone was found to induce an imprinting effect resulting in a polymer with a high affinity for bisphenol A. The prepared material was applied to the selective extraction of bisphenol A from milk. This presents a facile route to producing a low-cost and selective alternative to commercial SPE sorbents for food analysis.

Keywords: molecular imprinting, bisphenol A, SPE

M-13
ANALYSIS OF BISPHENOL A, BISPHENOL F, BADGE, BFDGE AND THEIR HYDROLYSIS AND CHLOROHYDROXY DERIVATIVES IN CANNED FOOD BY UPLC–MS/MS

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Epoxy resins are used to produce the inside coating for food containers and are synthesized from bisphenol A or F by condensation with epichlorhydrin. If this reaction is not carried out properly, by-products of the reaction (BADGE and BFDGE) can migrate into the preserved food in such containers, and the formation of hydrolysis and chlorohydroxy derivates occurs. Commission Regulation (EC) No.1898/2005 established the specific migration limits for the sum of BADGE and its hydrolysis derivatives at 9 mg/kg in food and the sum of BADGE chlorohydroxy derivates at 1mg/kg in food. The presence of BFDGE and their derivates is not permitted. The aim of this work is to develop a single extraction method of these compounds in different matrixes - oil-based and aqueous-based food -, such as oil, olives, cheese, fish (tuna, salmon), different kinds of sausage (bacon, ham, bologna), milk and baby food (vegetable or meat based, and also mixtures). Analytes are extracted using acetonitrile, followed by a defatting step using n-hexane. The extract is evaporated and reconstituted with water prior to solid phase extraction (SPE) using Bond Elut Plexa cartridge. After SPE, the organic eluent is evaporated and the extract reconstituted with mobile phase. These clean-up steps help to reduce matrix effect. The determination by UPLC-MS/MS involves two different methods; one method with ESI+ to detect ammonium adducts of BADGEs and BFDGEs, and another method with ESI- to detect bisphenols. Quantitation is based on the matrix matched surrogate calibration method. All samples are spiked before extraction with suitable internal standards (IS). Due to the unavailability of isotopic standards for BADGEs and BFDGEs, 3,4-Bis(4-(2-hydroxyethoxy)phenyl)hexane is used as internal standard for these analytes. It is a synthetic compound that will not be present in food and its structure and behaviour is similar to the analytes under study. Bisphenol A-d16 is the IS for bisphenols. The method has been fully validated; the limit of quantitation (LOQ) has been set at 0.040 mg/Kg. The procedure is accredited by the Spanish Accreditation Body (ENAC) according to the requirements of ISO 17025 standard.

Keywords: Bisphenol, BADGE, BFDGE, UPLC-MS/MS, canned-food

M-14
PHTHALATE CONTENT DETERMINATION IN SEASONED ITALIAN CHEESES BY LC(ESI) – MS/MS

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Phthalates are the most used additives for the plastics production (particularly, PVC), and they are considered "Persistent Organic Pollulants" (POPs). Humans are exposed to phthalates via food, air, water and other sources (such as cosmetics and pharmaceutical products). The risk of phthalate exposure for human health is mainly related to their activity of "Endocrine Disruptor Compounds" (EDCs). The evaluation of the phthalate contents of three Italian cheeses (grated Parmigiano Reggiano, grated Grana Padano, and Provolone slices) was performed by LC(ESI)-MS/MS, previous extraction by QuEChERS method [1].
Due to the different liposolubility and other physico-chemical properties of phthalates, they were divided into 3 groups. The QuEChERS extraction protocol was optimized for the three groups of phthalates, as well as for the milk samples and cheese samples.
A specific chromatographic run was set up per each group of phthalates, to be able to efficiently separate all analytes. An Agilent 1200 series binary HPLC pump coupled to a Phenomenex Luna C18 column (150 × 3 mm, 5 µm, 100 Å) was used. Sample elution was run under isocratic conditions, using a mobile phase made of CH_3OH (A) and H_2O (B), both buffered with 0.1% HCOOH and 1 mM CH_3COONH_4.
Quantitation of phthalates was carried out on API 3200 QqQ (Applied Biosystems) fitted with Turbo V Ionspray using Multiple Reaction Monitoring scan on 3 reaction ions per analyte, in positive polarity. LODs, LOQs and % recoveries were satisfactory.
The levels of some phthalates were higher than their corresponding German BfR limit [2] or EU SML [3] only in two out of six food chains of Italian cheeses, packed in printed plastic bags. Since the processing technologies of Parmigiano Reggiano, Grana Padano and Provolone are similar, the main source of volatile phthalates in the cheese-making industry is probably due to the environmental contamination; however, plastic materials used in processing, storage, transport, and packaging [4], may also give a meaningful contribution.

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[2] Bundesinstitut für Risikobewertung (BfR) (2007). Safeguards, 102, 7
[3] Commission Regulation (EU) No 10/2011 of 14 January 2011
[4] J.H. Petersen and L.K. Jensen (2010). Food Additives and Contaminants, 11, 1608-1616

Keywords: phthalates, Italian cheeses, LC-MS/MS

M-15
SIMULTANEOUS DETERMINATION OF MOSH AND MOAH FRACTIONS BY ON-LINE 2–CHANNEL NPLC–LV–GC–FID

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Swiss studies done by the canton laboratory in Zurich showed that mineral oils migrate at significant levels into food. They stem from recycled card board that is used for food packaging. These mineral oils consist of a saturated hydrocarbon fraction (MOSH: mineral oil saturated hydrocarbons) and a fraction of predominantly alkylated polycyclic aromatic hydrocarbons (MOAH: mineral oil aromatic hydrocarbons). The toxicology is not yet finally investigated. In general a contamination of food with mineral oils is not desirable and has hence to be minimised. For the determination of a mineral oil contamination (MOSH/MOAH) in food and packaging materials an optimised method was developed and tested. It is on the basis of the originally introduced method by M. Biedermann and K. Grob [1] with some optimisations regarding increased sample throughput by simultaneous detection of MOSH and MOAH fractions in a single HPLC and GC cycle time. In principal interfering fat and matrix components are initially removed during the normal phase HPLC step. By combinating retention gap technique and early vapor exit the HPLC fractions can be directly transferred to the GC and detected with FID. The original method [1] requires two separate HPLC injections and two distinct fraction transfers to the GC in order to analyse the amount of MOSH and MOAH in one sample. This is necessary, because the GC retention times of both fractions are too close to quantitate them both independently on only one separation column in a single GC run. The here presented method uses two autonomous channels to allow the simultaneous detection of MOSH and MOAH fractions. This becomes possible by using a second retention gap, a second GC separation column and FID. The total analysis time including GC detection, backflush and re-equilibration is 30 minutes. Since both fractions are not eluted in the same mobile phase, an adaption of the concentration parameters in the retention gap is important. The optimisation of the GC oven program and carrier gas pressures are of crucial importance crucial to analyze MOSH and MOAH components from one sample within 30 minutes. By using this technique the sample throughput can be doubled and the solvent consumption can be reduced.

[1] M. Biedermann, K. Fiselier, K. Grob, J. Agric. Food Chem., 2009, 57(19), 8711-8721

Keywords: MOSH, MOAH, Mineral oil, packaging material, food

M-16
GC–MS MULTIMETHOD FOR THE ANALYSIS OF PHOTOINITIATORS MIGRATING FROM PACKAGING MATERIALS INTO FOODSTUFFS

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Photoinitiators are used in UV-cured printing inks to start a polymerisation process that leads to the formation of a macromolecular network with embedded colour particles. Due to their technological advantages UV-cured inks are also widely used in the printing process of food packaging materials. Subsequently, the photoinitators can migrate from the packaging material into the enclosed foodstuff. A wide variety of chemical compounds may be used as photoinitiators; however, so far only a limited number of substances (mostly ITX and benzophenones) have been targeted in analytical methods reported for determining photoinitators in foodstuffs. We have developed a GC-MS multimethod that allows the screening for more than twenty different compounds used as photoinitiators (various substituted benzophenones, thioxanthones, benzoates, etc.). The various steps of method optimisation will be discussed and the characteristics of the final method will be described. The developed GC-MS method allows the sensitive and selective analysis of photoinitiators in both foodstuffs as well as packaging materials. Limits of quantification are mostly at or below 10 µg/kg food. The photoinitiator multimethod was employed for the screening of various cereal products and beverages as well as their respective packagings for the presence of photoinitiators. The observed substances and their concentrations in the foodstuffs and packaging materials will be presented.

Keywords: photoinitiators, packaging material, GC-MS, multimethod

M-17
FOOD PACKAGING MIGRATION – DIRECT INJECTION (ASAP) AND LC ANALYSES, USING QTOF MS

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Packaging has become an indispensible element in the food industry and food packaging companies are continuously developing new and innovative products that will protect the product from environmental contaminants during local and international transportation, and help provide a longer shelf life for the food product. Food packaging materials and packaging migration was named as one of the hot topics for 2010 and it is likely that this will remain to be the case. Whilst many of the packaging compounds used today are well documented (e.g BPA, BADGE…), there are other compounds that may also migrate and present a food safety issue. The challenge is that much knowledge around the packaging material is patented and this is further hampered by the complex matrices in food products. Use of a targeted approach will allow known compounds to be monitored but will undoubtedly miss new compounds that may be present. Here, two approaches using QTof MS are described. The first is a targeted approach using an atmospheric solids analysis probe (ASAP) in order to look at migration from kitchen utensils. The second approach is an untargeted analysis using ACQUITY UPLC & multivariate analysis (MVA) in order to monitor total migration observed over an 8 day period as specified in some of the legislation in Europe. These experiments looked at migration in infant formula packaging (tin & tin cap).

Keywords: Packaging migration, MVA, QTof MS,

M-18
CRITICAL COMPARISON OF TWO INSTRUMENTAL TECHNIQUES, GC-MS AND LC–MS, FOR THE FTOHS DETERMINATION IN FOOD CONTACT MATERIALS

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Fluorotelomer alcohols (FTOHs) represent an important part of a large group of the emerging perfluorinated alkylated substances (PFASs). FTOHs are used as starting compounds for synthesis of polyfluorinated surfactants (PFSs), especially their phosphate esters (PAPSs, resp. SN-diPAPS), which are commonly used to improve water and oil repelency of paper for food contact. Consequently, PAPSs are source of FTOHs, which are transformed to persistent perfluorinated carboxylic acids (PFCAs) with toxic potential. Since PAPSs are used in baking papers, muffin cups, popcorn bags or paper wraps, food prepared and/or stored in them could be important source of PFAS in human diet. Therefore it is important to develop sensitive and specific analytical methods to monitor FTOHs in food contact material. The aim of a presented study was to compare sensitivity, robustness and repeability of two different instrumental techniques, (i) gas chromatography coupled to mass spectrometry in chemical ionization (GC–MS) and (ii) ultra performance liquid chromatography hyphenated with tandem mass spectrometry with electrospray ionization (UPLC–MS/MS). Sensitivity GC–MS is highest for short chain FTOHs (C4–C8), however in the samples were detected also analytes with chain length C16–C20. Since sensitivity of UPLC–MS/MS increase with chain length, it is suitable detection technique for these long chain FTOHs. Finally, several commercially available food contact materials (baking paper, muffin cups and fast-food packaging) were examined for the FTOHs presence.

Keywords: FTOHs, GC–MS, LC–MS, food contact materials

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M-19
ANALYSIS OF POLYFLUORINATED SURFACTANTS IN FOOD AND FOOD CONTACT MATERIALS

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European Commission in the Recommendation 2010/161/EU advises the Member States to monitor the presence of perfluorinated alkylated substances (PFASs) in food. One of the groups of these anthropogenic compounds is polyfluoroalkyl phosphate surfactants (PAPSs) such as 8:2 diPAPS and 8:2 monoPAPS. Worth to notice, most of until now conducted studies have been focused on determination of perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) of different chain length in drinking water and food of animal origin, which are supposed to be the main sources of PFCs in human diet. However, another important source of PFASs in human diet can be packaging materials (paper and board), which are treated by polyfluorinated surfactants (PFSs) to improve their properties. The identified PFSs in the paper wraps are often ionic PAPSs, which degraded via fluorotelomer alcohols (FTOHs) to PFCAs, S-diPAPS and SN-diPAPS [1]. Since recently, non-ionic and polymeric PFSs represented by e.g. alkoxylates or acrylates, have been increasingly used [2]. Although data on detection and migration of PFSs into food stimulants are available, information on migration into real food are poor.

In the presented study, DART-MS (Direct Analysis in Real Time) was used for a rapid screening fluorotelomer patterns (series of ions differing by 99.99 Da, which correspond to C_2F_4) in paper and board for food packaging. In the positive paper samples especially PAPSs were identified. LC-MS/MS experiments, which show a number of isobaric isomers including fluorotelomer alcohols with different chain length followed. To extract PAPSs from paper samples utilising sonication with different solvent was tested. Methanol for ionic mono- and di-PAPSs and ethylacetate for neutral triPAPSs were chosen as the best compromise. Since PAPSs were found also in the muffin cups, migration into various food simulants and also to real muffins was tested, but a method for extraction of PAPSs from food had to be developed. The most promising procedure was chosen a modified QuEChERS method previously introduced for analysis of PFASs in food of animal origin [3], because of providing the same or better results for all ionic and neutral PAPSs to compare sonication with different solvents. To our surprise, migration into muffins was significantly higher than into the common food simulants (water, 3% acetic acid solution, 15% ethanol solution and olive oil). The concentration of PAPSs in the muffins, employing Zonyl FSE as calibration standard, was ≈ 5 μg g^{-1}.

[1] Begley T.H. et al. (2005); Food Addit Contam 22(10): 1023
[2] Trier X. et al. (2011); Environ Sci Pollut Res DOI 10.1007/s11356-010-0439-3
[3] Lacina O. et al. (2011); J Chromatogr A 1218: 4312

Keywords: polyfluoroalkyl phosphate surfactants, DART-MS, LC-MS/MS, migration, food

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M-20
SAFETY IN USE OF FOOD PACKAGING: MIGRATION FROM POLYURETHANE ADHESIVES OF MULTILAYER FOOD PACKAGING INTO FOOD SIMULANTS

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Polyurethane adhesives (PU) represent 90% of the adhesives market, used for preparing multilayer laminates for food contact materials. Adhesives are used in most of the food packaging to glue the different materials. Although a small amount (up to 5%) is used, the adhesives can provide more than 4000 different substances as potential migrants to be transferred into food in contact with the packaging. However the food contact materials are regulated, no specific legislation exists yet for adhesives. It must be highlighted that the risk of adhesives in food packaging materials is a real issue. It is well known that chemical compounds, which are included in the adhesive formulations and have a molecular weight lower than 1000 uma, diffuse through the different layers of materials and reach the food in contact with the packaging. However, very little is known about the composition and performance of their components in food packaging. The identification of potential migrants and the migration tests can allow the companies to improve the safety in use of adhesives, as the migrants could be replaced by others with a lower risk. This is one of the objectives of this research. Solid phase microextraction in headspace mode and gas chromatography coupled to mass spectrometer (HS-SPME-GC-MS) were used to identify the potential migrants in the adhesives (seven PU adhesives) and also in the individual plastic films (polyethylene terephthalate, polyamide, polypropylene, polyethylene and polyethylene/ethyl vinyl alcohol). The volatiles were extracted from 0.1 g of the pure cured adhesives and analyzed by HS-SPME-GC-MS. Cut-outs (1 dm^2) of the individual films were also analyzed. For migration tests, performed with Tenax® used as dry food simulant, pouches of 0.16 dm^2 were filled with 0.64 g of Tenax® (4g Tenax® per dm^2 laminate, according to UNE-EN 14338). The samples were stored at 40°C/10 days. After this period, the pouches were opened and the Tenax® was extracted 2 consecutive times using acetone. The solutions were put together, spiked with 10 μL of internal standard solution, concentrated under a stream of N^2 to 200 μL and finally analyzed by GC-MS. For migration tests, carried out with isooctane used as fatty food simulant, the pouches of 1 dm^2 were filled with 10 mL of isooctane. Three different contact conditions between the packaging and the food simulant were used: 20°C/2 days, 60°C/ 1.5h + 20°C/ 2 days and 60°C/2.5 h + 20ºC/2 days. The analyses of the migrants were also performed by GC–MS. Many volatile and semivolatile compounds were identified as potential migrants in the pure adhesives and in the individual films. There were compounds that migrated exclusively from the adhesives through the laminates into both simulants, and other compounds could have migrated from the adhesives but also from the plastic films. Comparison of the migration values between the simulants and conditions will be also shown and discussed.

Keywords: food safety, multilayer food packaging, polyurethane adhesives, migration

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M-21
SUBSTANCES MIGRATING FROM FOOD CONTACT MATERIALS INTO FOODSTUFFS: OVERVIEW AND SELECTED ANALYTICAL EXAMPLES

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Over the last decades organic trace analysis in foodstuffs has focussed mainly on residues (e.g. pesticides, veterinary drugs) and environmental contaminants (mycotoxins, heavy metals, dioxins and PCBs, etc.). However, unwanted and potentially harmful substances can also enter foodstuffs along the food chain from processing to storage and in the household. Every material that comes into contact with a foodstuff is a potential source for such contaminants. Food contact materials include inter alia packaging materials, kitchen utensils and tableware. This presentation will give an overview of the various food contact materials and the substances which might migrate from them into the foodstuff. Examples of analytical methods used to identify and quantify these contaminants and findings from recent investigations of samples from the Austrian market will be presented. The presented analyses will include both substances migrating from processing equipment and/or packaging materials (plasticisers, photoinitiators) as well as compounds leaching from kitchen utensils (melamine, primary aromatic amines).

Keywords: food contact materials, contaminants, packaging, kitchen utensils, analysis

PROCESSING CONTAMINANTS

(N-1 – N-19)

N-1
CHEMICAL CHANGES IN COFFEE ACCORDING TO THE PREPARATION PROCEDURES. PART A: PROCESSING CONTAMINANTS

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Acrylamide, furan, 3-MCPD and its esters are well-known processing contaminants of a wide range of foods, mainly those heat treated. High levels of acrylamide were found especially in starch rich foods such as potato and bakery products, or in coffee and coffee surrogates. Up to now, the highest levels of furan were determined in roasted coffee, caramel, vegetable based baby foods and canned foods. 3-MCPD is the most commonly found member of a group of chemical contaminants known as chloropropanols. Most of the 3-MCPD found in foods is present as the fatty acid esters. Both 3-MCPD and its esters can be formed in heat-processed fat-containing food in the presence of chloride ions from glycerol or acylglycerides. According to IARC, acrylamide and furan are classified as a probable (group 2A) and a possible (group 2B) carcinogen to humans, respectively. 3-MCPD is suspected genotoxic carcinogen. The content of free 3-MCPD is regulated in liquid acid-HVP and soy sauce with a limit of 0.02 $mg.kg^{-1}$, based on a 40% dry matter content, (EU Commission Regulation 466/2001). The toxicological significance and bioavailability of 3-MCPD esters is not yet known. Our aim within this study was to monitor the levels of the above mentioned processing contaminants in various types of coffee (roasted beans, instant coffee) available on the Czech retail market. To estimate their contribution to the total dietary intake, our research was also focused on the transfer of acrylamide and furan into the brew prepared by common procedures such as espresso, filtered coffee or so called “mud” coffee. The analytical procedure used for acrylamide determination was based on HPLC technique coupled to tandem in space mass spectrometry (MS/MS). Isotope dilution technique with $^{13}C_3$-acrylamide as an internal standard was used for quantification. The measurements of furan levels were carried out using a SPME-based sampling technique followed by GC-MS determination. 3-MCPD esters were analyzed using GC-HRTOF MS after acid hydrolysis and removal of liberated fatty acids; deuterated 1,2-dipalmitoyl-3-MCPD was used as an internal standard for quantification. Our results can contribute to the estimation of the dietary intake of acrylamide, furan and 3-MCPD esters from popular and widely consumed coffee products. The analysis of the processing contaminants in the same samples showed clear differences in their occurrence and behaviour. While almost the whole theoretically possible amount of acrylamide was detected in coffee brews, only less than 50% of this amount was found in the case of furan. The results have as well shown that the furan content in a brew standing in a cup decreases, whilst the content of acrylamide remains unchanged. The results presented clearly indicate the need to apply more complex approach in evaluating the impact of processing contaminants (and other compounds with protecting character, see Part B), instead of focusing only on one individual compound.

Keywords: coffee, acrylamide, furan, 3-MCPD esters

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N-2
MONITORING OF LIPID OXIDATION DURING CONVENTIONAL AND VACUUM FRYING BY DIRECT ANALYSIS IN REAL TIME–MASS SPECTROMETRY (DART–MS) TECHNIQUE

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The autooxidation of fats and oils is significantly accelerated at high temperatures, which are typically used during various food processing operations, such as deep frying. As lipids oxidize, various desirable and undesirable compounds, representing both the non-volatile and volatile species, are formed. While some of these compounds improve the flavor of final products, other can accumulate in processed foods and induce adverse health effects upon ingestion in the human gastrointestinal tract. Considering the need for minimization of the autooxidation, vacuum deep frying process represents a conceivable option, as it enables to obtain food with acceptable organoleptic properties while applying lower temperatures within the heat-treatment. Until now, numerous methods have been applied to monitor the degree of the autooxidation of oils and fats. Beside of the widely used titration methods, the chromatographic techniques based on either the liquid (LC), or gas chromatography (GC), coupled to various detectors, have been described. It should be noted that these conventional methods are typically time demanding and require a laborious sample pre-treatment. In this study, we demonstrate the potential of a novel desorption ionization technique, the direct analysis in real time (DART) hyphenated to the (ultra)high resolution mass spectrometry (HRMS), for a simple, high-throughput monitoring of the lipid oxidation dynamics during deep frying. The DART–HRMS technique was employed to compare the extent of oil oxidation that takes part during the conventional and vacuum frying of potato chips. In addition to the analysis of rapeseed oil which was used for frying, the DART–HRMS spectra obtained by the analysis of potato chip extracts were also acquired and examined. Only a minimal sample preparation (dilution and / or simple extraction) was employed to isolate both the non-polar and polar fraction of oxidized lipids prior to the instrumental analysis. To interpret the data, multivariate statistic! al analy sis (principal component analysis, PCA) was used. The advantages of high mass resolving power detection system for the tentative identification of lipid oxidation products was also demonstrated.

Keywords: Autooxidation, Oils, Deep-Frying, Direct Analysis in Real Time, Mass Spectrometry

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N-3
HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF REACTION PRODUCTS AND INTERMEDIATES FORMED IN CARBONYL-ASPARAGINE MODEL SYSTEM DURING HEATING

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This study aimed to investigate the formation of key intermediates and products in carbonyl-asparagine model systems during heating at elevated temperatures. Asparagine was heated with equimolar amount of glucose or hydroxymethylfurfural at 180°C for different times. The reaction products and intermediates formed in the model systems during heating at 180°C were analyzed by using high-resolution mass spectrometry by performing full scan ranging between *m/z* 50 and 300 with ultra-high resolving power. The analyses were performed by a liquid chromatography system (Thermo Scientific Accela) coupled to an orbitrap high-resolution mass spectrometry (Thermo Scientific Exactive) operated in positive atmospheric pressure chemical ionization mode. The chromatographic separations were performed on Atlantis T3 Column (250 mm × 4.6 mm id; 5 µm) using an isocratic mixture of 0.05% formic acid and methanol (70:30, *v/v*) as the mobile phase at a flow rate of 0.5 mL/min (30°C). The analytical conditions applied here successfully resolved the peaks of intermediates formed in the model system during heating. The observed masses were compared with corresponding theoretical masses to confirm the molecular structures of compounds identified in heated reaction mixtures. Orbitrap mass spectrometry analysis verified the formation of glucose dehydration products including hydroxymethylfurfural, and their involvement on Schiff base formation along with asparagine (Δ<1.5 ppm). The results revealed that decarboxylated Schiff bases simultaneously undergo both β-elimination and hydrolysis in asparagine-glucose model system during heating. As a key intermediate leading to acrylamide, 3-APA formed in the model system was detected with high mass accuracy. Considering the mechanism of acrylamide formation, there were strong indications that both pathways have certain contributions depending on the structure of carbonyl compound reacted with asparagine.

Keywords: Maillard reaction, acrylamide, 3-aminopropionamide, hydroxymethylfurfural, high-resolution mass spectrometry

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N-4
IMPROVED ANALYSIS OF TRANS FATTY ACIDS BY NEW IONIC LIQUID–BASED CAPILLARY GC COLUMNS

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Analyses of fatty acids (FAs) after transesterification to the corresponding FA methyl esters (FAMEs) are continuing to gain importance as more research is focusing on their biomedical impacts. This includes the analysis of saturated and polyunsaturated FAs along with the positional geometric (cis and trans) FA isomers. Unsaturated trans FAs (which are created e.g. during hydrogenation of vegetable oil) and saturated FAs are considered to have a negative impact on human health. Although bicyanopropyl polysilicone columns are the current standard for the separation of geometric FAME isomers there are still some limitations especially in the separation of both mono- and polyunsaturated FAMEs and conjugated linoleic acids (CLA). Ionic liquids are a class of non-molecular ionic solvents with low melting points. These compounds exhibit ideal properties for a stationary phase in gas chromatography such as very low vapor pressure and high thermal stability [1]. These liquids are unique combinations of cations and anions and can provide a variety of different selectivities when used as capillary GC phases. The application of new dicationic liquids as stationary phases improved the efficiency as well as the temperature stability [2]. This work introduces the technology of ionic liquids as stationary phases for GC and focuses on the challenging separation of selected geometric and positional isomers of the FAMEs 18:1, 18:2 and 18:3. Separations of several artificial and original multi-unsaturated fatty acid (MUFA) mixtures from partially hydrogenated vegetable oil on this new polar GC column based on ionic liquids will be shown and be compared with those obtained from traditional bicyanopropyl polysiloxane columns. The ionic liquid GC column provided resolution of some geometrical isomers that require complimentary techniques for their analysis using bicyanopropyl polysiloxane columns.

[1] A. Berthod et al., J. Chromatogr. A 2008, 1184, 6.
[2] D. W. Armstrong et al., J. Am. Chem. Soc. 2005, 127, 593-604

Keywords: Fatty Acid Analysis, Ionic Liquid GC columns

N-5
OPTIMIZATION OF SPE EXTRACTION AND CHROMATOGRAPHIC CONDITIONS FOR POLYCYCLIC AROMATIC HYDROCARBONS IN BARBEQUED MUSCLE FOODS

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Polycyclic aromatic hydrocarbons (PAHs) are an important class of toxicological compounds formed during the incomplete pyrolysis of carbon-containing materials, such as oil, wood, garbage or coal. In cells PAHs undergo metabolic activation to diol epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that start the carcinogenic process. Due to their carcinogenic activity, PAHs have been included in the European Union and the US Environmental Protection Agency (US-EPA) priority pollutant lists. Because PAHs are widely spread in the environment, their human exposure is unavoidable. In the non-tobacco smoking and non-occupationally-exposure population, diet is the major source of human exposure to PAHs. Their presence in the environment contribute to accidental contamination of many raw foods, however the traditional preservation practice of food with wood smoke or food cooked over an open flame induces PAHs generation and their presence in foodstuffs. The presence of PAHs in food is a matter of concern and requires continuous monitoring. However, extraction and quantification of PAHs in thermally processed meat and fish is difficult because these compounds occur in food at microtrace levels and many other organic components are co-extracted from the matrix. Additionally, most PAHs are structurally similar and occur as isomers, which make identification more difficult. Studies concerning the levels of grilled or barbecued food are scarce or quantified only B(a)P alone or more few PAHs. In this study a method for analysis of 15 PAHs in charcoal-grilled meat/fish was established by high performance liquid chromatography and fluorescence detection. Gradient elution was performed with methanol/water/ethyl acetate. Maxima excitation and emission wavelenghts were selected for each PAH. Retention times were very stable with coefficients of variation below 0.24% within analytical day and below 0.60 % across analytical days. Two different methods of cleanup and preconcentration steps were compared. Solvent extraction assisted by sonication carried out with n-hexane on 2 g of lyophilised meat or 1 g of lyophilised fish allowed to obtain high sensitivity, reproducibility and better extraction efficiency. Limits of quantification (LOQ, s/n = 10) were lower than 0.01 ng/g of meat wet weight and lower than 0.02 ng/g of fish wet weight for all PAHs (except for Na, Fl and IP that were lower than 0.1 ng/g). Two different quantification methods were compared. Standard addition method compensated PAHs losses due to incomplete extraction and it is recommended for analyses of grilled meat and fish samples allowing to collect data on PAHs contamination profile in this frequently consumed type of food, that usually contain very low amounts of the eight high molecular weight PAHs (BaA, Ch, BbF, BkF, BaP, IP, BgP, DhA), the only possible indicators for the carcinogenic potency of PAHs in food.

Keywords: Polycyclic aromatic hydrocarbons, charcoal muscle foods, SPE, HPLC/FLD, standard addition method

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N-6
FORMATION OF CHOLESTEROL OXIDATION PRODUCTS (COP) IN THERMALLY PROCESSED ANIMAL ORIGIN FOOD PRODUCTS – MODEL STUDIES

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Oxycholesterols are widely distributed in nature. They were found in foodstuffs of animal origin, in the blood and tissues of animals and humans. They exhibit many biological activities, which are of potential physiological, pathological or pharmacological importance. Oxysterols inhibit cell replication and have cytotoxic properties. Food technologists are interested particularly in oxysterols because they are formed during manufacturing and storage of foods of animal origin, and affect nutritional value of products. Nutritionists, physiologists and biochemists are interested in oxysterols because they can be easily absorbed from alimentary tract as well as they are formed in vivo through enzymatic and non-enzymatic processes. In the chain reaction, usually initiated by free radicals, epimeric hydroperoxides of cholesterol and cholesterol epoxides are formed. The presence of the tertiary atoms at position twenty (C20) and twenty-five (C25) in side chain adds to the centres sensitive to oxidation, forming thus many oxysterols which usually are termed as side-chain oxysterols. The objective of presented studies was to investigate in model studies the formation of COP under aerobic condition in different model media and heating in different temperatures in range 100–180°C. COPs were determined by means of transestryfication of extracted lipids, followed by formation of TMS derivatives and quantitative analysis by HRGC-MS – TIC and/or SIM modes.

Keywords: cholesterol, cholesterol oxidation products, thermally processed food, GC/MS

N-7
3-MCPD AND 3-MCPD ESTERS IN CANNED FISH AND SEAFOOD

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3-MCPD (3-monochloropropane-1,2-diol) or chlorohydrin, belongs to a group of chemicals known as chloropropanols, including 2-monochloro-1,3-propanediol (2-MCPD), 1,3-dichloro -2-propanol (1.3-DCP) and 2,3-dichloro-2-propanol (2,3-DCP). Commission Regulation (EC) No1881/2006 of 19 December 2006 prescribes maximum levels of 3-MCPD for hydrolysed vegetable proteína (HVP) and soy sauce. 3-MCPD and its esters can be formed during the thermal processing of fatty foods, from glycerol or acylglycerides in presence of chloride ions and acids such as citric or acetic acids at high temperatures. Therefore, 3-MCPD esters are an additional potential risk to human health, since they represent a reservoir in the human body that could release 3-MCPD after the action of digestive enzymes. The main sources of 3-MCPD in human dietary intake are sauces and bakery products. Currently, there is scarce information about the presence of 3-MCPD and its esters in canned fish. Therefore, this project aims to study a wide range of canned seafood (brine, pickle, oil, etc.) and the effect of different thermal conditions and storage in the presence of chloropropanols in final products. The method used for the analysis of 3-MCPD is based in the AOAC official method. Weigh 20 g of homogenized sample, add the internal standard 3-chloro-1,2-propanediol-d5 (3-MCPD-d5) and extract with a 5 M sodium chloride solution. The extract is mixed with a substrate based on diatomaceous earth and it is passed through a chromatography column through a solid support material. 1,3-DCP elutes during the first wash with hexane/diethyl ether (9:1) and 3-MCPD is extracted with diethyl ether. The extract was concentrated and redissolved in diethyl ether, which was dried and dissolved in 2,2,4-trimethylpentane. Finally, it is derivatized with heptafluorobutyrylimidazole (HFBI) in an organic solvent, after stirring and heating and it is analyzed by gas chromatography with mass spectrometric detection GC-MS-MS. Optimization of GC-MS-MS method was carried out. BPX-5 column was used. Chromatographic and MS detector conditions were: injector temperature applied was 250°C, column oven temperature program started at 50°C (hold 1 min.), 2°C/min to 90°C, and 30°C/min to 150°C (hold 3 min). 1,3-DCP eluted at 17.5 min, isolated parent ion was 275 *m/z* and fragmentation was carried out at 0.34 V, during 20 mse., obtaining mass 169. 3-MCPD-d5 and 3-MCPD eluted at 20.5 and 20.7 min, isolated parent ion were respectively 257 and 253 m/z and fragmentation was carried out at 0.44 and 0.42 V, during 50 msec, obtaining masses 229 and 225 as well as mass 169, used as qualifier ion for both analytes. Calibration was based on the isotopic dilution method. At the moment, we are carrying out the analysis of 3-MCPD levels and 1,3-DCP in canned seafood products; such as tuna, sardines, anchovies, mackerel, mussels and scallops prepared in different sauces. The obtained results will be shown in the conference.

Keywords: canned fish, 3-MCPD, GC-MS-MS

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N-8
GLYCIDOL FATTY ACID ESTERS IN FOODS: LC–MS/MS METHOD DEVELOPMENT

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Recent studies have identified the presence of glycidol (bound in form fatty acid esters) in many refined fats and oils and also products containing fats and oils, such as baby formulas. Glycidol esters are forming during processing/refining of commercials oils. Glycidol fatty acid esters could in theory be hydrolysed to the parent glycidol in the gastrointestinal tract. Glycidol is an epoxide with a Group 2A designation by IARC - probably carcinogenic to humans. Glycidol is a direct acting mutagen and multisite carcinogen in rodents, but no epidemiological or clinical studies on glycidol have been reported for humans. Current GC-MS methods for determination of bound glycidol require derivatization and are prone to artefact formation while current LC-MS methods suffer from the lack of robustness. A new method based on LC-MS/MS was developed. The method incorporates isotope dilution method for quantifying the five target analytes: glycidol esters of palmitic, stearic, oleic, linoleic and linolenic acid. For analysis, 10 mg oil samples are spiked with deuterated analogs of glycidol esters and purified by a two step chromatography on C18 and normal silica. A dried extract is redissolved in 250 µL of solvent and 15 µL is injected on a C18 column that is eluted in methanol. Detection of target glycidol fatty acid esters is by Multiple Reaction Monitoring in an APCI mode with 2 ion transitions for each analyte. The method was tested on replicates of virgin olive oil which was free of glycidol esters and the method detection limit is 0.1 ppm for each analyte. The major advantage of our method is that spurious peaks present in LC-MS chromatograms are absent from MS/MS chromatograms. The method will be applied to the survey of glycidol fatty acid esters in foods on the Canadian market. The method might be further refined by pre-concentration step on silica gel to remove most of tri-, di- and monoglycerides from the sample.

Keywords: Glycidol esters, Liquid chromatography – tandem mass spectrometry (LC-MS/MS), foods

N-9
OCCURRENCE OF FURAN IN CEREAL-BASED FOODS FROM THE BRAZILIAN MARKET

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Furan is a possible human carcinogen that can be formed during the thermal treatment of several processed foods. The exact mechanism of furan formation is not completely understood, but experiments carried out using model systems have shown that ascorbic acid, sugars, amino acids, and poly-unsaturated fatty acids could act as potential precursors. High levels of furan (>100 µg/kg) have been reported in some cereal-based products. Since this food category is commonly consumed by the population and no data is available in Brazil on furan content in these products, the objective of this work was to validate a method for furan determination in cereal-based foods and to investigate the levels of this contaminant in Brazilian commercial samples.Furan was determined by gas chromatography coupled to mass spectrometry preceded by headspace solid phase microextraction (HS-SPME-GC/MS). The SPME was carried out using a 75 µm carboxen-polydimethylsiloxane (CAR/PDMS) fiber, under previously optimized conditions, i.e. extraction temperature: 25°C and extraction time: 30 minutes. Quantification was performed by employing deuterated furan-d4 as internal standard. The method was validated by using a sample of whole bread according to the guidelines laid down by the Brazilian Institute of Metrology, Standardization and Industrial Quality. A total of 48 samples (including granola, cereal bars, corn flakes, breads, crackers, and toasts) were collected at supermarkets in the city of Campinas, SP, Brazil, and analyzed for furan content. The analytical method showed good linearity in the range of 0–100 µg/kg (r^2 = 0.998). No matrix effect was observed. Limits of detection (LOD) and quantification (LOQ) were calculated as 1.4 and 4.8 µg/kg, respectively. Mean recoveries varied from 92 to 123%, and coefficients of variation ranged from 3.8 to 13.7% for repeatability, and from 10.3 to 23.3% for within-laboratory reproducibility. Furan levels in the analyzed samples were from not detected to 171.9 µg/kg. Th e contaminant was found in quantifiable amounts in 42 samples, and levels higher than 100 µg/kg were observed in 4 of them. Corn flakes and crackers showed the higher mean furan levels, while white loaf bread presented the lower mean concentration. The results obtained in this study showed that most of the analyzed samples contained furan. In some products, high concentrations were observed. These data will be useful to estimate the exposure of the Brazilian population to furan due to the consumption of cereal-based foods.

Keywords: furan, cereal-based foods, SPME, GC-MS

Acknowledgement: CNPq - National Council for Scientific and Technological Development (Proc. 578381/2008-7).

N-10
DETERMINATION OF SIX COCCIDIOSTATS IN TARGETED FEED USING HPLC–UV/VIS–FLD WITH POST–COLUMN DERIVATISATION

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Coccidiostats are widely used as feed additives in poultry production. The control of their levels in targeted feeds plays important role for the assurance of efficiency of treatment, prevention of drug resistance and food safety. Considering above, a method for the determination six ionophore coccidiostats (lasalocid, maduramicin, monensin, narasin, salinomycin, semduramicin) in targeted feed was developed. Properly grinded and homogenized feed sample was spiked with internal standard (methyl monensin) and extracted with methanol. The extract was analysed with HPLC without any further purification. Analytes were separated on the Phenomenex Kinetex C18 (150 × 4.6 mm, 2.6 µm) column with 20 min gradient of methanol:0.02 M KH_2PO_4(pH 7.0) solution. Flow rate of mobile phase was 0.7 ml/min and oven temperature was 32°C. Eluate was pumped through fluorescence detector, used for lasalocid analysis (excitation and emission wavelength were 310 nm and 420 nm, respectively). Next, other ionophores were derivatised with vanillin in the presence of sulfuric acid at temperature 110°C. The obtained derivative was then detected at λ=520nm (UV-Vis detector). Fortified samples (spiked at 25, 50 and 100 mg/kg) and targeted feeds at authorized levels were used for method validation. Recovery was in the range of 85–99%, with within-laboratory reproducibilityin the range 5–15% (CV, %) depending on the analyte. LODs and LOQs were below 0.5 mg/kg and 2 mg/kg respectively. The method is quick, easy to perform, efficient. It enables the determination of all authorized ionophores with a single analytical protocol. The effectiveness of the presented method was proven in the analysis over 100 different feed samples. Additionally, developed procedure was successfully used in the proficiency tests.

Keywords: coccidiostats, targeted feed, post-column derivatisation

N-11
MONITORING OF ACRYLAMIDE IN THE COURSE OF MALTING AND IN BEER

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Acrylamide in food is produced in the course of Maillard's reaction and its precursors are reducing saccharides and amino acid asparagin. Reaction mechanism of the acrylamide formation in food depends on food composition and processing conditions. Acrylamide is created in a significant quantity by heat treatment of food above 120°C, the highest quantity of acrylamide is created at 150–180°C. At higher temperatures acrylamide creation is substantially lower as the elimination reaction is faster than that producing acrylamide. Raw material for malt production is barley, a plant with content of nitrogen compounds and high content of starch. During malting enzymes increase the content of reducing saccharides in malt, during kilning biochemical changes are induced by temperature, and melanoid substances originate. These conditions are favorable for acrylamide creation. Changes of acrylamide levels were followed in malt and subsequently in the produced beer. Acrylamide content varied depending on the type of the malt in the scope of 0.2–3.0 mg.kg^{-1} and thermal maximum of its origin (160–170°C) was confirmed. Despite its relatively high content in malt (from 0.2 to 3.0 mg.kg^{-1}), no acrylamide was detected in any of the analyzed samples of beer.

Keywords: acrylamide, GC/MSD, malt, beer

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N-12
COMPARISON OF DIRECT AND INDIRECT DETERMINATION OF 3-MONOCHLOROPROPANE-1,2-DIOL (3 MCPD) FATTY ACID ESTERS IN DIFFERENT FOODSTUFFS

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3-Chloropropane-1,2-diol (3-MCPD) is a well-known processing contaminant occurring in various foods such as acid hydrolyzed vegetable protein (HVB), and various food ingredients. Recently, 3-MCPD esters have been detected in a wide range of foodstuffs, especially in refined vegetable oils and products made thereof. The highest levels of these emerging lipophilic contaminants have been found in hydrogenated fats, palm oil and solid frying fats, at concentration levels largely exceeding those of free 3-MCPD. A conventional method for the determination of 3-MCPD esters is based on hydrolytic step followed by derivatization of released 3 MCPD by acidic hydrolysis for gas chromatography–mass spectrometry (GC/MS) analysis. The following performance parameters of this method were obtained: detection limit 100 μg kg^{-1}, recovery (96–99%) and repeatability (1–7%). In addition to method based on GC/MS, a novel alternative approach to analysis of 3-MCPD esters, which is able to determine individual native 3-MCPD diesters, was developed. The fraction of target analytes isolated from oil samples by fast silica gel mini-column chromatography was analyzed by ultrahigh pressure liquid chromatography (U-HPLC) coupled to a high-resolution spectrometry (HRMS) employing an Orbitrap mass analyser. The lowest calibration levels estimated for the respective analytes ranged from 2 to 5 μg kg^{-1}. Acceptable recoveries (89–120%) and repeatabilities (RSD 5–9%) were obtained for target analytes at spiking levels of 2 and 10 mg kg^{-1}. In this study, different foodstuffs as infant formula, purees, biscuits, canned fishes and oils were analyzed by both methods GC/MS and U-HPLC/HRMS and the obtained results were compared.

Keywords: 3-MCPD esters, vegetable oils, U-HPLC–MS

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N-13
INVESTIGATION OF CHLOROPROPANOLS LEVELS IN BRAZILIAN FOODS CONTAINING MALT INGREDIENTS

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Chloropropanols, such as 3-monochloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropan-2-ol (1,3-DCP), are a group of chemical contaminants with carcinogenic and genotoxic properties that can be found in several processed foods and ingredients, including malt-derived products. The formation of 3-MCPD in malt has been attributed to the dry-kilning process at temperatures above 170°C. Since malt ingredients are used for coloring and flavoring applications in baked cereal products, beers, sauces, gravies and malted drinks, and high levels of chloropropanols were already reported in dark brewing malted grains, roasted barley malt flours and dark malt extracts, it is important to evaluate if foods containing malt-derived ingredients may also contain the contaminants. For that, several foods containing malt, malt extract and/or malt flour were purchased at supermarkets in the city of Campinas, SP, Brazil, and analyzed for 3-MCPD and 1,3-DCP content. A total of 44 samples were selected, including granola, corn flakes, crackers, toasts, chocolate drinks (powder only) and beers. For chloropropanols analysis, saline solution and Extrelut NT20® were added to the samples, mixed and transferred to a glass column. Elution was performed with a mixture of hexane/ether and then with diethyl ether. Dried and concentrated extracts were derivatized with HFBI prior to analysis by gas chromatography-mass spectrometry (GC-MS). Quantification was carried out by employing deuterated 3-MCPD-d5 and 1,3-DCP-d5 as internal standards. The method was validated by using a sample of whole bread containing malt extract and malt flour according to the guidelines laid down by the Brazilian Institute of Metrology, Standardization and Industrial Quality. Good linearity over the range 0–312.5 µg/kg was obtained ($r^2 > 0.996$). There was no significant matrix effect for both 3-MCPD and 1,3-DCP. Limits of detection (LOD) and quantification (LOQ) were calculated as 3.2 and 10.7 µg/kg, respectively, for 3-MCPD and as 2.4 and 8.1 µg/kg, respectively, for 1,3-DCP. Mean recoveries varied from 96 to 133%, and coefficients of variation ranged from 3.7 to 10.1% for repeatability and from 4.6 to 10.3% for within-laboratory reproducibility. For most of the samples, 3-MCPD levels were below the LOQ. Only 11 samples (including granola, crackers and toast) showed quantifiable results, varying from 12.3 to 158.9 µg/kg. The 1,3-DCP was not detected in the analyzed samples. These results indicate that the use of malt-derived ingredients is not necessarily associated to the occurrence of chloropropanols in food, since most of the analyzed samples did not show quantifiable levels of these contaminants. The significant levels were generally observed in baked cereal products, which could be associated to the thermal process rather than the use of contaminated malt-derived ingredients.

Keywords: Chloropropanols, 3-MCPD, malt-derived products, GC-MS

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N-14
INVESTIGATION OF 3-APA AND ACRYLAMIDE FORMATION IN CARBONYL-ASPARAGINE MODEL SYSTEMS

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Acrylamide is classified as a probable human carcinogen hence it has been taking wide attention in food safety research area and it was revealed that the main pathway of acrylamide formation in foods is linked to the Maillard reaction (1). Studies to date clearly show that asparagine is mainly responsible for acrylamide formation in heated foods after condensation with a carbonyl source. Stadler et al. (2) have provided evidence that the Schiff base of asparagine is a direct precursor of acrylamide. Granvogl and Schieberle (3) have reported that 3-APA is an important transient intermediate giving rise to acrylamide formation in foods during heating also. This study aimed to investigate the formation of 3-APA and acrylamide in carbonyl-asparagine model system during heating. Different carbonyl compounds (vanillin and curcumin) having ketone and aldehyde functions were studied. Heat treatments were performed in a tightly closed glass tube at 180°C for different times. The reaction products were analyzed by an Agilent 1200 HPLC system, consisting of a binary pump, an autosampler and a temperature controlled column oven, coupled to an Agilent 6130 MS detector, equipped with an electrospray ionization (ESI) interface. The LC-MS system was operated in positive ionization mode using the following interface parameters: drying gas (N_2) flow rate of 13 mL/min, nebulizer pressure of 40 psig, drying gas temperature of 350°C, capillary voltage of 4 kV, and fragmentor voltage of 100 eV. The analytical separation was performed on an Atlantis T3 column using an isocratic mixture of 10 mM formic acid: methanol (70:30, *v/v*) as the mobile phase at a flow rate of 0.8 ml/min (40°C). Mass spectrum was recorded (*m/z* range 50–500) to determine 3-APA in the reaction mixtures recording signal in positive ionization mode. Presence of acrylamide and 3-APA were confirmed by comparing both mass spectra and retention time of corresponding standard compound. The vanillin-asparagine model generated significantly higher amounts of 3-APA than the curcumin-asparagine model.

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[2] Stadler, R. H.; Blank, I. et al Acrylamide from Maillard reaction products. Nature. 2002, 419, 449-450.
[3] Granvogl, M.; Schieberle, P. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. J. Agric. Food Chem. 2006, 54, 5933-5938.

Keywords: 3-APA, acrylamide, model system, asparagine, carbonyl

N-15
BENZO[A]PYRENE PHOTOLYSIS – QUEST TO IDENTIFY SOME OF PRODUCTS BY HPLC–MS–MS

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Benzo[a]pyrene (BaP) is a harmful micro-pollutant containing five fused aromatic rings and its photolysis in selected liquid media was studied by technique HPLC-MS-MS. This process plays an important role in BaP degradation in the environment and in foods. Oxidized intermediates with far more toxic effects to living organism in comparison to the mother compound (such as benzo[a]pyrenequinones or hydroxybenzo[a]pyrenes) were observed predominantly. The experimental results imply oxidation and/or degradation pathways and it was attempted to propose relevant structures based on their fragmentation pattern.

Keywords: photolysis, benzo[a]pyrene, oxidized products

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N-16
OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN BRAZILIAN ROASTED COFFEE

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Polycyclic aromatic hydrocarbons (PAHs) are stable contaminants that occur in food predominantly from processing and cooking. In coffee, the roasting process is responsible for the presence of PAHs in the final product and their concentration may vary due to the roasting degree and the composition of the coffee beans. Most of the ground roasted coffee commercially available in the Brazilian market is produced with a blend of Coffea arabica and Coffea canephora species. In view of the high consumption of coffee in Brazil and its importance as a dietary source of PAHs, this study was undertaken to determine the levels of these compounds in different brands of coffee commonly consumed in this country. For these purpose the 13 PAHs identified as being genotoxic and carcinogenic by the JECFA were chosen. A solid phase extraction (SPE) method for sample clean-up with C18 cartridge, followed by reversed-phase high performance liquid chromatography with fluorescence detector (HPLC-FLD) was proposed for the compounds determination. The method provides detection and quantification limits between 0.07–0.41 μg/kg and 0.22-1.39 μg/kg, respectively. Recoveries over 74% were obtained and the calibration curves were linear at the tested ranges (R^2 > 0.999). Considering all samples evaluated (18 brands and 6 types of coffee), the mean levels of summed PAHs varied between 0.35 and 362.94 μg/kg. Although no correlation has been established among coffee types and the levels of PAHs found, these data may be helpful in assessing the risks to public health arising from the presence of PAHs in the Brazilian diet.

Keywords: PAHs, coffee, contaminants, roasting

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N-17
AN ELISA FOR THE ROUTINE DETERMINATION OF ACRYLAMIDE IN SELECTED FRIED AND BAKED FOOD PRODUCTS

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Acrylamide is found in foods as a natural undesirable byproduct of the cooking process. This processed contaminant is created via Maillard reaction in which asparagine and reducing sugers (ingredients which occur naturely in potatoes, wheat and rye) react during baking, frying, grilling and toasting at temperatures above 120°C. To reduce acrylamide levels in foods, food safety regulatory bodies and food industry have been cooperating closely on approaches aimed at reducing acrylamide levels in processed foods. There is a clear need for an effective screening using rapid and reliable analysis of acrylamide in food matrices for quality control purposes. Currently, GC/MS and LC-MS/MS methods are primarily used for acrylamide analysis. In this presentation, ELISA method will be shown as an effective and viable screening means for acrylamide detection in food products. The method is based on sample clean up using two SPE columns, acrylamide derivatization and ELISA detection with LOQ around 30 µg/kg. An excellent correlation of results obtained by ELISA and GC/MS or LC-MS/MS within the concentration range 30-17 000 µg/kg was found in real samples. The ELISA method enables quantitative determination of acrylamide in snack and baked goods, and can be utilized for analysis of bread, toasted bread, crispbread, butter cookies, biscuits, potato pancakes, cereals, and other products. Further simplification of the method with regard to sample preparation and assay detection is in progress. The first commercial ELISA kit for monitoring of acrylamide in food products has been launched by Abraxis.

Keywords: rapid assay: acrylamide: simplification: validation: ELISA kit

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N-18
UPLC–ESI–MS/MS ANALYSIS OF ACRYLAMIDE IN COMPLEX FOOD MATRIXES: THE COFFEE CASE

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Acrylamide is a chemical compound generated in food by naturally occurring precursors as a result of thermal treatments like cooking or drying. Several studies have focussed on the development of a reliable, effective and quick method for detection of acrylamide in food, mostly in carbohydrate-rich foods, however, only a few reports centered on roasted and ground coffee. Coffee can be considered a troublesome matrix due to the low level of acrylamide formed during industrial coffee roasting and to the extractions of highly polar compounds during sample preparation that can act as potential interferents during analysis. Thus, for roasted and ground coffee sample preparation needs to be properly optimized in order to have a precise acrylamide quantification. As a matter of facts, available methods for cereal-based and/or potato-based foods do not apply sic et simpliciter to roasted and ground coffee. Several analytical methods can be used for acrylamide detection in food: GC–MS, GC–MS/MS, HPLC–FID, LC–MS/MS. Differently from other methods LC–MS/MS is particularly reliable for acrylamide determination, being capaple to directly analyse aqueous samples without prior and tedious derivatization steps or sophisticated detectors. The aim of this study is to compare different LC–MS/MS sample preparation methods. A wide range of clean-up procedures have been explored by using different SPE products, different solvents and different salt removal techniques. Sample clean-up by protein precipitation with Carrez reagents have also been tested. Furthermore different chromatographic column were tried, in order to achieve a better separation of the interest compound. The combination between clean-up procedures and chromatographic conditions strongly affects the quantitative determination of acrylamide in coffee. An inappropriate method would results in very different and unreliable acrylamide quantitation in the same coffee sample, for this reason the matrix specific optimization is highly recommended.

Keywords: acrylamide, UPLC-MS/MS, coffee

N-19
MULTIVARIATE EVALUATION OF BREAD COLOUR CHANGES AFFECTED BY SOME POTENTIAL ADDITIVES FOR ACRYLAMIDE MITIGATION

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Acrylamide in bread is formed predominantly in the crust of bread during baking and its creation is in a good correlation with brown pigments development. Acrylamide possible mitigation strategy in a bread crust matrix with an addition of inorganic salts into the basic powder formulation for bread making was studied with respect to colour evaluation and characterization by pattern recognition techniques. Impact of $CaCl_2$, $Ca2^+$ lactate, NH_4Cl, NaH_2PO_4, KH_2PO_4, $Na_2H_2P_2O_7$ and $Na_4P_2O^7$ additives on acrylamide creation in potato bread crust was evaluated and colour differences measured by 30 colour characteristics in CIEXYZ, CIEL*a*b*, CIEL*u*v*, CIEL*C*H° and HunterL*a*b* colour spaces. The reflection spectra were recorded using a Shimadzu UV–3600 spectrophotometer (Kyoto, Japan) with Large Integrating Sphere Assembly LISR 3100 (Shimadzu, Kyoto, Japan) employing quartz cell enabling reflection measurements from the surface of defined upper layer of dried and grounded bread crust samples. Each crust sample was measured at six different position on ground crust sample surface and the whole visible reflection spectrum (380–770 nm) was recorded. Colour values were calculated by using the Shimadzu Special Edition software ColorLite v3.1.16 (LabCognition, Shimadzu). Standard Illuminant D65 (representing average daylight) and 10° standard observer (perception angle of a human observer) were used in these calculations. Relative changes in colour of bread crust were not very intensive for human sensorial observation, perception and recognition. From all the examined colour variables only 7 significantly correlated with acrylamide content. Colour characteristics b* from CIELab and HunterLab colour space affirmed their relation to acrylamide creation, but the most intensive correlation with acrylamide was found for hue value v* derived from CIEL*u*v* colour space. Results showed that the addition of inorganic salts hasn't expected considerable elimination impact in the bread crust, but creation of acrylamide in a home bread maker's conditions was especially low in comparison to industrial conditions. This gives an additional study on potentiality of acrylamide reduction in some industrial foods.

*Keywords: acrylamide, additives, colour, CIELa*b*, CIELu*v**

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RESIDUES – PESTICIDES

(O-1 – O-53)

O-1
QUECHERS APPROACH FOR MONITORING SEVEN PESTICIDES RESIDUES IN BRAZILIAN HONEY SAMPLES USING GC–μECD

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The honey produced by the bees from the nectar´s plants and sugary secretions of the insects; can contain agrochemical products used in agricultural management. To evaluate these residues the QuEChERS method for sample preparation was approach and used for determination of 7 pesticides residues in Brazilian honey samples. The method involved 1% acetic acid- ethyl acetate extraction solvent with $MgSO_4$ and CH_3COONa followed by dSPE cleanup with PSA sorbent. The analyses were carried out with GC–μECD (Agilent 7890 A) equipment; an Agilent HP-5 30 m × 0.32mm × 0.25 μm column was used. The method showed linearity ≥ 0.99 and the LOQs for the pesticides studied are between 0.007 to 0.05 mg kg^{-1}. The method was validated using a blank sample of honey spiked at 0.07; 0.2 and 0.4 mg kg^{-1}; the percentage of recoveries were 85 to 128 with CV% 6 to 19; 85 to 125 CV% 2 to 12 and 75 to 119 with CV% 7 to 26 respectively for each spiked level. 26 representative honey samples from different parts of Brazil were analyzed, being that five honey samples showed pesticides residues of α Endosulfan 0.009 to 0.026; Dieldrin 0.010 to 0.011; and β Endosulfan 0.010 to 0.011 mgkg-1 respectively. The QuEChERS approach method showed adequate for chlorotalonil, heptachlor, captan, endosulfan alpha, endosulfan beta, endosulfan sulfate and dieldrin for analysis and quantification by GC–μECD.

Keywords: FOOD; VALIDATION; MULTIRESIDUE;

Acknowledgement: FAPESP, CNPq, CAPES

O-2
EFFECT OF THE PARTICLE SIZE OF QUINOA SAMPLE (CHENOPODIUM QUINOA WILLD) ON THE QUECHERS METHOD VALIDATION FOR SEVEN PESTICIDES USING GC μECD

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The particle size in the sample preparation process, improves the uniformity and representative of it, managing to be more accurate analysis results; the effect of three types of grinding for the sample preparation of the quinoa grains for pesticides residues analysis were tested (normal grinding whit food processor and two different sequences of cryogenic grinding) on the validation process of the QuEChERS method, were tested for seven pesticides: Chlorotalonil, heptachlor, captan, endosulfan alpha, endosulfan beta, endosulfan sulfate and dieldrin. The method involved extraction with ethyl acetate acetic acid 1%, solid–liquid partition with $MgSO_4$ and CH_3COONa followed by dSPE cleanup with PSA sorbent. The analyses were carried out with GC–μECD (Agilent 7890 A) equipment an Agilent 30 m HP-5 × 0.32 mm × 0.25 μm column was used. The method showed linearity ≥ 0.99 and the LOQs for the pesticides studied are between 0.02 to 0.115 mg.kg^{-1} for the grinding whit food processor; 0.025 to 0.138 and 0.027 to 0.144 mg.kg^{-1} respectively for the two sequences of cryogenic grinding. The method was validated using a quinoa sample without pesticides residues, used as blank, spiked at 0.08; 0.2 and 0.4 mg.kg^{-1}; the recoveries of the method were influenced by the particle size, being that for the level 0.08 mg.kg^{-1} for the two types of cryogenic grinding were between 78 until 145% and for the normal grinding 50 to 96%. There is an expressive matrix effect for the compounds Chlorotalonil, heptachlor, captan and endosulfan sulfate.

Keywords: Matrix effect, multiresidue, chromatography

Acknowledgement: FAPESP, CNPq, CAPES

O-3
QUECHERS AND GC–TOFMS WORKSHOPS IN SOUTH AFRICA FOR PESTICIDE RESIDUE ANALYSIS

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Developing countries such as South Africa have the same needs as any country as regards pesticide residue analysis for food. First, they want to protect internal consumer health. And, if they are to export their food, they must be able to prove that pesticides do not exist above maximum residue levels required by the import country. To accomplish these, they need fast, simple, repeatable, accurate, multi-residue sample preparation and analysis methods. QuEChERS, as a sample preparation technique, is very well suited to this task. We did "hands on" QuEChERS workshops as part of a training effort across South Africa at the University of Pretoria, the University of KwaZulu-Natal (Pietermaritzburg and Westville campuses), the Forensic Chemistry Laboratory in Cape Town, and the National Metrology Institute of South Africa. To match the fast sample preparation afforded by QuEChERS, we developed a fast 9.7 min GC–TOFMS analysis so the students could analyze their samples, and quantify and review the results, before the half-day course ended. Students analyzed fruits and vegetables, wine, blood, spices, seafood, and other samples.

Keywords: QuEChERS, GC–TOFMS, pesticides, training and education, fruits and vegetables

O-4
HIGH QUALITY ANALYSIS OF PESTICIDES IN MARIJUANA FOR FOOD AND MEDICINE USING QUECHERS, CARTRIDGE SPE CLEAN-UP, GC×GC–TOFMS, AND LC–MS/MS

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Over 15 states and the District of Columbia have enacted laws that legalize the use of medical marijuana. Potential therapeutic benefits of marijuana have been cited for pain relief, control of nausea and vomiting, stimulating appetite, and muscle relaxation. Unlike other prescribed medicines that are regulated by the US Food and Drug Administration, medical marijuana is still considered a Schedule 1 drug and is therefore illegal on the federal level. Patients have no assurances on the safety of the medicine due to potential harmful levels of pesticide residues. The Quick-Easy-Cheap-Effective-Rugged-Safe (QuEChERS) extraction approach was applied to marijuana. The complexity of the sample required cartridge solid phase extraction cleanup and comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC–TOFMS) for pesticide residue analysis. In addition, the sample extracts were diluted and analyzed by liquid chromatography-tandem mass spectrometry (LC–MS/MS) for comparison, and for determination of pesticides such as bifenazate and abamectin that cannot be done with GC.

Keywords: QuEChERS, GC×GC, cannabis, marijuana, pesticides

Acknowledgement: Randy Hoffman, The Pennsylvania State University Police Department

O-5
PESTICIDE ANALYSIS FOR ORGANIC CUT ROSES USING QUECHERS, GC-MS, AND GC×GC–TOFMS

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Currently, the United States Department of Agriculture (USDA) regulates only infestation and disease on imported cut flowers, representing approximately 70% of the rose market. This regulatory situation unintentionally encourages heavy use of pesticides, which are not regulated by USDA for cut flowers. Heavy pesticide use can have serious consequences for the health of workers in the flower industry, and their local environment. To address those issues, an organic flower industry exists that advertises agricultural sustainability practices that include limited pesticide use. In this contribution, the Quick–Easy–Cheap–Effective–Rugged–Safe (QuEChERS) sample preparation method was used with GC–MS and GC×GC-TOFMS to analyze pesticides on cut roses. Pesticide classes selected for investigation include organochlorine, organophosphorus, carbamate, pyrethroid, and triazole compounds that are routinely used by the flower industry. Development and optimization of sample preparation, including extraction and cleanup, will be highlighted. Baseline residue levels for organic certified roses and non-certified roses will be estimated in hopes of determining whether "a rose is a rose is a rose".

Keywords: QuEChERS, pesticides, cut flowers, roses, GC×GC

O-6
A COMPOUND-BASED SCANNING APPROACH FOR BROADBAND PESTICIDE RESIDUE ANALYSIS IN FRUITS AND VEGETABLES USING GC/MS/MS

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Traditionally pesticide residues in foods have been monitored with multi-residue methods using Gas Chromatography with either selective detectors (like ECD) or mass spectrometry. Compounds, which were amenable to analysis by these techniques, are now being withdrawn from use or replaced by compounds, which have better activity or less environmental impact. Many of these modern compounds are either not amenable to GC or are not detectable at sufficiently low levels in order to assess compliance with statutory maximum residue levels (low ppb levels). More and more compounds therefore have to be tested separately by specific methods, resulting in lengthy analysis times with high staff and consumable costs. Taking advantage of the selectivity provided by gas chromatography coupled with triple-quadrupole mass spectrometry and the advent of "Compound-Based Scanning" it is possible to develop a multi-class multi-residue procedure for the routine monitoring of pesticides at or below DG SANCO maximum residue levels (MRL's). The method presented has been validated in accordance with the EU Quality Control Procedures for Pesticide Residue Analysis (DG SANCO), giving good recovery and repeatability for over 300 pesticides and metabolites in a wide range of fruit and vegetable matrices.

Keywords: Residues, GC/MS/MS, DG SANCO, CBS, Fruits

O-7
EVALUATION METHOD FOR DETERMINATION OF PESTICIDE RESIDUES IN OLIVE AND OILSEED RAPE SAMPLES BY QUECHERS METHOD AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The aim of this paper is reports a method for determination of organochlorine, organophosphate and carbamate pesticides in fatty vegetables matrices such as olives (olea europaea cv. Picual) at different ripeness grade, and therefore different level of fat content, grown in Granada (Spain) and two oilseed rape (brassica napus) varieties grown in Prusy (Poland). The pesticides extraction were carried out by Quick, Easy, Cheap, Effective, Rugged and Safe QuEChERS method, adapted for the analysis of pesticide residues in food matrices with high fat content paying special attention the clean-up stage, playing with the capacity of different chemical sorbents such as PSA, GCB, C18, SAX and NH2 isolating the pesticide fraction from the whole fatty matrix in order to minimize matrix co-extractives and interferences and to allow a satisfactory long-term chromatography performance. Efficiency of the d-SPE clean-up step was evaluated by comparison adding different amounts as well as combinations of mentioned d-SPE sorbents. The analysis of pesticide residues were performed by Gas Chromatography Ion Trap Mass Spectrometry (GC/IT-MS) (using Varian 4000 GC/MS (Varian, Inc., USA), consisted of 3800 GC and 4000 Ion Trap MS detector). The linear relation was observed from 0 to 1000 $ngml^{-1}$ and the determination coefficient R^2>0.998 in all instances for all target analytes. The recoveries were in the range 70–120%, with RSD values lower than 17% at 0.020 $mgkg^{-1}$ spiking level for most pesticides. The proposed method was successfully applied analyzing pesticide residues in real olive and oilseed rape samples, detectable residues of OCP, OPP and carbamates pesticides were observed, but in all of the cases the contamination level were lower than the maximum residue levels (MRLs) set by the European Union (EU), Regulation (EC) N 396/2005, force since 1st September 2008.

Keywords: pesticide, GC/MS, QuEChERS, olive, oilseed rape

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O-8
STREAMLINED METHOD DEVELOPMENT FOR TRACE-LEVEL ANALYSIS OF ORGANOCHLORINE AND ORGANOPHOSPHORUS RESIDUES IN USP BOTANICAL ESSENTIAL OILS; ACHIEVING DETECTION-LIMITS WELL BELOW USP REQUIREMENTS

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High-performance liquid chromatography (HPLC) and Gas Chromatography (GC) coupled with tandem mass spectrometry (MS/MS) are routinely employed as the premiere analytical techniques for the selective quantitation of pesticide residues in essential oils. A number of studies have reported the derogatory effects caused by minimal sample preparations, which lead to co-extraction of endogenous/exogenous substances from the matrix. These matrix-interferences affect analyte ionization, sensitivity, and reproducibility; ultimately leading to inconsistent and unacceptable quantitative behaviors. We investigated the use of a “novel” GC Triple-Quadrupole Mass Spectrometer (GC/MS/MS) that allowed for minimal sample preparation; 2 µL injections of 1:100 dilutions of eucalyptus, lavender, peppermint, clove, tea tree and pine oils in hexane. Utilizing a new detection scheme “Compound-Based Scanning” our preliminary data indicate achievement of LOQ’s ≤20 µg/L for over 50-residues from the USP Botanical Pesticide Origin List. This approach demonstrates significant improvements over current techniques for the quantitative analysis of residues in essential oils.

Keywords: GC/MS/MS, Residues, Essential Oils, Pesticides

O-9
QUANTITATION AND CONFIRMATION OF PESTICIDES IN COMPLEX MATRICES USING TRIPLE QUADRUPOLE LC–MS/MS WITH TRIGGERED MRM (TMRM)

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All food commodities which are designated for human consumption need to be analyzed for pesticide residues. Maximum residue limits for more than 170,000 matrix-pesticide combinations are laid down in commission regulation (EC) 396/2005. The analysis of all pesticides typically requires a combination of GC–MS and LC–MS techniques. However, modern LC–MS/MS instruments are capable of analyzing an increasing number of pesticides with a single technique which is very interesting especially for control laboratories. However, matrix effects in electrospray ionization and the reporting of false positives are major concerns when using LC–MS/MS for quantitation in complex matrices. In this work we show the application of the new triggered MRM (tMRM) acquisition mode for the analysis of pesticides in complex matrices. When the primary transition of a pesticide is detected above a certain threshold additional confirmatory ions are acquired automatically for compound confirmation. The acquired product ion spectrum can be matched against a compound library resulting a library match score. An UHPLC-MS/MS method has been set up for the analysis of more than hundred pesticides with two primary transitions and up to 8 confirmatory ions per compound. Food samples have been extracted using the QuEChERS extraction protocol and commercially available extraction kits. A pesticide mixture containing all target compounds has been spiked to the final extracts at 5 different levels for method validation. Confirmation of pesticides has been done by comparison of qualifier/quantifier ratios, by comparison of the tMRM in-spectrum ratios and by library match scores. Several pesticide-matrix combinations are shown for which natural compounds exist which show high analogies to certain pesticides. This could result in false positive reportings by using a single qualifier/quantifier ratio. When using tMRM with library matching against a library with product ion spectra of more than 300 pesticides no false positives have been observed. In spectrum-ratios of the tMRM product ion spectra have been very stable across different concentration levels even in complex matrices like citron, tea, or black pepper. Excellent precision data for replicate injections show that quantitation has not been compromised when triggering additional transitions for confirmation allowing for quantitation and confirmation of pesticides in a single analytical run.

Keywords: Pesticide residues, Triple Quadrupole MS, Triggered MRM, Library matching, Confirmation

O-10
A COMPOUND-BASED SCREENING APPROACH TO SIMPLIFY METHOD DEVELOPMENT AND DATA PROCESSING FOR MULTI–RESIDUE ANALYSIS BY GC–MS/MS

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Introduction: Tandem mass spectrometry coupled to chromatography, such as GC-MS/MS, operated in the multiple reaction monitoring (MRM) mode, has become the method of choice for targeted screening of multi-residues analysis in complex food matrix samples due to its high specificity and capability of monitoring many product ions of a large number of compounds. Established regulations require to measure multiple MRMs for each target analyte, i.e., quantitation ion (one) and qualified ion(s), thus making it common to run several hundreds or even thousands MRMs in one method. MRM method development, in terms of setting up MRM acquisition table and processing results, is complicated and time consuming. In this study, a new compound-based screening (CBS) approach is introduced and demonstrated for analysis of 49 pesticides in MRM mode in pumpkin extraction on a Bruker Scion TQ GC-MS/MS system. CBS based algorithm significantly simplifies MRM method development. The results generated on Scion TQ show good reproducibility, robustness and linearity.
Experimental: All experiments were performed on Bruker's Scion TQ triple quadrupole mass spectrometer coupled to a Bruker 451 GC and CP 8400 Autosampler using the following conditions: Injection: Splitless, 2 µl, @ 260°C Column: BR-5ms 15 m × 250 µm id., film thickness 0.25 µm Oven Temp: 70°C (1 min) to 290°C (4 min) at 12°C/min EI source Temp: 260°C Transfer line Temp: 280°C Emission Current: 80 µA Q2 CID Gas Pressure: 4.5 psi A mixed pesticide standard of 49 pesticides was spiked into the pumpkin extract to make the final concentration of 1 ppb. Calibration using the mixed pesticide standard in 1:1 hexane : acetone from 1 to 100 ppb was performed before and after the injection of pumpkin matrix samples.
Results and Discussion: With the new CBS approach, a compound list with MRM transitions grouped under residues can be easily built by importing compounds from the default factory library or user libraries. The CBS based MRM method also automatically links data acquisition and processing together, which eliminates the need for a separate data processing method and simplifies the data processing. Moreover, this new approach implements an algorithm to optimize the dwell time for each compound without the use of chromatographic segments. In the current study, the CBS based GC-MS/MS method has shown excellent linearity from 1.0 to 100 ppb with r^2>0.995 for all pesticides before and after injections of matrix samples. The instrument robustness was demonstrated by 12 consecutive injections of 1 ppb spikes with RSD mostly between 4–8%. This result demonstrates good instrument and software performance of Scion TQ for fast analysis of multi-residue pesticide samples.

Keywords: GC–MS/MS, Triple Quadrupole, Compound based Screening, MRM

O-11
RAPID ANALYSIS OF PESTICIDES IN CITRUS OILS USING GC–MS/MS WITH PTV BACKFLUSH

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The analysis of citrus oil for pesticide contamination carries with it some very specific challenges. These challenges are a result of the fact that during production of these oils there is a large amount of highly concentrated matrix produced that causes difficultly for pesticide analysis. This highly complex matrix is known for its high background during GC-MS analysis and especially for high boiling point components. Complicated methods have been developed to deal with this in the past. Many of these include long sample preparation or significant effort on instrumentation to deal with any contamination. Detailed here is the analytical methodology for rapid multi-residue determination using timed selected reaction monitoring (timed-SRM) on a high performance GC–MS/MS system (TSQ Quantum XLS). Using timed SRM, the system automatically optimized MS duty cycle to ensure maximum sensitivity was reached and this allowed a simple sample dilution approach for this analysis. This, in addition to using backflushing on the GC system led to a sensitive and robust simultaneous analysis of more than 40 pesticides in citrus oil. The extracted standards showed better than 0.995 r^2 values for the correlation coefficient. Six matrix samples were spiked at 25 ppb level. These samples were analyzed repeatedly and the areas were used to assess the stability of the final method, including backflush. The relative standard deviations for the areas ranged from 0.69% for α-lindane to 3.68% for iprodione.

Keywords: pesticides, GC–MS/MS, citrus oil, SRM

O-12
PESTICIDE RESIDUE ANALYSIS IN VINE LEAVES – OFFICIAL CONTROL OF ORGANIC VITICULTURE IN THE CZECH REPUBLIC

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Central Institute for Supervising and Testing in Agriculture (UKZUZ) is responsible for official controls in various fields of agriculture production including viticulture in accordance with the Law No 147/2002 Coll. Organic farming currently represents 11 % of the total agriculture production in the Czech Republic. Basic requirements with regard to production, labelling and control of organic products are laid down in Regulation (EC) No 834/2007. In 2011 UKZUZ launched a programme of official controls of organic viticulture. Plant protection against fungal pests is crucial to ensure wine production. List of pesticides authorized for application on vine in the Czech Republic includes 43 active substances from which 31 pesticides were selected for initial testing. 20 samples of vine leaves from various organic vineyards, 3 negative and 6 positive control samples were collected and analyzed in July and August 2011. Sample preparation procedure for pesticide analysis based on the citrate-buffered QuEChERS (EN 15662:2008) has been accredited in the UKZUZ laboratory. Analysis of captan, folpet and iprodione was carried out using PTV-GC-MS/MS (3800GC – MS 1200, Varian) and a group of other 28 pesticides was determined using UPLC-MS/MS (Acquity UPLC–TQ MS Xevo, Waters). The method was validated using negative control samples of vine leaves spiked at two different levels (0.01 and 0.10 ppm) and has fulfilled performance criteria given by the SANCO/10684/2009 document. Levels of pesticide residues in 19/20 organic samples of vine leaves ranged from 0.011 to 9.76 ppm. The reporting limit of 0.01 ppm was exceeded for at least 2 or more pesticides in 11 organic samples. Folpet was the most frequently found and abundant pesticide (19/20 samples). Besides folpet residues of other 13 pesticides (azoxystrobin, dimethomorph, dinocap, fluopicolide, iprovalicarb, mandipropamid, metrafenone, proquinazid, pyrimethanil, quinoxyfen, spiroxamine, tebuconazole, triadimenol) were randomly found in those 11 samples. Simultaneously, positive controls were analyzed to get information about pesticide residue levels in vine leaves collected from conventionally treated vineyard. The levels in positive controls ranged up to 121 ppm and the most abundant pesticide was folpet. The initial findings pointed out the occurrence of pesticide residues in organic vineyards and confirmed the importance of establishing of official control system on organic viticulture. Upcoming study shall be focused on drawing up proper sampling methodology and suitable strategy for data assessment. This would allow Control Authority to make appropriate decisions whether found pesticides were unintentionally introduced to organic vineyard or they were misused in organic viticulture.

Keywords: Pesticides, vine, organic, viticulture, QuEChERS

O-13
GLYPHOSATE ANALYSIS – OLD FACTS AND NEW FINDINGS

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Glyphosate is a broad spectrum herbicide of global usage in agriculture, horticulture and silviculture applications and therefore among the largest-selling single crop-protection chemical products on the market today. However, this high load of glyphosate usage worldwide is in contradiction with the limited data on glyphosate residue levels reported in food and feed so far (e.g. Pesticides Online [1], RASFF portal [2] …). This can probably still be traced back in a large part to the lack of manageable analytical methods suitable for routine analysis. The methods published so far reveal several deficits, e.g. in terms of extraction conditions, tedious clean-up steps, time-consuming derivatization reactions as well as inconsistency in chromatography and detection. Nevertheless, since 2010 the monitoring of cereals for the widely used herbicide glyphosate is obligatory to all EU-member states, laid down in COMMISSION REGULATION (EC) No 1213/2008 and amending regulations. Hence, there is definitively a need for a reliable and easy-to-handle analytical method for routine monitoring of this compound. The main purpose of this work was to establish a rugged and easy-to-handle method for glyphosate analysis suitable for many labs dealing with food monitoring issues as it requires no tedious or expensive clean-up steps and can easily be performed with the same equipment as for LC-based multi-residue methods already established in the labs. The method consists of an aqueous extraction with liquid-liquid partition followed by protein precipitation and derivatization, finally performing UPLC-ESI-MS/MS determination using an Isotope dilution MS (IDMS) approach. The analytical performance of the method was evaluated according to SANCO/10684/2009 [3] criteria and demonstrated to be rugged, cost-effective and suitable for monitoring purposes as well as legislative enforcements within the European Union. Although the coordinated monitoring program so far limits glyphosate analysis to cereals this method has been proven to work also for relevant vegetable samples and more critical matrices with high fat content, e.g. soybeans, lentils and linseed. Finally, the suitability of the method has been tested by analysing monitoring samples from 2011, resulting in several positive findings for glyphosate.

[1] Pesticide ONLINE database (CVUA Stuttgart); www.pesticides-online.com
[2] RASFF portal; https://webgate.ec.europa.eu/rasff-window/portal/
[3] Document SANCO/10684/2009 Method validation and quality control procedures for pesticide residue analysis in food and feed. http://www.crl-pesticides.eu/library/docs/srm/AqcGuidance.pdf

Keywords: glyphosate analysis, derivatization, IDMS, UPLC-ESI-MS/MS

O-14
DEVELOPMENT OF CLEAN-UP MODULES FOR THE PURIFICATION OF COMPLEX MATRICES SUCH AS HOP AND HOP EXTRACTS TO DETERMINE PESTICIDES AT LOW LEVELS

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Currently, three multi-residue methods for the determination of pesticide residues in food are well known and in use worldwide: DFG S19 [1], Klein & Alder [2] und QuEChERS [3; 4]. Validation studies were executed and published as part of the official methods but mainly for foods with the highest consumption level and, therefore, the highest relevance such as fruits, vegetables, seeds, and cereals. However, results for complex matrices such as herbs, tea, and even hop are missing. For these materials with their high content of polyphenols, acidic compounds, and natural resin additional clean-up modules are necessary to obtain clean extracts for the measurement using GC/MS and LC/MS/MS with acceptable matrix interference. In this study, the purification power of several adsorbents for the analysis of pesticides in hop were investigated: aminopropyl (NH2), primary and secondary amines (PSA), Carbon/NH2, silica gel, cyanopropyl (CN), polyvinylpolypyrrolidone (PVPP), XAD, C18, and diatomic earth (ChemElut). Hop extracts were produced according to the three methods Klein & Alder [2] with methanol/water, DFG S19 using acetone/water, and the QuEChERS method using acetonitrile/water [3; 4]. As a fourth option, the extraction using ethyl acetate was also used as the first step. Afterwards, these extracts were purified using the listed adsorbents. To obtain quantitative information about the purification power of the adsorbents, aliquots of the purified extracts were dried and weighted. Additionally, the extracts were measured using HPLC with an analytical gel permeation column and diode array detection covering the wave length from UV to VIS. PSA, NH2, and Carbon/NH2 supplied the best extracts. Finally, the behavior of pesticides concerning these materials was checked. The best results for compounds analyzed by LC/MS/MS were achieved using a combination of ChemElut purification according to Klein & Alder with a post-purification step using PSA. The final method was applied to dried hop, hop extracts, and hop pellets to determine the recovery rates and the LOQs for up to 236 pesticides measured by LC/MS/MS and 171 pesticides measured by GC/MS. All the pesticides relevant and permitted for hop in Germany were part of the scope. The successful participation in an interlaboratory test organized by the German AHA (workgroup for hop analysis) proves that the clean-up method for hop is reliable.

[1] Amtliche Sammlung von Untersuchungsverfahren nach §64 LFGB L00.00-34
[2] Amtliche Sammlung von Untersuchungsverfahren nach §64 LFGB L00.00-113
[3] Amtliche Sammlung von Untersuchungsverfahren nach §64 LFGB L00.00-115
[4] Anastassiades, M.; Lehotary, S. J.; Stajnbaher, D.; Schenk, F.; J OAC Int. 86 (2003) 412-431

Keywords: pesticides, hop analysis, clean-up, SPE

O-15
DEVELOPMENT OF SPECIFIC LC–ESI–MS/MS METHODS FOR THE DETERMINATION OF SPINOSAD, THIACLOPRID AND PYRIDALYL AND STUDY OF THE DEGRADATION RATES AND THE PRE HARVEST INTERVALS IN SPRING ONIONS

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The ultimate aim of this study was the estimation of the degradation rates, the Pre Harvest Interval (PHI) and the t1/2 of spinosyn A and D, thiacloprid and pyridalyl in spring onions under the Egyptian field conditions. All the 3 pesticides were introduced more recently in the market. Spinosad has already been registered against a wide range of pests, and has been used with success in several crops. Nevertheless, thiacloprid and pyridalyl are new generation pesticides, thus not many studies were found in the literature. For this purpose a LC-ESI-MS/MS method was developed and validated to determine spinosyn A and D, thiacloprid and pyridalyl residues in spring onions. A specific LC-ESI-MS/MS method for the determination of pyridalyl was additionally developed, providing higher sensitivity and shorted analysis time, as there was no specific method in the literature, to our knowledge. QuEChERS procedure was used in the sample preparation. The chromatographic parameters (mobile phase, flow rate and gradient program) and the ionization parameters of the ESI were optimized for each pesticide to obtain maximum sensitivity. Linear dynamic range, limits of detection (LOD), limit of quantification (LOQ), precision, recovery and matrix effects were estimated. The method LODs were 0.02 μg/kg, for both spinosyn A and D, 0.035 μg/kg for thiacloprid and 0.05 μg/kg for pyridalyl. All the investigated pesticides showed high degradation rates. For spinosad, which is calculated as the sum of spinosyn A and D, the $t_{1/2}$ value was 1.2 days. For thiacloprid, it reached 2.2 days and for pyridalyl 4.4 days. Furthermore, the calculated PHI values, according to the Maximum Residues Limits (MRL) set by EU, were 1 day for spinosad, 12 days for thicaloprid and 40 days for pyridalyl.

Keywords: LC-ESI-MS/MS, pesticide residues, spring onions, degradation rate, PHI

O-16
QUALITY ASSURANCE TOOLS FOR PESTICIDE ANALYSIS – AN AMBITIOUS TASK

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The large number of existing pesticides makes it complicated for commercial and official control laboratories to comply with the quality specifications nowadays demanded for their analysis in food and feedstuff. The scope of the analytical methods developed ideally requires the coverage of not only the variety of active substances, but their combination with the matrices of interest, which in practice means an enormous task when it comes to proper validation of methods. Although justified from the legal and scientific point of view, the topic has been a matter of lively discussion during the recent years. Meanwhile guidance documents were elaborated[1], and recently revised[2], as to set the requirements for the methods to be used for the pre- and post-registration and control of pesticides by the competent authorities in the Member States of the European Union. In particular, for assessing the method performance in terms of accuracy, the guides propose validation experiments based on standard addition to the matrix for estimation of the recoveries. However, the use of reference materials as preferable option is already indicated1, despite the current lack of certified reference materials (CRMs) containing the relevant analytes at appropriate levels. It seems to be obvious for a producer or reference materials that covering all possible pesticide residue-matrix combinations is not feasible. The formal proposal for the classification of food and feedstuff of plant origin commodities into four groups2 including dry (high protein/high starch content), high water content, high oil content and high acid content matrices is reducing the complexity of the task to some extent in that particular area. Method validation is then necessary for representative commodities of these groups. With a similar view, the Institute for Reference Materials and Measurements initiated a set of parallel activities to evaluate the feasibility of producing reference materials for the analysis of pesticides in food, focusing on plant matrices for the time being. The two main lines covered are, on one hand, the development of highly accurate analytical methods for the determination of selected pesticide residues with the objective to obtain analytical methods for certifying pesticide values in the chosen commodity at a suitable level of uncertainty. On the other hand, practical aspects related to the physical processing of matrices from plant origin are investigated. For all the steps of the running venture, the contribution from the EURL on pesticide residues in fruits and vegetables has been crucial. These activities and the status of the project will be discussed during the presentation.

[1] SANCO/10684/2009: Method validation and quality control procedures for pesticide residues analysis in food and feed.
[2] SANCO/825/00 rev. 8.1: Guidance document on pesticide residue analytical methods

Keywords: certified reference materials, pesticide residues, food, method validation

O-17
QUALITATIVE ASPECTS OF PESTICIDES RESIDUE ANALYSIS IN VEGETABLES AND FRUITS USING LC WITH SINGLE STAGE HIGH RESOLUTION MASS SPECTROMETRY

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LC with full scan high resolution mass spectrometry is a promising option for simultaneous detection of very high numbers of pesticides in one run [1]. For application in routine practice, a high selectivity is of key importance. The selectivity of the overall analysis is determined by the measurement (resolving power/mass accuracy [2]) and the detection requirements and criteria that are set during data evaluation. In this work the selectivity and other qualitative aspects were investigated for detection of pesticides in vegetables and fruits using LC with full scan high resolution MS (Exactive Orbitrap). The measurements were performed in ESI+ mode using alternating scan events without fragmentation and with fragmentation (HCD cell, no precursor-ion selection, fixed collision energy), at a resolving power of 50,000 FWHM. Twenty-one different organic vegetables and fruits were analyzed as such and spiked with 132 pesticides at 0.01, 0.05 and 0.20 mg/kg. In the initial data processing, the blank samples were checked for signals occurring for the exact mass for three possible adducts ($[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$), in a retention time window of the expected retention time ± 30 s, for each of the 132 pesticides. The number of false detects obtained based on the exact mass of the adducts and the applied retention time restriction was considered too high for routine application. Various approaches to reduce the number of false detects were considered, including use of abundance of adducts, isotopes, fragment ions and response thresholds. The effectiveness regarding elimination of false detects and the impact on occurrence of false negatives in spiked samples will be discussed. In addition, the results will be evaluated against the identification criteria as currently set in SANCO/10684/2009.

[1] M. Mezcua, O. Malato, J.F. García-Reyes, A. Molina-Díaz,A.R. Fernández-Alba, Anal. Chem. 2009, 81, 913–929.
[2] M. Kellmann, H. Muenster, P. Zomer, H.G.J. Mol, J. Am. Soc. Mass Spectrom. 20 (2009) 1464

Keywords: high resolution MS, qualitative analysis, automated detection, identification, screening

O-18
FEASIBILITY OF FLOW INJECTION – MS/MS FOR RAPID DETERMINATION OF PESTICIDES REQUIRING SINGLE RESIDUE METHODS

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Recently the potential of flow injection – tandem mass spectrometry (i.e. without chromatography) for the direct fast analysis of extracts was investigated for sulfonylurea and carbamates [1,2]. The results reported were promising owing to the high sensitivity of the current MS/MS instruments combined with more freedom in optimizing eluent composition towards maximum MS sensitivity. This allowed strong dilution of the extracts thereby reducing matrix-induced ion suppression. Inspired by the results published by Nanita et al, we investigated the feasibility of this approach for rapid detection of pesticides that can be analyzed by LC-MS/MS, but that are not amenable to LC-MS/MS multi-methods because they require specific chromatographic conditions [3]. In general, these pesticides are highly polar small molecular weight compounds which are analyzed using ion exchange or special multi-functionalized stationary phases and/or special eluent additives. The robustness of such chromatography can be rather poor. Retention times are often affected by matrix and columns may deteriorate rapidly. Elimination of chromatography would eliminate these problems. Here we present preliminary results of the rapid detection of typical SRM-pesticides including glyphosate, glufosinate, fosethyl, ethephon, maleic hydrazide, chlormequat, mepiquat and others, in vegetable, fruit and cereal commodities.

[1] Nanita SC, Pentz AM, Bramble FQ, Anal. Chem. 2009, 81, 3134–3142
[2] Nanita SC, Stry JJ, Pentz AM, McClory JP, May JH, J. Agric. Food Chem. 2011, 59, 7557–7568
[3] Anastassiades M; Kolberg DI, Mack D, Sigalova I, Roux D, Fügel, D, http://www.crl-pesticides.eu/library/docs/srm/meth_PolarPesticides_CrlSrm.pdf

Keywords: flow injection-MS, polar pesticides, rapid methods, screening

O-19
MULTI-RESIDUE DETERMINATION OF PESTICIDES IN BABY FOODS OF ANIMAL ORIGIN BY TRIPLE QUADRUPOLE GC-MS/MS TECHNIQUE

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Products of animal origin may have been contaminated by pesticides through the food chain and by the use of pesticides as veterinary drugs. Therefore, animal origin baby foods could also be potentially contaminated. In order to protect children from the pesticide residues exposure through the diet, Maximum Residues Levels (MRLs) at general value of 0.01 mg/Kg are established by the Commission Directives 2006/125/EC and 2006/141/EC for infant foods. In the framework of the Italian National Reference Laboratory (NRL) for Pesticide Residues in Food of Animal Origin and Commodities with High Fat Content, our laboratory has developed a multiresidue method for the determination of pesticide residues in baby foods of animal origin. The main pesticides considered were those that accumulate in the fat as Organochlorine (OC), Organophosphorus (OP) and Pyrethroid (PYR) compounds. Meat, fish and cheese based baby food samples have been extracted by acetonitrile (ACN) at room temperature, filtered by Buckner under a vacuum system and cleaned by C18 cartridge and ACN; the compounds were determined by triple Quadrupole GC-MS/MS technique. The combination of the new generation of Triple-Quadrupole Mass Spectrometry system with Gas Chromatography, allows to detect a large number of pesticide in one analysis. By choosing transitions that are characteristic of the pesticides of interest, chemical noise is separated from signal, providing very high sensitivity and selectivity, even in very dirty matrices, as baby food of animal origin. The use of Programmed Temperature Vaporizer (PTV) injector increased the overall analytical sensitivity and allowed the analysis of pesticide residues even at the concentration less than 0.01 mg/Kg, as requested for some pesticides listed in the Annex VI and VII of Commission Directives 2006/125/EC for baby foods. The determination was performed by matrix matched standards in order to reduce the influence of matrix effects on the quantification. The method was tested for a total of 64 pesticides and the validation was performed according to the Document SANCO/10684/2009. Recoveries were studied at two levels: at the Limits of Quantitation (LOQs) in the range of 0.003–0.008 mg/Kg and at the high level in the range 0.028–0.1 mg/Kg. The results obtained are within the criteria of acceptability of 70–120% with RSD ≤20%. Linearity and LOQs were determined for all pesticides considered.

Keywords: Baby food, pesticide, residues, Gas Chromatography-Mass Spectrometry Triple Quadrupole Detector (GC-MS/MS-QQQ).

O-20
USING HIGH SENSITIVE BUT UNSELECTIVE MASS TRANSITIONS FOR THE RESIDUE ANALYSIS WITH QUADRUPOLE–TIME-OF-FLIGHT

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The elimination of carbon dioxide (CO_2) and water (H_2O) from organic molecules during the fragmentation process commonly requires low energy, and, therefore, the resulting fragments are received in a high yield. The drawback of these mass transitions is that the curves obtained with a conventional triple quadrupole system are often characterized by a high chemical noise, and the analyte signal is partly overlaid by interferences. Therefore, a quadrupole-time-of-flight-system (QToF) was tested, which combines a higher resolution with the high sensitivity of a triple quadrupole system.
The potential of these unselective mass transitions using a QToF system was exemplarily tested for two metabolites of plant protection compounds. One shows an elimination of H_2O, and the other one an elimination of CO_2.
The applicability of this approach was tested for these two molecules for a broad range of matrices from different crop groups. Control material was spiked with defined amounts of these two test compounds to obtain residue levels of 0.01 mg/kg and 0.10 mg/kg parent equivalent. Recoveries, standard deviations, and signal-to-noise ratios were used to determine the capability of these unselective mass transitions compared to more specific ones.
It could be shown that with a QToF system it is possible to use these unselective mass transitions for the quantitative and qualitative analysis of the two tested analytes at a residue level of 0.01 mg/kg and 0.10 mg/kg. Compared to more specific MRMs, lower limits of quantitation could be received due to the higher yield of the resulting fragments.

Keywords: Q-TOF, unselective mass transitions

O-21
ANALYSIS OF BITOXYBACILLIN AND THE BETA-EXOTOXIN THURINGIENSIN IN GREENHOUSE VEGETABLES

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The use of natural compounds as plant protection products (PPPs) gains much attention by the general public. An increase in selling figures of natural (PPPs) combined with a decrease of the chemical PPPs is noticeable. However natural products may also have toxic side-effects and must comply to European guidelines and regulations. Besides fully natural products also bacterial products are available as PPPs. One of these products is Bitoxybacillin, a bacterial product based on Bacillus Thuringiensis var Thuringiensis. Bacillus Thuringiensis var Thuringiensis produces the beta-exotoxin thuringiensin, a toxic compound inhibiting DNA-dependent RNA polymerases. Commercial products should be clearly free of thuringiensin as regarded by WHO (1). Because of the presence of thuringiensin the use of Bitoxybacillin is not allowed within the EU. As Bitoxybacillin is active against white-fly and thrips its possible use focusses on growers of greenhouse vegetables like cucumber, tomato, and paprika. Additional also growers of ornamentals, esp. roses, are possible users of Bitoxybacillin. However no official method is currently available for characterisation of the bacterial product Bitoxybacillin, as is the case for a quantitative, confirmative method for the residue analysis of the beta-exotoxin thuringiensin. Within this presentation the characterisation of Bitoxybacillin with biochemical and physical chemical techniques is described. Furthermore the development of a quantitative, confirmative residue analytical method based on LC-MSMS of the beta-exotoxin thuringiensin is described. Validation results will be presented including monitoring data for greenhouse vegetables and ornamentals in The Netherlands (2010-2011).

[1] Bacillus thuringiensis Environmental Health Criteria Series 217 ISBN 92 4 157217 5 World Health Organization 1999, Geneva, Switserland.

Keywords: Bacillus thuringiensis, beta-exotoxin, residue-analysis, greenhouse vegetables

O-22
ADVANCED LC–MS/MS TOOLS TO SCREEN FOR TARGETED AND NON-TARGETED CONTAMINANTS IN FOOD SAMPLES

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. Compound identification is typically performed by monitoring of two MRM transitions and calculating the area ratio of quantifier and qualifier ion. The potential risk of false positive results can be further minimized by the acquisition of full scan MS/MS for each compound detected. MS/MS spectra contain the complete molecular fingerprint of a molecule and thus providing an added degree of confidence for compound identification. However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run. Here the AB SCIEX TripleTOF™ 5600 system was used to acquire highly sensitive MS and information dependent MS/MS spectra throughout the LC run. Chromatograms were automatically explored and information on retention time, mass accuracy, isotopic pattern, and MS/MS library searching was used to quantify and identify target analytes with highest confidence. The LC-MS/MS data was further used to retrospectively search for non-targeted and unexpected compounds. A variety of food samples were analyzed for pesticides, veterinary drugs, mycotoxins and other chemical residues. A fast and generic procedure was used to extract contaminants of various classes. Extracts were subsequently analyzed by LC-MS/MS using a polar embedded C18 phase and different LC-MS/MS techniques. Different analytical setups will be presented and discussed with respect to targeted and non-targeted residue screening.

Keywords: LC-MS/MS, pesticides, veterinary drugs, accurate mass, high resolution

O-23
QUANTIFICATION OF ENDOCRINE DISRUPTORS AND PESTICIDES IN WATER USING WEIGHTED LINEAR REGRESSION SCHEMES

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The presence of pesticides in environmental matrices can have serious consequences such as financial, human health and environmental, being aquatic environment is particularly susceptible to this type of pollution. Unequivocal analytical data require a specific set of validation criteria and verification of method performance. The importance of validation, at least of routine analytical methods, has been hardly overestimated. In recent years this is particularly true in the context of quality management and accreditation, which has become subject of increased importance in analytical chemistry. Therefore, the present trend towards standardization of practices between countries has been at the genesis of the current need of international acceptation of analytical results and accurate validation of methods, which become increasingly important for ensuring a common level of quality. The aim of the present work was the validation of a multi-residue methodology based on a solid phase extraction followed by gas chromatography– tandem mass spectrometry for trace analysis of 31 compounds in water matrices. Heteroscedasticity of results has been overtaken by a weighted least squares linear regression (WLSLR) model application. As part of the assay validation a WLSLR model was used to obtain a calibration scheme once the assumption of homoscedasticity was not met for analytical data. WLSLR is an efficient method that provides unbiased estimative for prediction, calibration and optimization when standard deviation of the data random errors are not constant across all levels of the explanatory variables. It works by incorporating extra nonnegative constants, or weights, associated with each data point, into the fitting criterion and the size of the weight indicates the precision of the information contained in the associated observation. The other analytical performance and characteristics of the method were also studied and validated. The unweighted model overestimates the concentrations in the lower range of the calibration curve, near the limit of quantification. The weighted model presents a best %RE distribution scatter, a lower sum of %RE and, finally, none of the studied compounds showed relative errors greater than the acceptable limits of 15% and 20% for the different calibration line standards and the first pattern, respectively. Although weighted least squares regression is more complex and laborious than ordinary linear regression, involving the use of additional statistical tests and mathematical operations, it should be performed in order to obtain more realistic results and lower limits of quantification.

Keywords: "Endocrine disruptors" "Pesticides" "Water" "Weighted linear regression schemes" "Validation"

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O-24
A NEW COMPLETE SOLUTION FOR AUTOMATED, COMPREHENSIVE ESI– (Q–)TOF FULL SCAN ACCURATE MASS SCREENING OF PESTICIDES IN FOOD WITH HIGH CONFIDENCE

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Fast and comprehensive full scan accurate mass screening for hundreds of pesticides simultaneously has meanwhile found its way into routine use taking advantage of the high number of possible targets and additionally allowing for unknown evaluation and retrospective analysis. The screening procedure relies on full scan accurate mass data and a target compound database, which basically only needs information about name (-> identifier in the result table) and sum formula (-> accurate mass information) of a target. However, the use of comprehensive databases containing hundreds or even thousands of sum formula/name entries only is anything else than advisable. In the case of pesticide screening in food low signal intensities in highly complex matrix samples have to be evaluated to achieve the required reporting levels, thus potentially leading to a meaningless high number of false positive results. Inclusion of additional information and knowledge therefore is essential to obtain reliable results. The screening solution presented here makes use of multiple levels of confirmation and result rating for maximum confidence in the results and is characterized by: – A screening platform consisting of a defined UHPLC and ESI-(Q-)-TOF system, a dedicated screening software (TargetAnalysis) and a high quality/information rich pesticide database. – The ESI-(Q-)-TOF system provides data with high isotopic fidelity and excellent mass accuracy over a wide dynamic range. – Methods and conditions are used giving robust and reliable system performance, allowing to work with known and stable retention times. - TargetAnalysis uses retention time, mass accuracy and isopic pattern information for a first level confirmation and result rating according to customizable settings. - Certainty of results is increased with additional evaluation of known adducts and fragment ions as qualifier ions, also enabling unambigous compound assignment. - A further level of confirmation to avoid false positive results is obtained by combination of full scan and ISCID (Internal Source Collision Induced Dissociation) data in the same analytical run. The presence of (up to three) qualifier ions is evaluated together with their intensity ratios. Random or matrix related results will not pass this test and are thus filtered out. Number and quality of detected qualifier ions add a fourth rating criterion. – Various sorting options in the TargetAnalysis result table (e.g. name, retention time, registry number) help for easy result grouping (fragments, metabolites) and reviewing. – In case of ambiguous results detailed result evaluation is possible via easy linking to the standard data evaluation software (DataAnalysis). – If calibration data for detected compounds is available a quantitative result can be displayed in TargetAnalysis as well. – A fully automated setup and workflow is possible. Examples for the workflow and system performance will be given.

Keywords: pesticides, multi-target screening, ESI-(Q)TOF, result confirmation

O-25
COMPREHENSIVE CONFIRMATION WORKFLOW FOR FULL SCAN ACCURATE MASS MULTI-TARGET SCREENING OF PESTICIDES IN FOOD GIVING RESULTS WITH MAXIMUM CONFIDENCE

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Introduction: Fast and comprehensive full scan accurate mass screening for hundreds of pesticides in food simultaneously has found its way into routine use. Additionally to the high number of targets the technique takes advantage of unknown evaluation and retrospective analysis. However, especially at low levels and in highly complex food extracts interferences between target and matrix compounds can occur, preventing an unambiguous identification based solely on mass accuracy and isotope pattern. Here the usefulness of additional information from full scan, unselective MS/MS data by broad band CID (bbCID) or selective, data-dependent MS/MS for confirmation of tentative findings is evaluated.
Methods: From a base of >1000 pesticides a set of ~60 is selected based on relevance in routine monitoring. Individual MS/MS-spectra for all compounds were acquired for building an accurate-mass spectral library. Seven solvent and matrix based dilution series (QuEChERS: cucumber, strawberry, wheat flour, leek, orange, ginger) are evaluated using an UHPLC system coupled to an ESI-QTOF. Data acquisition is performed in alternating full scan resp. bbCID and in data-dependent MS/MS mode using a scheduled precursor list (SPL) for selected pesticides. Automatic data evaluation is performed using dedicated target screening software. Mass accuracy and isotope pattern quality information is collected for all detected compounds as a measure for matrix interferences.
Results: Confidence in pesticide detection declines with decreasing compound concentration and increasing extract complexity due to the higher probability for interferences. Mass errors increase and isotopic pattern quality decreases, resulting in only tentative identifications. In extreme cases those interferences lead to elevated mass errors even at high pesticide concentrations, requiring additional result confirmation. Nevertheless, tentative results can be accepted as true positive findings even at low concentration levels, if additional compound peaks are detectable. Those additional peaks can be adducts and/or fragments of the compound observed in the full scan MS data, fragment ions observed in bbCID data in the same analysis file with correct relative intensity ratios, and/or MS/MS fragments observed in a second data-dependent MS/MS run which confirmed the compound identity by spectral library search. The ideal screening workflow therefore consists of one or max. two analytical runs: 1) run in alternating full scan/bbCID mode: compound detection (plus quantitation) based on retention time, accurate mass and isotope pattern. Confirmation by additional full scan or bbCID diagnostic ions (including relative intensity ratios for bbCID fragments). 2) Second run in data-dependent MS/MS mode with an adjusted SPL containing retention time and precursor mass information of residual tentative findings. Confirmation by MS/MS data search in an accurate mass spectral library.
Keywords: pesticides, multi-target screening, ESI-QTOF, result confirmation

O-26
MULTI-RESIDUE ANALYSIS STUDY OF 61 PESTICIDES BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–ELECTROSPRAY–TANDEM MASS SPECTROMETRY

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This research has been studied to apply Ultra performance liquid chromatography-electrospray ionization tandem mass spectrometry(UPLC-MS/MS) to multi-residue analysis of a total 61 pesticides. Monitored cone voltage (CV), collision energy (CE) and selected reaction monitoring (SRM) transition which show the most intense in each of pesticides and nine groups were divided for retention time (RT) established multi-residue analysis method. Five pesticides (Aldicarb, Butocarboxim, Etofenprox, Cycloprothrin, Oxamyl) produced sodium or ammonium Adducts ($[M+Na]^+$ or $[M+NH_4]^+$) known as an irreversible by-product. Lufenuron has been optimized under deprotonated $[M-H]^-$. In the UPLC–MS/MS method, most of pesticides were graphically separated except for Isoprocarb, 2,3,5-trimethacarb and 3,4,5-trimethacarb. According to repeat test carried out on each pesticides, the range of fluctuation in retention times and carrier solvent were narrow as relative standard deviation (RSD) in percentage. The instrumental LOQ, generally, was computed at about 1–30 ng/L. Result of representative samples that contaminated with pesticide of about 200 cases examined samples(vegetables and fruits) in the laboratory from August to September, 2009 that used UPLC–UV analysis was compared with UPLC–MS/MS. UPLC MS/MS method could find not only the pesticides detected under UPLC–UV analysis but also the pesticides that had not been found under UPLC–UV analysis. Therefore, these results recommend that the UPLC–MS/MS could be effective means as a screening method for the analysis of pesticides in agricultural products.

Keywords: Multi-residue analysis, UPLC–MS/MS, Pesticides, Adducts, UPLC-UV analysis

O-27
EVALUATION OF THE PERFORMANCE IMPROVEMENTS NEEDED IN AN ESI-QTOF-MS SYSTEM FOR QUALITATIVE AND QUANTITATIVE MULTI-TARGET PESTICIDE SCREENING IN FOOD

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Introduction: Fast and comprehensive full scan accurate mass screening becomes a valid alternative in food control in particular if hundreds of pesticides have to be proved in a short time frame. Additionally to the high number of targets the technique takes advantage of unknown evaluation and retrospective analysis. However, practical studies of the performance of current high mass accuracy mass spectrometers reveal certain limitations. Due to matrix background, unambiguous identification and correct quantitation is not always achievable at required trace levels. Besides of additional MS/MS-based result confirmation workflows, the general improvement of the instrument performance itself is of interest. This study evaluates the performance enhancement achieved using a redesigned QTOF which is improved with regard to requirements for food safety analysis.
Methods: A representative set of ~60 out of >1000 pesticides was chosen, with regard to their relevance for routine monitoring. Seven solvent and matrix based dilution series (QuEChERS: cucumber, strawberry, wheat flour, leek, orange, ginger) of the pesticide-mix were analyzed by an UHPLC–QTOF system. Data acquisition is performed in alternating full scan resp. bbCID mode using different acquisition rates up to 50H z. Automatic data evaluation is performed using dedicated target screening software. The basic instrument performance criteria mass accuracy, resolution, detection limits, linearity, dynamic range and reproducibility of the system are evaluated for the solvent based pesticide samples.
Results: The novel instrument provides enhanced performance especially with regard to mass accuracy (better than 1 ppm), detection limits (usually fg/µL range) and accessible mass band width, which allows for screening methods, covering a significantly wider scope of analytes in one method (m/z range <100 to >1000). Especially the performance for low (*m/z* <200) and very low *m/z* signals (*m/z* <100) is substantially improved. This is important for confirmation experiments in broad band CID (bbCID) mode, since often low mass fragments are significant for the unambiguous identification of compounds (like e.g. for distinguishing the coeluting pair aldicarb/butocarboxim) For most compounds calibration curves are linear up to ~4 orders of magnitude. Peak area reproducibility is in the range of a few percent, sometimes below 1%. The mass accuracy stability of the system enables the use of selective high resolution extracted ion chromatograms (hrEIC) with trace widths below 1mDa. We found that mass accuracy performance and isotope pattern quality are widely independent of the acquisition rate. Performance criteria for screening protocols given in the EU decision 2002/657/EC require a mass resolution of 20,000 for the assignment of identification points for LC–hrMS data. This requirement is met for the complete *m/z* range that was evaluated.

Keywords: pesticides, multi-target screening, ESI–QTOF

O-28
AUTOMATIC SCREENING AND IDENTIFICATION OF FOOD RESIDUES WITH HIGH CONFIDENCE BASED ON HIGH RESOLUTION AND ACCURATE MASS LC–MS/MS

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Liquid Chromatography coupled to Tandem Mass Spectrometry (LC–MS/MS) is a widely used analytical tool for the screening of food residues and contaminants. Triple quadrupole based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results, but are limited to targeted screening only. With an increasing demand for retrospective and non-targeted analyses high resolution and accurate mass instruments gain popularity. Information dependent acquisition using a TripleTOF™ 5600 system enables the acquisition of highly sensitive TOF-MS and TOF–MS/MS spectra simultaneously. The information saved into the high resolution MS can be used to screen for and quantify targeted analytes. MS/MS spectra can be searched against mass spectral libraries for compound identification with highest confidence. In addition, the information saved into each data file can be explored for non-targeted and unexpected compounds. The complexity of such data requires powerful data mining tools. The XIC Manager software can be used for targeted and non-targeted processing of high resolution MS and MS/MS data allowing for screening and identification with the highest confidence based on retention time, accurate mass molecular ion, isotopic pattern, and automatic MS/MS library searching. Here we present examples of LC–MS/MS screening and identification of pesticides in extracts of fruit and vegetable samples after simple QuEChERS extraction. Features of the XIC Manager software for targeted and non-targeted screening are highlighted.

Keywords: LC–MS/MS, pesticides, accurate mass, high resolution, software

O-29
DETERMINATION OF ACIDIC PESTICIDES: OPTIMISATION OF ALKALINE HYDROLYSIS USED FOR CONVERSION OF THEIR CONJUGATES (ESTERS) TO FREE ACIDS

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Nowadays, maximum levels for pesticide residues in food commodities are unified in the EU member state by the Regulation (EC) No 396/2005. Maximum residue limits (MRLs) are controlled not only within the frame of the EU coordinated programme but also by national monitoring. Taking into account that about 2–5% of samples exceed the EU MRLs [1], it is obvious that development of methods for the determination of pesticides is still needed, especially if novel active ingredients or metabolites are included in the pesticide definition. Acidic herbicides represent a group of problematic and notably polar analytes and it is rather difficult to determine their residues within the common multiresidue methods. The aim of our study was to develop a simple and reliable LC-MS method for the determination of 20 most common acidic herbicides. Three versions of the QuEChERS sample preparation method have been compared: original [2], citrate-buffering [3] and acetate-buffering [4]. Clean-up step using PSA during dispersive-SPE had to be excluded because free acids are removed by this sorbent. Therefore, acetonitrile extracts were analyzed directly without any clean-up step. Satisfactory recoveries meeting requirements of SANCO/10684/2009 document were achieved by original and citrate-buffering versions whereas acetate-buffering version provided lower recoveries, especially in case of food with higher pH. Residue definition of acidic herbicides involves not only the free form of these compounds, but also their esters or conjugates. For this reason the extraction step has to be modified to release those bounded compound, in this study, performed by alkaline hydrolysis. Initial approach was based on EURL (Reference Laboratories for Residues of Pesticides) procedure [5]: (i) 2.5 g of wheat, (ii) 10 ml of water, (iii) 200 µl NaOH, (iv) shaking within 30 minutes, (v) neutralization step and then (vi) original QuEChERS extraction followed. During the optimisation of alkaline hydrolysis the amount of added NaOH, time of hydrolysis and amount of added water in relation to the sample weight was assessed. Recovery of each experiment was evaluated from samples spiked with haloxyfop-2-ethoxyethyl and expressed as a recovery of haloxyfop acid. The recovery of initial approach did not reach 50%. The most important factor that influenced its value was the ratio between the sample weight and added water. In case of five time lower sample weight, the recovery increased to 92%, but it was accompanied with increased quantitation limit. Thus the sample weight was increased along with higher water addition. Finally, 2.5 g of wheat with 20 ml of water was the optimal ratio that allows satisfactory recovery and reliable control of low MRLs.

[1] EFSA Journal 2010; 8(6):1646
[2] M. Anastassiades: Journal of AOAC 2, 86 (2003)
[3] EN 15662:2008 (2008).
[4] S.J. Lehotay, J. AOAC Int. 90, 485 (2007).
[5] http://www.eurl-pesticides.eu/docs/public/home.asp?LabID=100&Lang=EN

Keywords: acidic herbicide, QuEChERS, alkaline hydrolysis

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O-30
OPTIMISATION OF GC/MS MULTIRESIDUE METHOD FOR DETERMINATION OF PESTICIDES IN FRUIT AND VEGETABLE

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Pesticides are chemical substances used to protect food crops against various pests. Under certain conditions their residues may enter food chain and pose a health risk for consumers. To avoid / minimise such negative effects MRLs (maximum residue limits) for various commodities are set for a wide range of active ingredients of pesticide preparations by Regulation (EC) No 396/20051. Multidetection methods capable to determine a large number of pesticides simultaneously—in a single run—are used for the monitoring of pesticide residues and/or MRL control. The determination of GC-amenable pesticides in food samples is traditionally performed by gas chromatography (GC) coupled to mass spectrometry (MS). The aim of this study was to optimise and validate a multiresidue method based on “QuEChERS” (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach that consists from (i) acetonitrile–water partition (transfer of analytes into an organic phase is supported by inorganic salts) and (ii) dispersive SPE clean-up of the crude extracts. For identification and quantification of target pesticides, GC–HRTOFMS and GC–MS/MS systems were used. Within our experiments, the amount and the type of sorbents in dispersive SPE were optimised for two common types of fruit and vegetable matrices such as apples and lettuce. For the purpose of validation, homogenised samples were spiked at two concentration levels – 0.1 and 0.01 mg/kg. The obtained recoveries were, in most cases, in the range 70–120% and RSDs were below 20%. The limits of quantification (LOQ) for most pesticides were below 0.01 mg/kg. Matrix effects were compensated by the use of matrix-matched standards for each matrix. The obtained performance characteristics were compared with those of “classic” method employing ethyl acetate for the extraction and gel permeation chromatography for extract purification.

[1] http://ec.europa.eu/sanco_pesticides/public/index.cfm (20th April 2011)
[2] Method validation and quality control procedures for pesticide residues analysis in food and feed. Document SANCO/10684/2009
[3] Lehotay SJ, Maštovská K, Lightfield AR (2005) J AOAC Int 88:615–629

Keywords: pesticides, QuEChERS,

Acknowledgement: Financial Support from Specific University Research (MSMT No. 21/2011)

O-31
AUSTRALIAN GRAINS RESIDUE MONITORING PROGRAM – SAMPLE COLLECTION AND ANALYTICAL TECHNIQUES

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To preserve Australia's status as an exporter of high quality grain, primary producers rely on pesticides to protect their crops from pests and diseases. The Australian Pesticides and Veterinary Medicines Authority registers crop protection products where use in accordance with good agricultural practice (GAP) is unlikely to prejudice trade. GAP is confirmed through Australian Government National Residue Survey (NRS) residue monitoring programs which in turn support market access. Last year, 20,000 grain, meat and horticultural commodity samples were collected for analyses. Each year, 4,000 grain samples are collected, in accordance with formal NRS sampling procedures, with most from the bulk export program. Samples are also collected from container export, maltsters, and oat, oilseed and chickpea processors. The Grains Program provides an independent verification of the residue status of Australian grain with residue testing results reported against both Australian and international MRLs. Grain marketers receive NRS residue testing reports for each grain consignment. Each grain sample is subjected to a fast and efficient multi-residue screen (MRS), covering insecticides, fungicides and herbicides, which has been developed by the contract laboratory for routine screening and quantification. The reliability of the Australian analyses must be assured. The NRS laboratory performance evaluation system has been developed to ensure requisite standards are met, using a range of proficiency tests and other techniques in the selection of laboratories. NRS has been accredited against the relevant international standard (ILAC G:13 2007 as a proficiency test provider since July 2005. Formal laboratory PT takes place regularly to ensure the analytical performance of all contract laboratories continues to meet NRS requirements. Other performance evaluation processes used include an intra-laboratory check sample program; assessment of analytical methods; on-site audits and use of blind samples. For the MRS, the laboratory receives a 1 kg of grain sample and a 20g subsample is taken for acetone extraction by sonication. The sample extract is then split and further processed for triple-quad LC/MS and GC/MS analysis. Confirmation techniques employed are consistent with the EC Decision 2002/657/EC with at least 3 identification points (minimum 1 precursor ion and 2 transactional ions) plus ion ratio to be met for LC/MS or GC/MS detections. If this fails, a secondary analytical column of different polarity coupled with a different detector must be used. If a chemical residue is detected at levels equal to or exceeding the MRL, re-analysis must be performed on a separate portion of the original. Additional screens for herbicides, rodenticides and mycotoxins have been developed cooperatively between NRS and the contract laboratory.

Keywords: pesticide, residue, Australia, grain, analytical

O-32
COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GC×GC) COUPLED TO A FAST QUADRUPOLE MASS SPECTROMETER FOR THE RELIABLE QUANTIFICATION OF PESTICIDES IN WATER

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Comprehensive two-dimensional gas chromatography (GC×GC) is a novel approach for the analyses of complex samples. The bidimensional approach is based on the connection of two columns, with independent selectivities. The heart of the system is the interface, called modulator, which has the function is to cut, re-concentrate and launch chromatography bands from a primary conventional column, onto a short micro-bore column, generating very narrow second dimension peaks. Therefore, GC×GC requires fast detector acquisition rates to provide sufficient data density for accurate definition of the narrow 2D peaks, which are often reported to be less than about 100 ms wide. In the present work the performance of a novel rapid-scanning (20 000 amu/s) quadrupole mass spectrometer (qMS) has been evaluated in the comprehensive 2D gas chromatography analysis of pesticides contained in water. Analytes extraction was performed by using direct solid-phase microextraction (SPME). The qMS performance was evaluated using a 50-440 *m/z* mass range and a 33 Hz scan frequency, considering: a) number of data points per peak; b) mass spectral quality; c) extent of peak skewing; d) consistency of retention times. External calibration curves were built for 28 pesticides, limit of detection and quantification, precision, accuracy and intra-day precision were evaluated as well.

Keywords: GC×GC, quadrupole mass spectrometer, solid-phase microextraction, pesticide

O-33
ANALYSIS OF PESTICIDES IN VEGETABLES AT 1 PPB LEVELS USING BACKFLUSH PTV GC–MS/MS

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In this poster we present results on pesticide analysis in Quechers extracts by GC-MS/MS showing reproducible detection limits below 5 ppb, along with excellent calibration curves going up to 100 ppb. The measurements were performed using timed selected reaction monitoring in EI mode. The hyperbolic quadrupoles used in the Q1 and Q3 mass filters allow for increased mass resolution, thus achieving highest signal to noise values with low pesticide levels. A short comparison is made between nominal resolution (0.7 Da) and Ultra resolution at 0.1 Da. Various matrices were spiked at different levels and in total over 150 pesticides were analyzed in a single run. All compounds were analyzed using two transitions, one for quantitation and the second for confirmation. The ion ratio's were calculated and automatically monitored, and for a select number of compounds, the ion ratio deviation has been calculated. The PTV injector was optimized for minimizing compound breakdown, while backflush offers protection to the analytical column at the same time.

Keywords: pesticide residues, GC-MS/MS, PTV

O-34
AUTOMATED RAW EXTRACT ANALYSER FOR PESTICIDES – DETERMINATION OF 300 PESTICIDES FROM DIFFERENT FOODS WITHOUT SAMPLE PREPARATION BY 2D–LC–MS/MS

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The automated raw extract analyser for pesticides is a fully automated system for the determination of up to 300 different class pesticides from various food commodities. For the measurement we inject an aliquot of an acetonitrile raw extract into the system. The following clean-up is carried out by a multidimensional liquid chromatography. The pesticides are separated from the matrix compounds and transferred to the analytical column. Because of the chromatographic approach we get a much better cleaning effect compared to the classical methods. With our system we are able to determine all compounds of the established methods with analogous sensitivity but, with only one injection of the raw extract.

Keywords: pesticides, 2D-LC, LC-MSMS, food, multimethod

O-35
MULTIRESIDUES PESTICIDE ANALYSIS IN MILK, HONEY BEE AND WAX USING QUECHERS METHOD AND GC MS TECHNIQUE

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The AOAC official method 2007.01 [1] has been used for the determination of pesticides belonging to organochloride, organophosphate and pyretroid group, in honeybee, wax and milk. The extraction and purification of the samples has been performed using QuEChERS (Quick, Easy, Cheap Effective, Rugged, Safe) methodology which consist in shaking a well homogenized sample with the solvent in a centrifuge tube, partitioning with magnesium sulfate and sodium acetate, followed by a cleanup step using dispersive solid phase extraction. The analysis is done using Gas Chromatography-Mass Spectrometry with negative chemical ionisation and selected ion monitoring. The method is single laboratory validated following the ISO/IEC 17025 [2] and the Document SANCO N° 10684/2009 [3] by using fortified samples, of honeybee, wax and milk, at three different concentration levels. The limit of detection (LOD) has been calculated for each pesticides from the calibration curve performed in matrix, in the range between 0.02 µg/ml and 0.4 µg/ml and it is less than 0.01 mg/kg for most of the analytes. The suitability of the calibration curves has also been checked by the residual plot and by the calculation of the quality coefficient g, as defined by Miller [4] and further by De Beer et al. [5]. The accuracy, expressed in terms of trueness and precision, was verified by performing repeated analysis of spiked samples. The trueness was expressed as bias and the precision was evaluated as relative standard deviation and HorRat ratio [6]. The bias, expressed as recovery, for the major of pesticides ranged between 50 and 120% for the three matrices. The precision, expressed as HorRat ratio,is less than 2 in all cases. Another common expression of accuracy is the measurements of uncertainty. It has been estimated following the bottom-up approach, as reported by Eurachem Guide [7], starting from the equation of measurand and applying the error propagation law. The estimation of variances was performed from the single laboratory validation data. Uncertainty average obtained did not exceed the value of 50% suggested by Document SANCO [3]

[1] AOAC 2007.01: “Pesticide residues in foods by acetonitrile extraction and partioning with magnesium sulfate.”
[2] EN ISO/IEC 17025: 2005. ISO Geneva.
[3] Document No. SANCO/10684/2009 “ http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf 4) Miller, J.N., 1991.. Analyst 116:3-14. 5) De Beer, J.O., De Beer, T.R. and Goeyens, L., 2007. Analytica Chimica Acta 584: 57-65. 6) Hortwitz W.,. Albert R., 2006 J. AOAC Int, vol 89 n.4, 1095-1109 7) Eurachem/Citac Guide 2000_" Quantifying Uncertainty in Analytical Measurement" Second Edition

Keywords: QuEChERS, GC MS, pesticides

O-36
UTILIZATION OF HIGH RESOLUTION LC–MS FOR SCREENING AND QUANTITATIVE ANALYSIS OF PESTICIDES IN FOOD MATRIX USING A Q–EXACTIVE BENCH TOP ORBITRAP PLATFORM

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Current methodologies for quantitation of pesticides in food revolve around using triple quadrupole platforms. The method described here utilizes LC-MS/MS with QExactive using Selective High Resolution Accurate Mass. The QExactive is capable of resolutions of up to 140,000K FWHM. A Thermo Hypersil Gold aQ 50×2.1 mm 1.9 µm column was utilized with a run time 12 mins. A standard curve spiked with 110 pesticides in neat matrix ranging from 0.05 to 250 ng/mL was injected in triplicate. Analysis of hot peppers and bell peppers was screened for targeted and unknown pesticides. A full scan was set to 70,000K resolution to minimize matrix interference while Data Dependant MS/MS was collected and compared to spectra library for confirmation and help prevent false positives. Calibration lines show r^2 better than 0.99, limits of detection varies from 50 to 250 pg/mL depending individual compounds. The poster will show online searching and quantitation capabilities with one streamline software.

Keywords: QExactive, Food, Pesticide, Screening, High Resolution

O-37
NEW SCREENING AND QUANTIFICATION STRATEGIES APPLIED TO THE ANALYSIS OF MYCOTOXINS AND PESTICIDES

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The presence of residues and contaminants in food and animal feed is strictly monitored to protect consumers from exposure to toxicants. Fast and highly selective methods are necessary to screen, confirm and quantify different classes of contaminants, in where the complexity of the matrix and the analyte concentration plays an important role. High resolution mass spectrometry – using full scan analysis mode – has been described as the preferred screening tool due to the possibility to look for a large number of compounds and also because it enables retrospective analysis [1]. Furthermore, the use of a resolving power ≥ 50,000 FWHM is reported as being as selective as 2 SRM transitions when using QqQ instruments [2]. In this work, two different strategies have been applied to the screening of mycotoxins and pesticides in different matrices using a quadrupole-Orbitrap analyser – Q-Exactive™. The first experiment consisted on combining full scan mode at different resolving powers (35,000; 70,000 and 140,000 FWHM) with data dependent MS/MS spectra acquired at a resolving power of 17,500 FWHM. The MS/MS spectra were generated by making use of a high energy collision induced dissociation cell (HCD). The second experiment consisted on using timed targeted SIM at a resolving power of 35,000, 70,000 and 140,000 FWHM in combination with data dependent MS/MS mode at 17,500 FWHM. Both experiments were tested and evaluated in terms of quantification and confirmatory capabilities for the analyses of mycotoxins and pesticides in different food and feed matrices.

Keywords: HRMS, MS/MS mode, screening, pesticides, mycotoxines

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O-38
ROUTINE APPLICATION OF UPLC QTOF MS FOR THE QUANTITATIVE DETERMINATION OF MULTIPLE PESTICIDE RESIDUES THAT MAY REMAIN IN OR ON OUR FOOD

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SASA participates in annual UK and EU statutory surveillance programmes that monitor various UK and imported food & drink for the presence of residues of pesticides, their metabolites and other degradation products. Monitoring is essential in order to support enforcement of legislation, ensure good agricultural practice and to assess the pesticide load in our diet. Analytical methodologies employed in the determination of multiple pesticide residues in foodstuffs must be capable of quantifying very low levels of incurred residues and confirming the identity and magnitude of these residues in compliance with rigorous Analytical Quality Control (AQC) guidelines. We have utilised UPLC MSMS using both ±ESI modes as a front-line technique since 2006. However, the procurement of a UPLC QToF system in 2009 has significantly enhanced our surveillance capabilities. In particular, we now perform post-acquisition screening and/or confirmation using our in-house accurate mass database, examine data retrospectively for candidate analytes for which we have no reference materials and perform parallel UPLC QToF experiments in order to rapidly confirm or refute screen results obtained using UPLC MSMS. A comparison of results obtained using UPLC MSMS and UPLC QToF MS operated in ToF only 'full mass range' data acquisition mode for the quantitative determination of over 145 pesticides that could be present in or on leek, strawberry and green bean samples is presented. The samples were received as part of the EU and UK surveillance and proficiency testing programmes.

Keywords: pesticides, food, uplc, accurate mass, time-of-flight

O-39
COUPLED TURBOFLOW CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR THE ANALYSIS OF PESTICIDE RESIDUES IN GRAPES, BABY FOOD AND WHEAT FLOUR

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In this poster an online sample preparation method for simultaneous determination of 48 pesticides in grapes, baby food and wheat flour matrices will be presented. Target pesticides were selected to represent a wide variety of chemical structures and three typical matrices (solid, liquid, semi-solid) were selected. Turbulent flow chromatography was applied for online sample clean-up directly coupled to liquid chromatography-tandem mass spectrometry analysis. The aim was on one hand side to demonstrate the efficiency of a new online sample preparation method for pesticide analysis and on the other hand to reduce total analysis time, eliminate manual lab work and provide cleaner extracts with more reproducible results. The in-house validated method with 13 min total run time was proven as convenient, fast and fit-for-purpose in meeting regulatory requirements for pesticide residue monitoring. This poster puts focus on method selectivity as well as the efficiency of the online sample clean up step demonstrated by matrix effect as main validation parameter. The method has a potential to be an attractive alternative to the widely used QuEChERS method replacing manual sample preparation steps and also can readily be extended to a larger number and wider range of pesticide residues.

Keywords: turbulent flow chromatography, pesticides, baby food, online sample preparation

O-40
THE BRAZILIAN LABORATORY NETWORK: PROGRESS TOWARDS THE EVOLUTION OF THE NATIONAL RESIDUE AND CONTAMINANTS CONTROL PLAN ON PLANT PRODUCTS

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The Brazilian National Residue and Contaminants Control Plan has gained more focus, resources and expansion year after year. Especially in the Plant Area, which is responsible for monitoring the production of plant origin foodstuffs, one can see a fair increase in the analytical scope, in the amount of samples collected and analyzed, and in the variety of products tested. All of that was brought about with an also increasing laboratory capability, which had to be developed with suitable instrumentation, fit-for-purpose methods, and a quality management system based and accredited on ISO 17025. These very laboratories comprise the Brazilian Agricultural Laboratory Network, and therefore are monitored by audits and performance in interlaboratory comparisons, in a way to assure that the analytical results provided by them are technically valid, giving the inspection bodies the necessary information for them to proceed with investigatory actions whenever necessary. In this sense, the laboratories have an important role in the whole control system of the Ministry of Agriculture of Brasil, which is responsible for assuring consumer's safety – both domestic and from importing countries. In this work, data is presented showing the evolution of PNCRC in the Plant products area from recent years for both pesticide residues and mycotoxins contamination, and the aspects concerning the laboratory network role to make this evolution possible. Number of samples tested in a monthly basis and turnaround times are presented for the 2nd half of crop year 2010/2011, when laboratory activity began to have a closer overview, thus providing the central competent authority the information necessary for a better management. A comparison of the volume of samples and number of analytes tested for is made between crop year 2010/2011 and previous seasons. These laboratories are required to pursue methods validated according to SANCO 10684/2009, and to regularly take part in proficiency tests, especially those provided by t he EURLs. The results on those exercises are also briefly presented.

Keywords: Residue, mycotoxins, laboratory network

O-41
A COMPARISON OF QUECHERS (QUICK, EASY, CHEAP, EFFECTIVE, RUGGED AND SAFE APPROACH FOR DETERMINING PESTICIDE RESIDUES) PRODUCTS PREPARED "IN HOUSE" VERSUS COMMERCIALLY AVAILABLE QUECHERS PRODUCTS

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The QuEChERS approach to agricultural pesticide testing was developed at the USDA in Wyndmoor, PA USA. This extraction technique has been accepted world-wide as an effective method for pesticide testing in food and other agricultural matrices. Since many laboratories assemble their own QuEChERS products for this analysis, a comparison study was conducted at the USDA to determine if commercially prepared QuEChERS products could be as effective as products prepared in the users lab. Bulk magnesium sulfate anhydrous, primary secondary amine (PSA) and endcapped C18 were purchased from a commercial source and compared to the commercial product assembled using the same lot of bulk sorbents. The ratio of magnesium sulfate, PSA and C18 was 3:1:1. The clean-up products were tested on un-spiked extracts of milk, honey and soybean. Efficacy of clean-up was determined by GC/MS and compared the number of peaks above threshold values. Results determined that the commercially prepared products provided superior clean-up to the products assembled in the lab in all three matrices. The extra peaks observed in the lab prepared products were probably caused by contamination from the lab environment. The commercially assembled products were prepared under controlled conditions minimizing potential contamination. These results, coupled with the obvious time and labor savings for assembly, indicate that commercially available QuEChERS products are preferable to products made "in house".

Keywords: QuEChERS, pesticides

O-42
DETERMINATION OF ORGANOCHLORINE PESTICIDES IN CARROTS FROM PORTUGUESE REGIONS

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Organochlorine pesticides (OCP) were applied in the second half of the twentieth century worldwide as insecticides and fungicides against pests in fruit growing, horticultural and arable crops [1]. These compounds are considered to be one of the most prevalent contaminants [2] and it is known that pesticides may cause potential health risks to human life if harmful residues appear in food compromising food safety and quality [3-5] Although the Human health effects after exposure to OCPs are not adequately understood it has been considered that these contaminants have an endocrine-disrupting activity and that they have also been implicated in the etiology of various diseases and endocrine-related disorders [6]. The majority of the OCPs are also part of the larger group of persistent organic pollutants (POPs) generally characterized by high lipophilicity, bioaccumulation potential, long half-life in the environment and potential long-range transport [2, 7, 8]. Their presence in the environment, decades after being applied, has led to the ingress of OCPs into growing plants and the persistence of their residues [9]. Therefore the determination of pesticides residues in food matrices has become a necessity in view of the toxicity and stability of these xenobiotics [10]. The aims of this work were the determination of 14 OCPs in carrots, from Portuguese Regions, and the evaluation of its biomagnification potential. The analyzed compounds include: HCH (α,β,δ), HCB, lindane, aldrin, 4.4'-DCBP, α-endosulfan, dieldrin, *p-p'*-DDE, endrin, β-endosulfan *p-p'*-DDD, *o-p'*-DDT and methoxychlor. The extraction was performed with QuEChERS and the analyses were carried out with the GC-ECD and confirmation by GC-MS. The recoveries for all pesticides studied were from 8% (HCB) to 106% (β-endosulfan) at concentration of 60 µg/kg. The limit of detection (LOD) except for HCH (α,β,δ), endrin and methoxychlor, met maximum residue limits determined by EU by GC-ECD which presented LODs bellow 15 µg/kg.

Keywords: QuEChERS, GC-ECD, Organochlorine Pesticides, Carrots.

O-43
EVALUATING POROUS MATERIALS FOR SAMPLING PESTICIDES FROM SURFACES USING DIRECT ANALYSIS IN REAL TIME (DART) –MASS SPECTROMETRY

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Rapid screening of pesticides present on the surface of fruits and vegetables has been facilitated by using direct analysis in real time (DART) open air high resolution accurate mass mass spectrometry. These experiments focus on the use of various materials to collect pesticides from large objects including plants and produce commodities by using a vacuum-assisted sampling approach. Evaluation of the efficiency of various polymeric foams, cotton swabs and wire mesh for capture of analytes with and without the use of solvents will also be examined. Suitability of different materials as both sampling and desorption ionization support will be reported. These experiments build on the original pesticide screening experiments where polyethylene foam was used as both the collection and desorption substrate. Small fruits and nuts were examined for pesticides using "Transmission-mode" DART-MS analysis [1].

[1] Edison, S., et al., Rapid Commun. Mass Spectrom.,2011, 25, 127-139

Keywords: Pesticide Screening, DART, HR/AM

O-44
ANALYSIS OF 400+ PESTICIDES IN A SINGLE RUN USING TRIPLE QUADRUPOLE MASS SPECTROMETER

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Increasing food safety concerns and the growing agricultural trade has resulted in stringent pesticide regulations globally. To comply with strict food safety standards, a screening and quantitative method for large numbers of pesticides is becoming important. This poster describes a method for analysis of multi-class pesticides in food samples using liquid chromatography coupled with triple quadrupole mass spectrometer. A multi-residue method was developed for screening and quantitation of approximately 400 pesticides in one 45 minute run. One or two ion ratios were used to confirm each analyte. In addition, the Quantitation Enhanced Data-Dependent (QED) scan that delivers an information rich mass spectrum while undergoing quantification could also be used for structural confirmation. The method was applied to pesticide analysis in orange, asparagus and apple extract. Furthermore, the method was developed using software with built-in workflows for streamlining method development and routine analysis. The experiment results will be discussed in detail.

Keywords: Pesticides analysis, food safety, triple quadrupole mass spectrometer

O-45
COMPREHENSIVE GC×GC(QMS) PESTICIDE ANALYIS: QUALITATIVE AND QUANTITATIVE ANALYSIS WITH AN ULTRA FAST QUADRUPOL MASS SPECTROMETER

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The QuEChERS preparation method for pesticide analysis is well established as it reduces sample preparation effort drastically compared to the former used method with a final GPC clean up step. On the other hand when injecting the extracts prepared by QuEChERS many matrix signals can be observed in the GCMS chromatogram.Therefore full scan modes are necessary to prevent false positive or false negative determination of target pesticides which could easily happen when running the GCMS in the more sensitive selected ion monitoring (SIM). To reach a high sensitivity for routine work in full scan here comprehensive GCxGC(q MS) was combined with Rapid large volume injection of 30 µl into the Optic PTV with a sintered glas liner i.e. a liner which has a rough inner wall surface and which was desactivated by a SILTEK desactivation. No additional packing material was necessary for the large volume injection due to the capacity of the large inner liner surface. A syringe was used with a side hole needle in order to spray the 30µl onto the liner wall. µThe solvent (ACN) was vented through the split line and this was monitored with a TCD in the split flow (solvent monitor). Best results were achieved with 55°C initial PTV temperature followd by a 15°C/sec ramp to 280 °C and a resulting venting time of 38 sec. For comprehensive GC×GC with thermal modulation a RTX-1 30 m, 0.25 mm, 0.25 µm was coupled to a BPX-50 1 m, 0.15 mm, 0.15 µm with a loop of 1.6 m. The ZOEX ZX2 Modulator was used (ZOEX corporation, USA) which allows thermal modulation without the use of liquid nitrogen. As MS detector, the high speed GCMS–QP2010 Ultra was used (max 100 spectra/sec, 20,000 amu/sec). The GC program started at 100°C with a ramp of 2.5°C/min to 280°C for 20 minutes. The modulation frequency was set to 8 sec which results in 3 peaks per compound. The mass spectrometric detector was run in full scan mode with a mass range from 80–390 amu and a sampling frequency of 50 scans/sec. This resulted in more than 15 data points across each modulated peak which ensured quantitative analysis with sufficient precision. For method validation a clean pesticide standard diluted in acetonitrile was measured and the image was compared to the data recorded with an apple matrix spiked with 54 seleted pesticides. The concentrations range was 0.01 to 0.2 mg/kg. The limit of detection was below 1 µg/kg. In a different experiment a SCAN-SIM acquisition was performed. In this experiment for the selected ion signal aquired after the full scan an increase of signal to noise of about 10 was observed. Also in this experiment each peak was aquired with more than 10 data points to ensure quantitative relyability

Keywords: comprehensive GC×GC (qMS),QuEChERS, Pesticides

O-46
A RAPID SOLUTION FOR COMBINED QUALITATIVE AND QUANTITATIVE ANALYSIS OF KNOWN AND UNKNOWN PESTICIDES IN WATER, USING E QUAN WITH EXACTIVE

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The demand for quick and simple analysis of large numbers of samples in environmental analysis is growing year by year. While the limits of quantification requested by governmental authorities are lowered nearly on a yearly basis, the number of analytes of interest is growing exponentially. Mass spectrometric detection with HRAM technology using full scan experiments can deliver the ability to detect as many analytes as necessary in combination with screening for an unlimited number of compounds in a targeted as well as untargeted approach, using only one chromatographic run. A single stage benchtop Orbitrap mass analyzer in combination with online solid phase extraction and a novel software application for unified qualitative and quantitative data processing fulfills these demands with higher confidence and precision. For this experiment, an EQuan MAX™ online solid phase extraction system was coupled to an Exactive™ benchtop Orbitrap mass spectrometer. For online SPE two extraction columns were directly coupled (Hypersil Gold C18, 20×2.1 mm, 12 µm particle size and Hypercarb Javellin column, 10×2.1 mm, 5 µm particle size). Analytical separation was carried out on a Hypersil Gold C18 column (50×2.1 mm, 1.90 µm particle size). For analysis different samples of surface water were spiked with a mixture of pesticides commonly occurring in environmental samples. From each of these samples 1 mL was loaded directly and without any preliminary treatment onto the stack of trapping columns. Mass spectrometric detection was carried out in full scan mode alternating positive and negative ionization. A Group of 20 pesticides representing a broad range of polarities from very polar to very non-polar was taken to represent the whole range of polarity patterns usually occurring in environmental analyses. The coupling of two trapping columns of different polarity yielded in highly sensitive detection of all analytes with excellent precision and reproducibility. The samples were injected onto the columns without any preliminary treatment, but thorough washing of the trapping columns after sample loading was applied to remove unbound matrix components and salts. The application of a 7 minute gradient for separation of the analytes yielded in a chromatographic cycle of 20 minutes, including all washing and equilibration steps. Parallel detection in positive and negative ionization mode opens the perspective of easy enhancement of this method to a far greater number of analytes, All analytes yielded good linear behavior over the full range of calibration and showed good reproducibility in repeated injections. Analytes of very polar to very un-polar behavior could be trapped and pre-concentrated with no significant difference. In addition, further fully automated untargeted and unknown screening could be applied to the same data set within the same software application.

Keywords: Exactive, E Quan, pesticides, water

O-47
5975-SMB – A NEW TYPE OF GC–MS WITH ADVANCED CAPABILITIES FOR IMPROVED FOOD SAFETY

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Gas chromatography mass spectrometry although widely used can benefit from a new design that will provide major improvements in: a) Increased range of low volatility and thermally labile samples amenable for analysis including those "LC-MS pesticides"; b) Provision of enhanced molecular ions while retaining library search capability; c) Significantly improved sensitivity, particularly for bottleneck pesticides that are hard to analyze; c) Reduced analysis cycle time to few minutes or less. In order to address these challenges we have combined the benefits of a supersonic molecular beam (SMB) interface and its fly-through EI ion source with the Agilent 7890 GC + 5975 MSD, resulting in a new and powerful GC-MS platform named 5975-SMB with record setting performance. The 5975-SMB is based on GC and MS interface with SMB and on electron ionization of sample compounds in a dual cage fly-through ion source. The GC eluting sample compounds are mixed with helium make up gas, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, collimated into a SMB and pass a contact-free EI ion source where they are ionized by 70 eV electrons and mass analyzed. The 5975-SMB Supersonic GC-MS is characterized by few unique features including: A) The detection sensitivity is significantly improved. The combination of high ionization yields, enhanced molecular ions, elimination of vacuum background and ion source peak tailing and degradation resulted in record signal to noise ratio (S/N) and low LOD, particularly for pesticides and compounds that are hard to analyze. B) Improved sample identification is achieved through having enhanced and trustworthy molecular ions (and isomer MS effects) combined with the usual library searchable EI fragments. Isotope Abundance Analysis (IAA) software is seamlessly integrated and complements the availability of trustworthy molecular ions through the provision of elemental formulas and automated confirmation and/or rejection of NIST library identification. C) The range of thermally labile pesticides and low volatility samples that are amenable for analysis is significantly extended via the use of short columns with high column flow rates. D) Effective fast and ultra fast GC-MS is provided by the 5975-SMB Supersonic GC-MS via the use of high column flow rates in combination with a unique low thermal mass fast GC inlet. Sub one minute full analysis cycle time can be routinely achieved including for pesticides in complex agricultural matrices. The use of the Supersonic GC-MS will be demonstrated with emphasis on food safety and pesticide analysis applications and a new approach and method for improved universal pesticide analysis will be described, explained and demonstrated.

Keywords: Supersonic Molecular Beams, GC-MS, Pesticide Analysis

O-48
DETERMINATION OF HERBICIDES AT LOW TRACE LEVEL (PPT), USING WATER SAMPLE DIRECT INJECTION IN UHPLC/MS/MS COUPLE WITH RP AMIDE AND F5 ASCENTIS EXPRESS FUSED CORE HPLC COLUMN

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The purpose of the experiment was to test the possibility to inject, without any extraction or purification process samples directly in LCMSMS of drinking water or groundwater. To have a simple and robust system for the rapid recovery and improving the reproducibility of the method to use routinely. Furthermore to compare columns of different polarity and selectivity to improve the chromatographic profiles of metabolites of atrazine, in particular the desethyl desisopropyl atrazine.

Keywords: Erbicides, HPLC/MS/MS, Fused core HPLC columns

O-49
NEW PERSPECTIVES FOR THE ANALYSIS OF TRIAZOLE-BASED METABOLITES: DIFFERENTIAL MOBILITY SPECTROMETRY & TIME OF FLIGHT

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1,2,4-Triazole and their major conjugates triazole acetic acid, triazole lactic acid, and triazole alanine are degradation products of triazole fungicides such as triadimefon or tebuconazole. These molecules commonly referred to as "triazole derivative metabolites" can occur in plant, animal, and soil materials. Their high polarity and small molecular weight make the qualitative and quantitative analysis in matrix difficult. LC–MS/MS curves of these molecules obtained from plant extracts are characterized by a high chemical noise. Additionally, the analyte signals are often overlaid with interferences caused by co-extracted matrix compounds. In order to achieve 0.01 mg/kg the selectivity needs to be greatly enhanced in comparison to previously tested LC–MS/MS techniques. Therefore, the differential mobility spectrometer as a chemical pre-filter before MS/MS and the accurate mass measurement using a Q–TOF were tested for the analysis of the four triazole derivative metabolites in plant materials. 5 g of plant material was weighed in and extracted with a mixture of methanol/water (4/1, *v/v*) and then homogenized with a common Ultra Turrax system. After filtration, the extract was made up to a final volume of 50 mL with extraction solvent. 2.5 mL of this raw extract were evaporated to dryness and reconstituted in 1 mL of Milli-Q-water, which contained the isotopically labeled internal standard of each analyte. The measurement of the samples was performed on LC–DMS–MS/MS using the AB SCIEX SelexION™ with the AB SCIEX QTRAP® 5500 and on LC–Q–TOF using the AB SCIEX TripleTOF™ 5600. The applicability of both technologies was tested for a broad range of matrices. Materials with a high water, acid, oil, starch, and protein content were investigated. Control materials were spiked with defined amounts of standard solution to obtain residue levels of 0.01 mg/kg and 0.10 mg/kg. Recoveries, standard deviations, signal-to-noise ratios, and the evaluation ability of the obtained analyte signals were used to determine the capability of both technologies. Finally, both the LC–DMS–MS/MS and the LC–Q-TOF provide a high degree of selectivity. Interferences are eliminated, and a significant reduction of the chemical noise in the obtained chromatograms can be observed. With both technologies, the quantitative and qualitative analysis of the four triazole derivative metabolites at a residue level of 0.01 mg/kg and 0.10 mg/kg was achieved for a broad range of plant materials. Compared to the conventional LC–MS/MS analysis, no additional clean-up or derivatization processes were required, which enabled a higher sample throughput.

Keywords: triazole derivative metabolites, plant materials, differential mobility spectrometry, Q–TOF

O-50
GC–μECD ANALYSIS AND CONFIRMATION OF CLP PESTICIDES IN OLIVE OIL

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An olive oil sample was analyzed for 20 contract laboratory protocol (CLP) pesticides. A QuEChERS extraction with dSPE cleaned the sample prior to analysis. A dual μECD and dual capillary GC column approach accomplished simultaneous primary and confirmatory analysis. The primary column, DB-35ms 30 m × 0.25 mm × 0.25 μm and confirmatory column DB-XLB 30 m × 0.25 mm × 0.50 μm effectively resolve all 20 CLP pesticides with sensitivity in the low ppm range. An un-purged two-way capillary flow technology splitter divided the flow from a single injection port to the two analytical GC columns. Endosulfan sulfate and endosulfan 1 residues are confirmed to be present in the olive oil sample. In previous studies of pesticides residue analysis in olive oil, extraction procedures varied from LLE/SPE, GPC, and more recently to the use of QuEChERS methodology. QuEChERS extraction followed by dSPE is a convenient way to clean up sample matrixes just enough to remove chromatographic interferences and at the same time retain sensitivity for analytes of interest.

Keywords: CLP Pesticides, QuEChERS, GC analysis

O-51
DEVELOPMENT OF AN ELECTROCHEMICAL IMMUNOSENSOR BASED ON SPECIFIC ANTIBODIES LABELLED WITH CDS NANOPARTICLES FOR IN-SITU PARAQUAT MONITORING IN SPIKED POTATO SAMPLES

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The analysis of the presence of potentially hazardous chemicals (e.g. pesticides, antibiotics) in food remains a major concern in the European Community. However, to ensure quality and traceability, there is a great need to increase the continuous control and monitoring of foodstuff at critical steps in the food chain, such as for example after the recollection of the raw materials, after the food processing (monitoring of storage and logistics), as well as of final products. Fast, reliable and cost-effective analytical methods are necessary to ensure the safety of the food products. Following the flexibility, sensitivity, specificity and efficiency of analysis demonstrated by the numerous immunochemical and biological tests today available, research is now intending to go forward by developing devices capable of working out of laboratory, i.e. in the different steps of the food chain. With this idea the concept arises of biosensor as a miniaturized analytical devices, consisting of an immobilized biological component (antibody, enzyme, receptor, DNA, etc.) in intimate contact with a transducer (optic, electrochemical, piezoelectric, etc.) that may convert the biorecognition process into a quantifiable electrical signal. In this work, a new electrochemical immunosensor to detect residual amounts of paraquat (PQ) in a complex matrix, such as potatoes, is presented. The immunosensor is based on graphite composite electrodes (GECs), immunoreagents specifically developed to detect paraquat, magnetic µ-particles, and CdS nanoparticles labelled to the specific antibodies. In this approach, CdS nanoparticles will be used as specific non-interfering labels and selective amplifiers. Likewise, the use of magnetic particles as a solid support for the PQ antigen, let us to reduce the matrix effect and also offers a simplified way to perform the electrochemical assay. By means of the well-known anodic stripping techniques, CdS nanoparticles are read, and the amounts of its metal ions are expressed as a signal of current or charge. Due to the high detectability of the immunosensor, the results obtained showed that after the extraction and dilution of the matrix, PQ can be determined in potato samples achieving limits of detection below 0.001 mg Kg^{-1}, and therefore, far below the MRL required by EC for paraquat (0.02 mg Kg^{-1}). Hence, the results obtained open the door to commercial sensors of simple manipulation, transportable and economics.

Keywords: paraquat, CdS nanoparticles, specific antibodies, immunosensors

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O-52
OVERCOMING MATRIX EFFECTS USING THE DILUTION APPROACH IN MULTIRESIDUE METHODS FOR FRUITS AND VEGETABLES

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During recent years matrix effects in liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) have quickly become a major concern in food analysis. The phenomenon of ion suppression can lead to errors in the quantification of the analytes of interest, as well as can affect detection capability, precision, and accuracy of the method. Sample dilution is an easy and effective method to reduce interfering compounds, and so, to diminish matrix effects. For this work, different dilutions of the matrix were tested in order to study the signal suppression of fifty three pesticides in three different matrices: orange, tomato and leek. Several dilutions of the matrix were tested in order to study the evolution of signal suppression. Dilution of the extracts led to a reduction of the signal suppression in most of the cases. A dilution factor of 15 demonstrated to be enough to eliminate most of the matrix effects, opening the possibility to perform quantification with solvent based standards in the majority of the cases. With the aim of checking the possible differences when quantifying with solvent based or with diluted matrix-matched standards in different commodities, one hundred and ten samples of diverse types of fruits and vegetables were analysed in both ways. In most of the cases the differences were not significative, although for some specific examples quantification with solvent based calibration curves should be avoided, as the errors could be significant. From the results obtained we can say that it's not possible to generalize, and specific problems should be approached individually in order to achieve the best results. In those cases signal suppression could not be reduced, a possible solution would be to use stable isotope-labeled internal standards for quantification of the problematic pesticides. On the basis of the results, it can be concluded that sample dilution proved to be very advantageous, as it was easy to implement and very effective regarding diminishing of signal suppression.

Keywords: Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), Pesticides, Matrix effect, Fruits and Vegetables, Dilutions.

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O-53
THE HALF-LIVES OF BIOLOGICAL ACTIVITY OF ETHABOXAM AND SPINOSAD ON LETTUCE

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This study was performed to investigate pre-harvest residue limit (PHRL) in lettuce and estimate biological half-life for residue of each pesticide. The lettuce was sprayed with the pesticide ethaboxam and insecticide spinosad of standard and double application rates. Ethaboxam and spinosad were sprayed once at 10 days before harvest on lettuce under greenhouse conditions. The lettuces of standard and double were sampled 10 times from ethaboxam and 7 times from spinosad. The pesticides from lettuce samples were extracted with acetonitrile, n-hexan, methanol and analyzed by LC-DAD. Their quantitation limit of ethaboxam was 0.0006 mg/kg and spinosad was 0.006 mg/kg. Recoveries of ethaboxam at fortification levels of 0.1 and 0.5 mg/kg were 8711% and 86.13%, respectively. Recoveries of spinosad at fortification levels of 1 and 5 mg/kg were 96.1Ï3.5% and 95.83.1%, respectively. The biological half-lives of ethaboxam were about 4.8 days at standard application rate, and 4.7 days at doupble application rate. The biological half-lives of spinosad were about 1.1 days at standard application rate, and 1.3 days at doupble application rate.

Keywords: Biological half-lives, Ethaboxam, Spinosad

RESIDUES – VETERINARY DRUGS ET AL.

(P-1 – P-62)

P-1
ACCURATE MASS SCREENING OF PHARMACEUTICALS AND FUNGICIDES IN WATER BY UHPLC–EXACTIVE ORBITRAP MS

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The use of pharmaceuticals in livestock production is really a potential source of environmental contamination. Possible impacts of those compounds in the environment include toxicity and the emergence of antibiotic resistance. Continuous monitoring is required to record variations in concentrations of these substances in the environment. A rapid, versatile and selective multi-method was develop and validated for screening purpose for 43 pharmaceuticals, biocides and fungicides compounds, in surface and ground water, in one single full scan MS method. Sample volume, pH condition, elution, and rinsing solvents were optimized in this work, to improve SPE clean-up efficiency. Superior chromatographic resolution was provided by UHPLC system. Analyses was carries out by full-scan-mass spectrometric detection using Exacte Orbitrap technology (FWHM 50,000). Powerful resolution of the Exactive was a very important parameter, in combination with mass accuracy, for significant sensitivity and selectivity improvement. The recovery, detection limit and matrix effects was evaluated together to determine the performance of the method. Detection was based on accurate masses and on retention time. The evaluation of recovery of the final method, concluded that 74% of compounds show recoveries higher than 80%, 15% of compounds that show recoveries between 60% and 80%, 7% of compounds show recoveries between 40% and 50%, and itraconazole had a recovery lower than 10%. The level of detection was 10 ng/l for 61% of compound, 50 ng/l for 32%, 100 ng/l for 5%. Nystatin cannot be detected in water sample. Average mass accuracy in this experiment ranged within 0.2-2.9 ppm with a standard deviation ranged within 0.4 -3. Validation study, based on EU guidelines, proves that the detection capability CCβ is lower than 10 ng/l (for β error 5%) for 37% of compounds, than 50 ng/l for 33% of compounds, and then 100 ng/l for 16% of compounds. The development method was used to water and groundwater samples collected throughout the Netherlands and different residues like sulfamethoxazole, carabamazepine, ketoconazole, diclofenac, carbendazim, fluconazole and propiconazole were detected and confirmed. This study demonstrate than Exactive Orbitrap MS is enable to detect the pharmaceutical and fungicide residues in water samples, in a concentration range on 10 ng/l to 100 ng/l, in the multimethod condition.

Keywords: antifungal compounds, water contamination, ultra high resolution, mass accuracy, multiresidue analysis.

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P-2
DETERMINATION OF CHLORAMPHENICOL BY VALIDATED LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD IN CROATIAN HONEY

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Honey is generally considered a natural and healthy product. Chloramphenicol (CAP) is a bacteriostatic antimicrobial bannded for food-producing animals use in EU since 1994. because of heavy side effects in humans and by Croatian low in 2005. The aim of this study was to investigate the presence of CAP in honey in Croatian market by validated and confirmative method based on liquid chromatography – tandem mass spectrometry with negative electrospray ionisation. The method was applied for analyses of honey samples collected from whole Croatian area. There were 269 honey samples in total in period from 2005. till 2011. Method quantification limit was 0.3 μg kg^{-1} and only two positive samples were found. These results proved very good quality of Croatian honey.

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Keywords: antibiotics, chloramphenicol, honey, mass spectrometry

P-3
A STEP FORWARD THE DETECTION OF BOVINE RECOMBINANT SOMATOTROPIN IN MILK

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Recombinant bovine somatotropin (rbST) is a protein hormone that may be used to increase milk yield in lactating cows. As for all growth promoters, its administration has been banned in European Union. Nevertheless, it is allowed to use in many countries including USA, Brazil and South Africa. Due to the import of dairy products from these countries and possible illegal use of somatotropin on black market, sensitive and reliable methods for the detection of rbST treated animals should be implemented, with the milk being the matrix of choice for official control. A generic protocol using SPE C4 purification and tandem mass spectrometry detection of N-terminal tryptic peptide has been developed in LABERCA and successfully implemented in the analyses of plasma samples [1,2,3]. The milk, however, is a more complex matrix containing huge amounts of possibly interfering proteins of similar physicochemical properties. Therefore, new analytical strategies had to be developed to reach expected physiological recombinant somatotropin levels below 1.0 ng/mL. The presented work was performed within the FP7-IAPP Marie Curie UNIQUE-Check project. Novel approaches were tested for both final analyte detection and sample preparation. The application of MS3 on Q-Trap hybrid ion trap-triple quadruple instrument slightly improved the sensitivity by the almost complete elimination of background noise. Still, the expected physiological levels could not be achieved (LOQ 2 ng/mL). The improvements in sample preparation protocol were therefore necessary and they concerned the isolation of somatotropin from milk samples with anti-rbST antibodies. Different separation techniques were tested covering immunoaffinity chromatography and magnetic immunoprecipitation. The application of selective purification protocol increased method recovery and significantly reduced ion suppression, which led to the enhanced detection limit of 0.5 ng/mL. The use of magnetic beads for sample purification has additionally the advantage of being environmental friendly and labour-effective.

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Keywords: bovine somatotropin, mass spectrometry, milk

P-4
DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE ANALYSIS OF CHLORAMPHENICOL IN HONEY

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Chloramphenicol (CAP) is a broad spectrum antibiotic with excellent antibacterial and pharmacokinetic properties. Because of its side effects (e.g. aplastic anemia and hypersensitivity) the European union banned its use in food producing animals. The aim of this study was to develop and validate method for qualification and quantification of CAP in honey based on liquid chromatography – tandem mass spectrometry with negative electrospray ionisation. The target antibiotic was separated using reversed-phase liquid chromatography on chromatographic column Zorbax SB C18 (150 mm × 2.1 mm, 3.5 μm) with a gradient elution using acetonitrile – 0.1% formic acid mobile phase at a flow rate of 0.3 mL min-1, with column temperature 35 oC for CAP and 5D-CAP as internal standard. Homogenised honey samples were diluted with acetate buffer solution at pH 6 and extracted on Oasis HLB sorbens. Recoveries for real (acacia, chestnut, linden and flower) honey samples (n=36) were 102% with RSD 8.4%. Method quantification limit for CAP was 0.3 μg kg^{-1}. For detection and quantitative determination selected reaction monitoring was used. The method was applied for analyses of honey samples collected from whole Croatian area.

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Keywords: antibiotics, chloramphenicol, honey, mass spectrometry

P-5
DEVELOPMENT OF AN EVIDENCE BIOCHIP ARRAY FOR THE MULTIPLEX DETERMINATION OF MORE THAN TWENTY ANTHELMINTIC DRUGS

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Anthelmintic drugs are used in clinical and veterinary practice for the treatment of infections caused by parasitic worms. Their extensive use in food-producing animals can cause the presence of residues in food. For consumer protection is relevant to monitor the levels of anthelmintic residues to ensure that they remain within the legally permitted maximum acceptable concentrations. For this purpose, the use of multiplex screening methodologies is advantageous. Biochip array technology allows the simultaneous determination of multiple analytes from a single sample at a single point in time. This increases the result output and has implications in the cost-effectiveness of the tests. This study reports the development of an Evidence biochip array for the multiplex screening of amino benzimidazoles, avermectins, benzimidazoles, levamisole, moxidectin, thiabendazole, triclabendazole. Simultaneous competitive chemiluminescent immunoassays are employed. The assays were applied to the semi-automated bench top analyser Evidence Investigator where 54 samples can be handled at a time. The solid support and vessel is the biochip (9 mm × 9 mm), which contains an array of discrete test sites. The instrument incorporates dedicated software with capability to process, report and archive the multiple data generated. The amino benzimidazoles assay detected amino-mebendazole, albendazole 2-amino sulphone, amino-flubendazole (%cross-reactivity: 141%, 100%, 99%), the avermectins assay detected emamectin benzoate, eprinomectin, abamectin, ivermectin and doramectin (% cross-reactivity: 254%, 191%, 178%, 100%, 75% respectively), the benzimidazoles assay detected albendazole sulphone, albendazole, albendazole sulphoxide, oxibendazole, oxfendazole, flubendazole (% cross-reactivity: 178%, 100%, 99%, 48%, 40%, 29% respectively), the thiabendazole assay detected cambendazole, thiabendazole, 5-hydroxythiabendazole (%cross-reactivity: 800%, 100% and 91% respectively) and the triclabendazole assay detected (keto-triclabendazole, triclabendazole, triclabendazole sulphoxide (%cross-reactivity: 150%, 100% and 40% respectively). The Limits of Detection (LOD) values ranged from <0.1 ppb (amino benzimidazole) to 3.0 ppb (levamisole) in milk and from <0.2 ppb (amino benzimidazoles) to 6.5 ppb (levamisole) in beef muscle. For all the immunoassays the intra-assay precision expressed as %CV was typically <12% for different concentration levels. This multi-analytical approach on biochip platform is applicable to the screening of more than twenty anthelmintics in different food matrices leading to consolidation of tests and enhancement of test result output.

Keywords: anthelmintic drugs, biochip array, multiplex screening, immunoassay

P-6
COMMUTABILITY AND USE OF BLANK MATRIX MATERIALS – TWO IMPORTANT, BUT OFTEN FORGOTTEN ASPECTS FOR PROPER USE OF CERTIFIED REFERENCE MATERIALS IN FOOD ANALYSIS

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Certified reference materials (CRMs) are important tools to enable and safeguard reliable analytical measurements and are typically used for calibration, method validation purposes, and method performance verification. Key requirements such as appropriate homogeneity and stability, and establishment of a certified value with stated uncertainty and metrological traceability are usually addressed during the production of a CRM. Two other important issues however, the commutability of the material with routine samples among results from different measurement procedures ("replaceability", "substitutability"), and the certification and use of appropriate blank matrix materials, are often not taken into account accordingly, especially in the food science community. The presentation reviews the definition of commutability and highlights the importance of this material property for the correct use of a CRM. Moreover, it emphasizes the challenges and considerations for certification of blank matrix materials, which are important tools in determining the recovery as well as the decision limit (CCα) and detection capability (CCβ) of analytical methods. All presented data make reference to examples from the area of veterinary drug residue analysis: a commutability study for the certified reference material "chloramphenicol in pork" as well as the certification of the blank materials "dimetridazole in pork" and "oxytetracycline in milk" are reviewed.

Keywords: certified reference materials (CRMs), commutability, blank material, veterinary drug residues, quality assurance

P-7
SCREENING OF CARBADOX IN FEED AND MEAT THROUGH RAPID LIQUID CHROMATOGRAPHY METHODOLOGY

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The abuse in the use of antibiotics in farm animals has created an alarm in recent years due to the observed increased antibiotic resistance for some microorganisms. This includes resistence in the gastrointestinal tract and destruction of the existing flora, increasing the susceptibility to pathogen microorganisms like Salmonella spp. or Escherichia coli. Some allergic reactions or undesirable problems may also appear. The control of the absence of these substances in foods was regulated in the Directive 96/23/EC and Decision 2002/657/EC provided rules for the analytical methods to be used in testing of official samples. Due to the large number of samples to be analyzed, it is necessary to develop effective screening methodologies. The objective of this work was to develop a rapid HPLC method for the screening of carbadox in feed and the detection of its metabolite quinoxaline 2-carboxilic acid (QCA) in meat. This method implied the use of new columns with packagings of lower size and shorter lenghts. In the case of feed, the column was a Superspher RP 18 (Phenomenex) 4 µm (125 × 4 mm) eluted with 82.5 % 0.01 M acetate buffer, pH 6 and 17.5 % of acetonitrile. The flow rate was kept at 0.5 mL/min. UV detection at 365 nm. In the case of meat, the column was a Zorbax Eclipse Plus C18 (Agilent) 1.8 µm (50 × 4.6 mm) eluted with 65% 0.01 M phosphate buffer + 0.05 M tetrabutilamonia sulphate, pH 7 and 35% of methanol. The flow rate was 1.5 mL/min, at 40°C and detection at 320 nm. The decision limit (CCα) in feed was 5 µg/g and the detection capacity (CCβ) of 6 µg/g and in the case of the QCA metabolite in meat they were 2.24 µg/kg and 2.26 µg/kg, respectively. This technique shows a good alternative to routine screening analysis of such antibiotic in feed and its metabolite in meat.

Keywords: Carbadox, residues, HPLC, meat, feed

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P-8
A SEMI–AUTOMATED METHOD FOR THE MULTICLASS ANALYSIS OF VETERINARY DRUGS IN HONEY BASED ON TURBULENT–FLOW LIQUID CHROMATOGRAPHY COUPLED TO ULTRA–HIGH PRESSURE LIQUID CHROMATOGRAPHY–ORBITRAP MASS SPECTROMETRY (TFC–UHPLC–ORBITRAP–MS)

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Honey is a complex product rich in sugars, vitamins and minerals, and it is very popular and highly consumed. It has been considered as natural and healthy product of animal origin, free of impurities. However, in recent years, a number of publications dealing with the determination of antimicrobial residues in bee products have been reported. In apiculture, veterinary drugs (VD) are mainly used for the treatment of bacterial brood diseases, such as American foulbrood (Bacillus larvae) and European foulbrood (Streptococcus pluton). Therefore, VD residue control represents an important issue to ensure consumer protection. In general, there is a zero-tolerance policy to VD residues in honey, so it is necessary to develop methods allowing the determination of these compounds at trace levels. Furthermore, different classes of antibiotics can be present in honey samples, and therefore, multi-class methods are necessary for the simultaneous determination of this type of compounds. Although some studies have developed multi-class analysis methods for VD in honey, they are based on laborious, time-consuming and complex multi-step sample preparation procedures, which do not allow high sample throughput. In this sense, a simple, rapid and semi-automated method for the determination of 33 VD residues belonging to 8 families (tetracyclines, macrolides, sulfonamides, quinolones, avermectins, benzimidazoles, aminoglicoside and penicillins) in honey samples using turbulent flow chromatography on-line extraction combined with ultra-high pressure liquid chromatography coupled Orbitrap analyzer (TFC–UHPLC-Orbitrap-MS) has been developed and validated. Once the honey samples were diluted with an aqueous solution of EDTA, they were passed through a TFC column, applying a fully automated system. Then, the analytes were retained whereas high-molecular weight matrix compounds eluted. The analytes were subsequently eluted and determined by UHPLC-Orbitrap-MS. The high resolution power of the Orbitrap analyzer was essential to obtain sufficient selectivity to enable the detection and quantification of the target compounds. The method was validated and mean recoveries were evaluated at three concentration levels (5, 10 and 50 µg/kg), ranging from 70 to 120% except for sulfonamides and penicillins, which were validated at 10 and 50 µg/kg. Intra-day and inter-day precision (expressed as relative standard deviation, RSD) were lower than 20% and 25% respectively. Limits of quantification (LOQs) ranged from 0.1 µg/kg (sulfadimidine, thiabendazole, danofloxacin, enrofloxacin, difloxacin, sarafloxacin and ivermectin) to 50 µg/kg (sulfadimethoxine, chlorotetracycline, oxfendazole, oxacillin, cloxacillin and albendazole). Finally, the proposed method was successfully applied to quantify VD in real honey samples and it demonstrated to be a fast, non-laborious, robust and sensitive method, which can be applied in routine analysis.

Keywords: Veterinary drugs, honey, semi-automated sample extraction, turbulent flow, Orbitrap

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P-9
OCCURRENCE OF ETHOXYQUINE AND ITS MAJOR METABOLITE, ETHOXYQUIN DIMER, IN AQUACULTURE PRODUCTS

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Nowadays, aquaculture or fish farming contributes to about one third to the total world production of fisheries. Aquaculture involves cultivating freshwater and saltwater populations under controlled conditions, and required large amounts of animal origin fish feed ingredients. The latter are prepared in form of meals and are applied by targeting fish carnivores species like salmons. These raw materials are very sensitive because of their high content of poly-unsaturated fatty acids. Thus, fish meals require the addition of antioxidant, not only in order to preserve their high nutritional quality, but also to avoid any risk of oxidation (and consequently any explosion risk) during their transport or storage. Ethoxyquine (EQ; 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is the most used feed additives in EU. This synthetic antioxidant is usually added in fish meals due to its ability to scavenge lipid peroxide radicals and thus terminate the spontaneous oxidation of unsaturated lipids. Therefore, EQ is subsequently accumulated in aquaculture fish products and could also consequently be absorbed by consumers. An analytical method used for screening of around 180 veterinary drugs and metabolites was used to detect and quantified EQ and its major metabolite, ethoxyquine dimer (EQDM) in fish products. An easy sample preparation with acetonitrile extraction was hyphenated to ultra high performance liquid chromatography (UHPLC) coupled to TOF or MSMS mass spectrometry. Identification of EQ, EQDM and others contaminants is based on accurate mass measurement, confirmation on MRM transitions, and quantification was performed with the standard addition technique in order to correct unavoidable matrix effects. An in-house validation procedure was conducted and allowed to fixe a limit of detection (LOD) at 10 µg/kg for EQ and EQDM. The screening method was applied to around 150 samples intended for human consumption found on the Swiss market including a large assortment of products including various fish species and with different preparations (whole, fresh, smoked, fillet) and also some fish eggs. Results obtained confirms the occurrence of EQ and EQDM in almost all tested samples. EQ was generally found in low concentrations whereas EQDM was detected in high concentrations (ppm level). However, there is still a gap in CH or EU legislation as no maximal residue limit is set due to the absence of toxicological reference values for EQ and EQDM. Therefore, the consumer risk assessment could not be conducted. The data obtained and compiled in the present work shows the important focus on food safety, particularly in relation to animals feeds. The results reported illustrate the transfer and impact of EQ from feeds to the edible parts of fish products with low EQ levels and high or very high EQDM levels.

Keywords: Ethoxyquin, Aquaculture, UPLC-MSMS, UPLC-TOF

P-10
THE PRODUCTIVE SECTOR OF HONEY IN BRAZIL AND THE PRESENCE OF RESIDUES AND CONTAMINANTS ACCORDING THE PNCRC/MAPA

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The honey produced in Brazil is recognized worldwide for its unique sensory, physicochemical and microbiological characteristics, which gives it safety, quality and guaranteed presence at the table of the most demanding consumers in Brazil, also conferring prominent position in the global agribusiness. The presence of veterinary products residues in honey may compromise its marketing, consumer health and cause damage throughout the production chain, being primordial the official control and monitoring to ensure the guarantee of food safety. The production in native areas, the presence of Africanized bees and the low prevalence of diseases in the hives, incur practically no use of veterinary products. To this end, the Ministry of Agriculture, Livestock and Supply (MAPA) established the National Control Plan for Residues and Contaminants (PNCRC). Therefore, this study sought to evaluate the presence of residues / contaminants at honey in Brazil, analyzing 1021 samples at different establishments registered at the Federal Inspection Service – SIF, from official data from PNCRC in the period from 2006 to 2010, not being detected in any of the samples the presence of residues / contaminants above established safety levels.

Keywords: Residues, contaminants, honey, quality

P-11
VALIDATION OF A MULTI-RESIDUE METHOD FOR THE DETERMINATION OF SEVERAL ANTIBIOTIC SUBSTANCE GROUPS IN HONEY BY LC–MS/MS

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Multi-methods allowing the determination of several antibiotic substance groups in different matrices in only one analytical run are of growing importance in the residue control of food. The validated antibiotic substance groups macrolides, lincosamides, quinolones, tetracyclines, sulfonamides, pleuromutilines as well as the single substances trimethoprim and dapsone are broad-spectrum antibiotics with different activities against gram-positive and gram-negative bacteria, includ-ing some anaerobes. They have been widely used in the treatment of infectious diseases. Low-level doses consumed for long periods of time, e. g. in foodstuffs, can lead to the spreading of drug-resistant micro-organisms. Maximum residue limits (MRLs) in different food matrices were established by European Regulation (EC) No 470/2009 and subsequent modifications, but no MRLs were set for honey. To ensure human food safety, antibiotics are not allowed in the treatment of bees in honey production. However, only recommended concentrations for some substances exist for the validation in honey. The method was specifically developed for the determination and confirmation of the above-mentioned substance groups in honey by LC-MS/MS in the ESI+ mode, and is based on different methods for single groups or other matrices. The method was validated mainly in a concentration range of 5 – 50 ng/g, because no MRLs are established and in such cases the spike concentrations should be as low as reasonably achievable. The validation showed that the method was applicable to samples of differ-ent kinds of honey. The validation was performed in accordance with Commission Decision 2002/657/EC on the basis of an in-house validation approach. Different influencing factors were selected with regard to the re-quirements of different samples and varying conditions in the laboratory. Therefore an efficient valida-tion approach is necessary. The validation was performed on the basis of a factor-comprehensive in-house validation concept by means of an orthogonal experimental design realised with InterVal. Using this concept the validation of the multi-method was successfully accomplished with a limited number of experiments within one validation study. The relevant validation parameters, e.g. the analytical limits (CC-alpha and CC-beta), are presented and discussed.

Keywords: multi-residue method, honey, antibiotic substance groups, orthogonal design validation, LC–MS/MS

P-12
ANALYSIS OF ESTROGENS COMPOUNDS, A CLASS OF ENDOCRINE DISRUPTING CHEMICALS USING SOLID PHASE EXTRACTION BASED ON MOLECULARLY IMPRINTED POLYMER FOR SELECTIVE EXTRACTION

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Endocrine disruptors are exogenous substances that act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones. Endocrine disrupting compounds encompass a broad class of molecules, including steroid hormones (17β-Estradiol, 17α-Ethynyl Estradiol,…). Most studies have focused on the impact of environmental compounds with hormone-like action on human development and reproductive health. However, an alternative but neglected source of these compounds is hormone residues in food for instance eggs, milk, serum and meat. To analyse the endocrine disrupting compounds, a new method is proposed for the clean-up and pre-concentration of natural and synthetic estrogens employing molecularly imprinted polymer (MIP) as selective sorbent for solid-phase extraction (SPE) suitable for complex matrices. A MIP is a synthetic material with artificially generated three-dimensional network, able to specifically rebind a target molecule. Based on this technology, we have developed a powerful technique of selective solid-phase extraction and clean-up before analysis of this class of compounds. The method was applied to the analysis of biological samples (food from animal origin or fluids) and water and compared to classical clean up. Good recoveries for Estrone, 17β-Estradiol, 17α-Estradiol, Estriol and 17α-Ethinylestradiol were obtained. These results showed the suitability of the MIP-SPE method for the selective extraction of a class of structurally related compounds such as natural and synthetic estrogens.

Keywords: solid phase extraction, molecularly imprinted polymers, Endocrine disruptors, Estrogens, food from animal origin

P-13
A SIMPLE AND RAPID UPLC–MS/MS METHOD FOR THE DETERMINATION OF CEMICAL AND IONOPHORIC COCCIDIOSTATS IN VEGETABLES

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In poultry farming anticoccidial drugs are widely used as feed additives for the prevention and treatment of coccidiosis. Coccidiostats, and veterinary medicines in general, are often poorly absorbed. As a result manure from treated animals may contain high concentrations of coccidiostats, unmodified as well as metabolized. For instance, of the administered dose of diclazuril, 98% is excreted within 10 days, of which the parent compound accounts for 85-95%. Experimental studies have shown that the uptake of veterinary medicines into vegetables from soil containing contaminated manure can occur. This gives rise to several questions regarding the impact on the environment, resistance problems and public health and allergy issues. This work will describe the development of a rapid UPLC-MS/MS method for the detection both ionophoric and chemical coccidiostats in vegetables. During method development two approaches, SPE and QuEChERS, were compared and for each method, possible critical factors (e.g. extraction solvent, volume of eluent) were statistically examined by linear regression with the use of a Plackett-Burman and a full factorial design. Final extracts were analyzed with UPLC-MS/MS operating in MRM mode. In order to optimize the chromatographic run and separation, different mobile phases were tested. Compared to the QuEChERS method, SPE resulted in higher MS responses, due to the concentration step in the SPE procedure. Matrix components however were also present in higher concentrations with the SPE method. The most efficient method was obtained by extracting with 100% MeOH. The samples were then vortexed, shaken and centrifuged. Supernatant was applied to a C18 SPE column and eluted with MeOH. After drying at 60°C under N_2 atmosphere the compounds were dissolved in MeOH/H_2O (50/50; *v/v*). A gradient elution of H_2O + 0.1% FA to MeOH + 0.1% FA on a C18 UPLC column was able to adequately separate the coccidiostats and to complete the run within 5 minutes. As this method in a later stage will be used in an experimental study enclosing different genera (roots, leaves or fruits as consumed part) and vegetables with different chemical characteristics (high water, starch or lipid content), validation of the method is performed on zucchini, tomato, carrot, potato and lettuce for following compounds: monensin, narasin, lasalocid A, salinomycin, diclazuril and nicarbazin.

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Keywords: coccidiostats, vegetables, UPLC-MS/MS, residues, uptake

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P-14
THE DEVELOPMENT OF A NEW MULTIPLEX DIPSTICK FOR THE SIMULTANEOUS DETECTION OF SULFONAMIDES, FLUOROQUINOLONES, TYLOSIN AND CHLORAMPHENICOL IN HONEY

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At present the use of antibiotics in apiculture is not permitted in Europe. However, between 2004-2009 approximately 60% of all alerts for drug residues in food of animal origin reported on the European Commission's Rapid Alert System on Food and Feed alerts (RASFFs) related to antibiotics with a high proportion being in honey. The majority of monitoring for veterinary drug residues is conducted using sophisticated laboratory instrumental equipment e.g., LC-MS/MS. Whilst this type of analysis provides quantitative and confirmatory results the associated turnaround times/costs may be unacceptable for routine screening. This situation underlines the need to develop rapid and inexpensive multiplex screening tests. An indirect competitive multiplex dipstick was developed within the EC funded Conffidence project with the aim of detecting some of the most frequently confirmed antibiotics in honey including fluoroquinolones, sulfonamides, tylosin and chloramphenicol. The dipstick was formulated as follows; freeze-dried antibodies were labelled with gold particles and the competitor conjugates were immobilized on a nitrocellulose membrane. A generic extraction was developed combining an acidic hydrolysis to release the sugar-conjugated residues with an ethyl acetate extraction/concentration step prior to reconstitution in assay buffer. The dipstick is inserted into the test sample to initiate the immunochromatographic separation. The dipstick result can be determined by visual observation or optical measurement of the appearance of four test lines. The test detects more than 10 sulfonamides (≤25 µg kg-1), 7 (fluoro) quinolones (≤25 µg kg-1) and tylosin (<10 µg kg-1). This multiplex dipstick is applicable for the rapid and simultaneous detection of the target antibiotic residues in a variety of honey types at industry relevant concentrations. An alternative format of assay for field-testing without any need of sample extraction and with a higher level of detection is also under evaluation.

Keywords: Antibiotics, Multiplex, Dipstick, Screening, Honey.

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P-15
DEVELOPMENT OF TRIAMINOSENSOR® DIPSTICK ASSAY, THE FIRST TEST DETECTING THE MAIN AMINOGLYCOSIDES IN MILK IN 6 MINUTES

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Aminoglycosides are one of the major classes of antibiotics used in veterinary medicine to treat animal infections (1, 2, 3). They are especially effective against gram negative bacteria and are frequently used alone and in association with other antibiotics under intramammarry administration to treat mastisis of dairy lactating cows (3). The detection of residual antibiotics such as Aminoglycosides in milk is of great concern to farmers, milk industry, regulatory agencies and consumers. The consumption of Aminoglicosides can indeed have many adverse effects including allergic reactions, nephrotoxicity and ototoxicity (4, 5, 6) and may induce the emergence of multi-resistant bacteria that does not respond to commonly used treatments for human illnesses (7, 8, 9). Moreover, residual antibiotics could alter the efficiency of industrial processing of raw milk toward cheese or other fermented dairy products preparation (9, 11, 12). Although some microbial inhibition screening tests have a broad spectrum of antibiotics detection in milk, they are usually unable to detect Aminoglycosides at their European Maximum Residue Limit (MRL) concentrations. In contrast, some existing immunoassays such as ELISA are very sensitive but are time-consuming and specific for only one single Aminoglycoside compound due to the very limited structural similarities between the members of this antibiotic family. We have thus developed a rapid multiplex dipstick assay for the detection of the most relevant Aminoglycosides in milk, including at least Gentamycin, Neomycin, Streptomycin and Dihydrostreptomycin at or below their respective MRLs. The dipstick membrane shows 3 specific test lines and one control line. An appropriate combination of freeze-dried antibodies labelled with gold nanoparticles induces the appearance of a strong red coloured signal to each specific test line after migration of an uncontaminated milk sample along the dipstick. On the opposite way, the presence of residual Aminoglycosides in the milk will prevent the specific antibodies to bind the test lines and will not induce any red signal to appear on the dipstick membrane. The results can be quickly interpreted by visual observation or by an optical reader measurement. In this work, we are presenting the first multiplex dipstick assay that can detect/discriminate Gentamycin, Dihydro/Streptomycin and Neomycin contamination (≤ MRL) in one single milk analysis of 6 minutes. This "Triaminosensor®" dipstick assay is commercialized at Unisensor S.A. since July 2011 under reference KIT048.

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Keywords: Antibiotics, Aminoglycosides, Dipstick, Screening, Milk.

P-16
ANALYSIS OF AMINOGLYCOSIDES IN HONEY BY HILIC/MS/MS

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Aminoglycosides are a class of antimicrobials used in veterinary practice and also in apiculture to treat gram-negative bacterial infections. Streptomycin is one of the most used antibiotics in beekeeping. In EU, there are no MRLs set for the presence of aminoglycosides in honey, and in some European countries they are not allowed to be used for treating honey bees. From an analytical perspective, aminoglycosides are basic and highly polar compounds which contain several amino groups with different pKa values. The chromatographic separation of aminoglycosides in a reverse phase column requires the use of strong ion pairing agents, which cause severe ion suppression and also contaminate the instrument. As an alternative, Hydrophilic Interaction Liquid Chromatography (HILIC) is used to avoid the use of ion pairing agents. In this work, the chromatographic behaviour of 10 aminoglycosides in different HILIC stationary phases (bare silica, amide, amino and zwitterionic) has been systematically studied. Among these stationary phases, the zwitterionic phase provided better separation of aminoglycosides and the effect of pH, ionic concentration and column temperature on retention time, peak shape and sensitivity was studied using an experimental design. During the method development, crosstalk between MS/MS channels of the analytes was observed and resolved. Concerning sample preparation, Weak cation exchange (WCX) solid phase extraction (SPE) and strong cation exchange (SCX) SPE were assessed for extracting aminoglycosides from honey. WCX SPE proved to be adequate for all the aminoglycosides studied as it provided better recoveries than SCX SPE. Four different brands of WCX cartridges were assessed and considerable differences were observed in recoveries. The highest recoveries (69−95%) were obtained with Waters Accell Plus CM (WCX) cartridges. This method is validated according to the European Commission Decision 2002/657/EC.

Keywords: Aminoglycosides, HILIC, Experimental design, Honey, Weak Cation Exchange SPE

P-17
DEVELOPMENT AN ENZYME-LINKED IMMUNOSORBENT ASSAY SCREENING FOR FLUOROQUINOLONES IN MILK, EGGS AND FISH

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Fluoroquinolones (FQs), such as enrofloxacin and ciprofloxacin, are among the most commonly used antibiotics for the treatment of bacterial infection. They are widely used both for animals and for humans. The potential contamination of animal origin foodstuffs with FQs made it necessary for monitoring the quality of these materials and to develop a method to screen for the possible presence of the drug residues. An indirect competitive enzyme-linked immunosorbent assay (ELISA) was developed for the detection of enrofloxacin and ciprofloxacin using polyclonal rabbit's antibody. The limits of detection (LOD) of the ELISA for enrofloxacin and ciprofloxacin in phosphate buffer were 0.1 and 1 ng/mL, respectively. The assay showed little cross-reactivity (<0.1%) with enrofloxacin structural analogues, except for ofloxacin (15%). Development technique was optimized for eggs, milk and fish analysis. The sample preparation method was developed. The average recoveries for enrofloxacin from fortified eggs, milk and fish samples, at three concentrations of 10, 50, 100 ng/g, were 80−105, 82−97 and 84−115%, respectively. The LOD in eggs, milk and fish for enrofloxacin was 1, 0,5 and 2 ng/g. The 10 milk samples and 10 eggs and 10 fish samples were analyzed and results were submitted by HPLC-MS.

Keywords: fluoroquinolones, ELISA, limits of detection, drug residues

P-18
THE ESTABLISHMENT OF AN ANALYTICAL METHOD FOR THE RESIDUE OF DICYCLANIL IN MUSCLE TISSUE OF CATTLE

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High performance liquid chromatography (HPLC) is the method of choice in checking purity of new drug candidates, synthetic procedures, evaluating new formulations, etc. HPLC method development provides in particular an interesting application in the evaluation of analytical methods of drugs. Presently, a simple and rapid method using high performance liquid chromatography (HPLC) based on solid phase extraction (SPE) for the determination of veterinary drug dicyclanil in cattle muscle was developed. Sample workup involved homogenizing cattle muscle tissues, solid-liquid extraction with acetonitrile and the extracts were analyzed by HPLC with gradient elution on a C18 column and C8 guard column with ultraviolet detector at 267 nm. The calibration graphs were rectilinear from 0 to 750 ppb. The limit of detection, (LOD) and the limit of quantification, (LOQ) of the dicyclanil in the cattle muscle (following maximum residual limit standards, (MRLs) viz., 150 ppb) were 1.08 ppb and 3.26 ppb respectively, also the cattle muscle showed recoveries between 96.4 and 98.5%. The method is regulatory one and suitable for determination of residues of dicyclanil in cattle muscle tissue. To validate the established method, calibration curve, linearity and precision were examined and found to be suitable and comparable with reference to Codex standards.

Keywords: Dicyclanil, Veterinary Drug, Residue, Cattle

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P-19
VALIDATION OF TRISENSOR® ASSAY, THE FIRST DIPSTICK TEST DETECTING THREE OF THE MOST IMPORTANT ANTIBIOTICS FAMILIES IN MILK IN 6 MINUTES

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Beta-lactams, sulfonamides and tetracyclines are the major classes of antibiotics widely used by veterinaries and farmers to treat animal infections. The risks due to the presence of antibiotic residues in milk were quickly identified as well by the health authorities as by industry. To preserve the health of the consumers and to respect the EU regulation, protocols of quality control of milk for its payment were developed in many countries, supplemented by a monitoring of the sensitive molecules, either prohibited or not detected by the analytical protocol. These protocols, generally based on microbiological tests generating a global inhibition result, are often time consuming. Also, the introduction of the "fast" tests into these protocols has an undeniable advantage: obtaining the results more quickly, qualitative information (identification of the antibiotics family) and quantitative. Moreover, these fast tests have their place in monitoring. For example, the monitoring results obtained in Belgium in 2006, show the importance of the detection of the tetracyclines in the tank milk. Before their official use (payment or monitoring), the rapid tests must be evaluated and validated. This validation is based on the European regulation and the guide lines of the European Union Refers Laboratory for Antimicrobial and Dye Residues in Food (Laboratory of Fougeres, France). In this work, we are presenting the evaluation of the TriSensor® Assay (Unisensor SA*, Belgium), a multiplex dipstick assay using specific receptors and generic monoclonal antibodies for simultaneous detection of all Betalactam, Tetracycline and Sulfonamides compounds. The results, quickly interpreted by visual observation or by an optical reader measurement, are visualized at the 3 specific capture lines by the use of colloidal gold-conjugates. This validation includes the determination of the Limitis Of Detection (LOD) for 29 molecules from the three antibiotic families and the comparison with their respective Maximum Residue Limits (MRL). The interferences caused by 15 molecules belonging to other antibiotics or therapeutics families (10 * LMR) were also evaluated (including false-positive and false-negative results). The effect of natural milk parameters (composition, quality, status of the cows) on the test was assessed. An international interlaboratory study completes this evaluation.

Keywords: Antibiotics, Sulfonamides, Tetracyclins, beta-lactams, Validation, Screening, Milk

P-20
BRAZILIAN PROFICIENCY TESTING SCHEME FOR THE SCREENING AND CONFIRMATION OF TETRACYCLINES RESIDUES IN MILK

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Proficiency Testing (PT) by interlaboratory comparisons is used as a powerful tool to evaluate and demonstrate the reliability of laboratory test results. The offer of PT schemes, as well as reference materials (RMs)/certified reference materials (CRMs), is still scarce in the field of analysis of veterinary drug residues. The cost of imported RMs/CRMs, the expensive participation fees in European PT schemes and bureaucracy in customs clearance are additional problems for Brazilian laboratories, reinforcing the need of national RM/CRM producers and PT providers. For this reason, an in-house RM of oxytetracycline (OTC) in milk was prepared [1] and samples used for the realization of a PT. Although LC-MS/MS is the technique of choice for residue analysis, several laboratories belonging to the Brazilian network of public health laboratories employ ELISA commercial test kits. Therefore, this PT was designed to include laboratories in charge of screening and/or confirmatory analysis. After the treatment of an animal (crossbred cow Dutch/zebu) with OTC, milk was collected, partially skimmed and stored at <-70°C. A negative control was also produced. Two lots of lyophilized samples were obtained and both showed to be homogeneous and stable. Four sample flasks (two of the OTC-free material and two of the incurred milks) were freely distributed to each laboratory. All 15 participants that reported screening data accomplished satisfying results, with the exception of one laboratory, that presented a false positive for one of the incurred samples. The assigned values and their standard deviations were obtained by the robust mean of the results issued by all participant laboratories of the quantitative PT that reported recovery values and showed no outliers (8 laboratories), besides INCQS. According to the z-scores, 4 of the 8 laboratories obtained questionable or unsatisfactory results for at least one of the analyzed lots. Of a total of 16 reported results, 10 were considered satisfactory, 2 questionable and 4 unsatisfactory [2].

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[2] INCQS. Abril 2011. Relatório do Ensaio de Proficiência para Determinação de Medicamentos Veterinários em Alimentos 3ª Rodada – Matriz Leite em Pó.

Keywords: proficiency testing, tetracyclines, milk

Acknowledgement: ANVISA; all participants in the PT; CNPq and FINEP (financial support)

P-21
EFFECTIVE SAMPLE PREPARATION FOR MULTI-RESIDUE LC–MS DETERMINATION OF VETERINARY DRUGS IN MEAT AND MILK

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To ensure food safety, there is a need for multiresidue UPLCMS methods that can identify and quantify a wide range of veterinary drug residues from many drug classes. Solvent extraction can be effective for many of these compounds in meat and milk. However, highly water soluble drugs such as sulfanilamide and salbutamol may not be well recovered using this approach. If, instead, an aqueous buffer is used for extraction then there is poor recovery of fat soluble compounds such as phenylbutazone and dexamethasone. In this poster we will discuss effective sample preparation to maximize recovery of the widest possible range of veterinary residues in meat or milk. Also, we will present effective SPE cleanup protocols for reduction of matrix effects detrimental to good UPLC-MS performance. Representative compounds from seven classes of veterinary drugs (tetracycline, macrolide, fluoroquinolone, sulfonamide, beta-lactam, steroid and beta-adrenergic) were selected to demonstrate the effectiveness of the methodology.

Keywords: UPLCMS, SPE, veterinary residues

P-22
DISTRIBUTION OF TYLOSIN RESIDUES IN HONEY MATURATION TANK

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Tylosin is an antibacterial illegally used in EU for the treatment of Paenicillus larvae infections of honeybees. Treatment of bees with tylosin could cause a contamination of honey. This study would demonstrate that this veterinary drug showed a different distribution in honey stored in tanks. Two different varieties of honey (acacia and wildflower) from two tanks (200 litres each one) were collected at 13 different depths of each thanks and analyzed. In order to help tylosin residues stratification, honey were stored at room temperature for 3 months and the week before the sampling, they were stored at 4±2°C. To make sampling as a standard procedure, each tank was divided into 13 hypothetic layers (about 5 cm at each rate) and 13 samples were collected from each layer: every sample was collected in duplicate. Visual differences among honeys layers were observed: Acacia honey in the first tank was homogeneous, clear and smooth; instead, in the second tank, wildflower honey was not homogeneous, dark, crystallized and compact, especially in the deeper layers. Samples were extracted with a buffer solution, cleaned up by SPE cartridge and tylosin residues were analyzed by high performance liquid chromatography coupled with mass spectrometry. Concentrations of tylosin residues among layers of each tank resulted quite different. In the first one, upper layer presented higher levels of tylosin (2.0−3.8 µg/kg) than the lower layers. Otherwise, the second tank showed the highest level of tylosin in deeper layers (0.9−3.2 µg/kg), where honey was more compact and not homogeneous. These results could be related to the different density and crystallization degree of analyzed honeys. This study shows that different concentrations of tylosin residues could be detected into layers tank, after honey stratification and suggests other investigations to improve the procedure of honey sampling.

Keywords: Honey, maturation tank, stratification, tylosin

P-23
LOW LEVEL DETERMINATION OF VOLATILE NITROSAMINES IN SMOKELESS TOBACCO USING GC–MS/MS

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Volatile nitrosamines (VNA) are a class of compounds that are known to pose significant health risks in tobacco. Measuring VNAs has traditionally been accomplished using gas chromatography (GC) and thermal energy analyzer (TEA) detection. This technique supports the detection of the VNAs, but poses many challenges. First, regulations continue to drive detection limits lower than what are achievable using GC-TEA. Second, TEA detection is not as specific as other detection techniques. Third, GC-TEA does not allow further analysis of the tobacco for other contaminants. GC-MS/MS (TSQ Quantum XLS) in timed selected reaction monitoring (t-SRM) mode was used for the sensitive analysis of VNA in smokeless tobacco. This method achieved detection limits to 1 ng/mL and limits of quantitation at 2 ng/mL for six VNAs, using a 1 µL injection volume, 0.5 µg/kg and 1 µg/kg in sample respectively. Injecting small volumes was important to avoid contamination of the system. All method development and calibration curves were performed using spiked uncontaminated tobacco.

Keywords: VNA, nitrosamines, GC-MS/MS, tobacco

P-24
DISTRIBUTION OF TETRACYCLINES RESIDUES IN BEEHIVE

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European and American foulbrood are an important and contagious disease of honeybees. Beekeepers often manage them with an early, but illegal treatment in beehive with veterinary drugs of tetracyclines family during wintertime. In order to evaluate distribution and persistence of tetracycline drugs group into a beehive, a bees colony was treated with two different drugs, chlortetracycline (CTCC) and oxytetracycline (OTC) at one year apart. The first treatment was done with 0.25 g of oxytetracycline dissolved in sugar syrup to feed the bees: the second was realized in the same beehive one year after, using 0.6 g of chlortetracycline mixed with powdered sugar spread on the frames. Two years after the last treatment, a large number of samples were collected from the beehive (portions of the comb, bees, wooden walls of the beehive, beeswax, honey and propolis). To analyze the different matrices a change of method used for the analysis of honey was applied. Samples were extracted with a solution of succinic acid, cleaned up by SPE cartridge and analyzed by high performance liquid chromatography coupled with mass spectrometry. Results showed a high contamination of chlortetracycline (CTCC), oxytetracycline (OTC) and tetracycline residues (TC) in wooden walls of the beehive (1397 µg/kg of CTCC, 160 µg/kg of OTC and 1232 µg/kg of TC). The mean concentrations of the beehive cap were 1109 µg/kg of CTCC, 1278 µg/kg of OTC and 366 µg/kg TC, while the down side of the hive showed the higher concentrations of chlortetracycline (99958 µg/kg): otherwise the concentrations of oxytetracycline and tetracycline were lower than other sites (8 µg/kg OTC and 73 µg/kg TC). Significant concentrations of CTCC and TC were also detected in beeswax (2119 µg/kg of CTCC and 2274 µg/kg of TC) and propolis (23065 µg/kg of CTCC and 2079 µg/kg of TC) collected from the beeframes. The tetracyclines residues in brood-combs were higher than those in the honey-combs. Otherwise, relatively low concentrations of tetracyclines were detected in bees. The presence of tetracycline residues although the molecule wasn't used during the treatment, is probably due to the presence as impurity of the drug or to degradation processes. The higher concentrations of chlortetracycline in the down side of the hive was probably because the contaminated powdered sugar spread on the frames used in excess for the treatment had deposited at the bottom causing a persistent contamination source, while the oxytetracycline used in sugar syrup caused a more homogeneous distribution of the drug. The study shows that tetracyclines residues in beehive are persistent even after many years from the antibiotic treatment: furthermore the different residues distribution into the hive and its products is probably due to various causes, including the method of drug administration

Keywords: Tetracyclines distribution, beehive, chlortetracycline, oxytetracycline

P-25
DEVELOPMENT OF FLUORESCENCE POLARIZATION IMMUNOASSAY FOR FLUOROQUINOLONES

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Wide varieties of chromatography methods are available for the determination of fluoroquinolones in food samples. Immunochemical methods of analysis like ELISA, immunochromatographic strip-test and biosensors are more commonly used now. Another immunochemical method is fluorescence polarization immunoassay (FPIA). FPIA is homogeneous immunoassay based on competition between the test component (antigen) and a tracer (antigen labeled with a fluorescent label) for a limited number of antibody binding sites. The high value of tracer fluorescence polarization is reached upon the binding of the tracer in a complex with antibodies. An increase in the analyte concentration in solution resulted in a decrease in the concentration of the tracer–antibody immune complex and a corresponding decrease in the fluorescence polarization. Consequently, the results obtained by FPIA strongly depend on the quality of the tracer and the antibodies and, especially, on their specificity with respect to each other. Thus, an important stage in the development of sensetive FPIA is a search for an appropriate pair of immunoreagents. Several tracers were synthesized using FITC and fluoroquinolones with second amino-group. The optimal tracers were synthesized from Sarafloxacin or Clinafloxacin. The tracers QF-FITC were purified and three main bands from TLC (Rf: 0.1, 0.2, and 0.4) were obtained. One band (Rf: 0.1) of them was tracer, which showed good binding to anti-FQ monoclonal antibodies. The optimized FPIA had a detection limit of 1 ng/mL, linear working range from 10 to 1000 ng/mL and within-assay coefficient of variation less than 4%. Cross-reactivity studies demonstrated this method is capable of detection simultaneously of several FQ as sum. The total time of determination of FQ in 10 samples was no longer than 10 min. The FPIA is a useful alternative approach to chromatography and ELISA because it is a homogeneous method without any separation and washing steps and with the advantage of simplicity and high p! recision.

Keywords: fluorescence polarization immunoassay, fluoroquinolones

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P-26
SIMULTANEOUS FLOW CYTOMETRIC DETECTION OF RESIDUES OF TETRACYCLINES, FLUOROQUINOLONES AND AMPHENICOLS IN MEAT AND KIDNEY SAMPLES

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There is an ongoing demand for effective screening assays for residues of veterinary drugs to monitor their use in livestock and to secure the safety of food from these animals. So-called suspension array is a new trend in (immuno)affinity screening methods enabling the detection of multiple groups of different veterinary drugs simultaneously while still revealing the identity of each group, i.e. so-called multi-analyte or multiplex methods. A bead-based flow cytometric affinity assay for the simultaneous detection of residues of amphenicols (bead A), fluoroquinolones (bead F) and tetracyclines (bead T) in meat and kidney samples was developed and validated according to the European Commission Decision 2002/657/CE. Validation was performed using blank meat and kidney samples spiked with antibiotic constituents from the three antibiotic families that are relevant in the veterinary field. The blank samples were analyzed by a confirmatory liquid chromatographic-mass spectrometric method to confirm the blank status of the meat and kidney samples prior to validation of the method. Using the three beads together, residues of tetracyclines, fluoroquinolones and amphenicols could be detected at or below their respective maximum residue limits (MRLs) set in the Commission Regulation 37/2010. Chloramphenicol could be detected at the Minimum Required Performance Level (MRPL) set in Belgium (0.1 µg kg^{-1}). The principle of the bead-based flow cytometric method, sample preparation procedure, performance parameters, validation of the method and pros and cons of the method in comparison to other multi-analyte screening methods are presented and discussed. The method will be discussed, particularly, with focus on the increase of sample throughput and its potential to extend the range of analytes that can be determined simultaneously.

Keywords: Multi-analyte, bead-based assay, flow cytometry, veterinary drug residues

P-27
MULTI-RESIDUE DETERMINATION OF VETERINARY DRUGS AND PHARMACEUTICAL RESIDUES IN DAIRY PRODUCTS AND EGG USING LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Veterinary drugs are widely used in dairy cattle management for the treatment and prevention of diseases. Their extended use may result in drug residues being present in milk, especially if they are not used according to label directions. The presence of residues and its associated harmful health effects on humans makes the control of veterinary drug residue an important measure in ensuring consumer protection. Moreover, general pharmaceuticals for human medication are considered as widespread emerging pollutants with potential to enter the food chain. Therefore, multiclass, multiresidue methods for the determination of veterinary drugs and general pharmaceuticals in milk and dairy products are of great need. In this work, a method was developed for the simultaneous determination of a large number of veterinary drugs and other pharmaceutical residues in milk, butter, cheese and egg. The determined analytes belong to different classes of drugs (sulfonamides, tetracyclines, quinolones, beta-lactams, macrolides, lincosamide antibiotics, NSAIDs, anthelmintics, coccidiostats, benzimidazoles, phenicols, beta blockers, diuretics, statins). Determination was performed in both positive and negative ionization mode and the separation was held on Atlantis T3 C18 column (100 mm × 2.1 mm, 3 μm). In both cases gradient elution was used with the mobile phases being methanol − 0.01% formic acid for the positive determination and methanol-acetonitrile-ammonium formate 1mM for the negative determination. For the sample preparation two different strategies were tested. In the first one a generic extraction was performed with no further cleanup step, while the second one consists of a Solid Phase Extraction approach using Strata-X cartridges. In both cases validation data were obtained.

Keywords: multiresidue-multiclass method, veterinary drugs, pharmaceutical residues, LC-ESI-MS/MS, dairy products

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P-28
PHARMACEUTICAL PRODUCTS IN SURFACE AND DRINKING WATER: A BELGIAN SURVEY

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Pharmaceutical products represent an emerging group of environmental contaminants. As urban wastewater treatment plants show actually poor removal efficiency for several veterinary and human drugs, these compounds are widely present in the aquatic environment and could therefore be potentially present in both surface and drinking waters. The present work is a survey conducted in Wallonia (region located in the South of Belgium) to identify the most present and problematic compounds in surface and drinking water. An analytical method was first developed to simultaneously extract, detect and confirm 130 pharmaceutical products (PPs) and pesticides in water, including a purification/concentration step on an Oasis HLB column and analysis by UPLC-ESI-MS/MS. Targeted PPs were selected from Belgian consumption data, previously published works and from the list of relevant PPs of the European KNAPPE project. Persistent and/or problematic pesticides were also included in our work. The sampling of surface water was performed from September 2010 to January 2011. Our strategy was to select 68 sites, corresponding to wastewater treatment plants (WWTP) discharge points into a stream or a river. For most of the WWTP, two water samples were collected from the river bank, upstream and downstream of the discharge point. A manual sampling was performed, leading to 132 water samples stored in 2-liters glass bottles until analysis. 36 drinking water samples were also collected, mainly from Wallonia but also from Brussel. Our results show that pharmaceutical products and pesticides are present in both surface and drinking water. Non-steroidal anti-inflammatory drug (NSAID), analgesic, antibiotic, antihelmintic, anti-epileptic and bronchodilator are present in both water types when additional drugs classes were detected in surface water, such psychotropic, statin, diuretic, antihypertensive, lipid regulator and beta-blocker.

Keywords: water, pharmaceutical products, pesticides, UPLC-MS/MS

P-29
DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND THEIR METABOLITES IN MILK BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Non-steroidal anti-inflammatory drugs are widely used in the treatment of animals. According to Council Directive 96/23 residues of these drugs have to be monitored due to the potential risk they pose to the consumers' health. Differences in chemical properties and low target concentrations in milk (values of maximum residue limits or concentrations recommended by European Union Reference Laboratory) make liquid chromatography-tandem mass spectrometry (LC-MS/MS) a preferable technique for both screening and confirmatory purposes. Considering above, the method for the detection of wide range of NSAIDs was developed. It included both "acidic" NSAIDs (carprofen, diclofenac, flunixin, meloxicam, phenylbutazone, oxyphenbutazone, tolfenamic acid, mefenamic acid, naproxen, ketoprofen, ibuprofen, firocoxib, rofecoxib, celecoxib) and "basic" NSAIDs (four metamizole metabolites). Analytes were extracted from milk samples with acetonitrile in the presence of ammonium acetate. One portion of extract was directly analysed for the presence of metamizole metabolites, whereas the second one was additionally cleaned-up with amino cartridge. All NSAIDs were separated on Phenomenex Luna C8(2) column with 30 min gradient of methanol : acetonitrile (and 0.05 M ammonium formate (pH 5.0) solution and analysed by LC-MS/MS technique in negative (acidic NSAIDs) and positive (metamizole metabolites) ionization. Method was validated according to the requirements described in the Commission Decision 2002/657/EC. Within-laboratory reproducibility was in the range of 5−35%, with recovery in the range 50−105%. The method enabled the detection of all analytes with expected sensitivity, below the recommended concentrations. The developed method fulfils the criteria for confirmatory methods and thanks to its labour efficiency; it may be used also for screening purposes. The developed procedure was also successfully verified in the proficiency test organized by EU-RL in 2010. According to authors' knowledge, this is one of the first methods able to detect diclofenac residues below MRL value in milk (0.1 µg/kg). Its additional advantage is the possibility of simultaneous determination of "acidic" NSAIDs and metamizole metabolites.

Keywords: NSAIDs.metamizole.milk.residues.2002/657/EC

P-30
I'SCREEN SULFA QL: A NEW QUALITATIVE ENZYME IMMUNOASSAY FOR A RAPID AND SENSITIVE DETECTION OF THIRTEEN SULFONAMIDES IN FOOD

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Sulfonamides are a group of broad antibacterial drugs widely used in veterinary medicine for the treatment of bacterial infections and as growth-promoting feed additives. As a consequence, residues can be present in food from animal origin, causing allergic reactions, hepato- and nefrotoxicity and selection of resistant bacteria in exposed consumers. Maximum Residue Limits (MRL) for sulfonamides have been established in many countries. In the European Union, in the USA and in Canada an MRL of 100 µg/Kg was set for total sulfonamides in edible tissue and in milk, while the use of these antibiotics is unauthorised in eggs producing animals. No safe limits have been established for honey, that should contain "zero" level of antimicrobials. I'screen Sulfa QL, a qualitative enzyme immunoassay for the broad range screening of sulfonamides in tissue, milk, honey and eggs was developed. For this purpose, a polyclonal antibody was arisen in rabbit against the sulfanilamidic moiety of the molecule, which is shared by all sulfonamides. Thanks to antibody cross-reactivity, at least thirteen of these drugs can be detected by the assay. Positive controls were built in order to identify contaminated samples at different cut-offs: 10 or 50 ppb of sulfamethazine in muscle, 15 ppb of sulfamethazine in eggs, 20 ppb of sufamethazine in milk and 8 ppb of sulfathiazole in honey. According to the assay specificity, about eight other sulfonamides can be detected with sensitivities up to 5 ppb or less. Moreover, high specificity was obtained for blank samples. Validation results show that I'screen Sulfa QL is a reliable tool for a rapid and cost effective screening of sulfonamide residues in food of animal origin. The assay sensitivity is compliant with the detection capabilities required by EU regulation (37/2010/EC). At the moment, I'Screen SULFA QL is the only enzyme immunoassay on the market that can detect with high sensitivity such a large number of the most frequently used among these antimicrobial drugs.

Keywords: Sulfonamides, enzyme immunoassay, screening

P-31
FIVEPLEX FLOW CYTOMETRIC IMMUNOASSAY FOR THE SIMULTANEOUS DETECTION OF SIX COCCIDIOSTATS IN FEED AND EGG

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Different coccidiostats are used as feed additive to control coccidiosis in poultry. For consumers protection, maximum levels (ML's) have been set by the European Union (regulation 124/2009 and directive 2009/8/EC) and monitoring has to be performed. A fiveplex flow cytometry-based immunoassay (FCIA) was developed within the EU-project CONffIDENCE for the simultaneous detection of 6 frequently used coccidiostats. This FCIA uses carboxylated polystyrene microspheres (xMAP technology, Luminex®), internally dyed with a red and orange fluorophore. By using different ratio's of these two fluorophores, 100 different microsphere sets are created which can be chemically coupled with antigens (drug or drug-protein conjugates). At the moment only 5 microsphere sets are being used for this assay but new developed assays can be added, at any time, to broaden the scope of this multiplex. The assay uses new and previously developed coccidiostat-specific polyclonal antibodies and R-phycoerythrin labeled second antibodies and their binding to the microspheres is analyzed in a dual laser reader containing a red laser for identification of the microsphere sets and a green laser for the quantification of the amount of antibodies bound to the beads. All coccidiostat assays developed in this multiplex format are competitive inhibition immunoassays. Results will be presented about this application for the simultaneous detection of narasin, salinomycin, diclazuril, lasalocid, monensin and nicarbazin in feed and egg extract.

Keywords: Coccidiostats, Multiplex flow cytometric immunoassay, Luminex, Egg, Feed

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P-32
FAST, WIDE–RANGE SCREENING OF BANNED VETERINARY DRUGS IN URINE BY LIQUID CHROMATOGRAPHY COUPLED TO HIGHVRESOLUTION MASS SPECTROMETRY

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The use of some pharmacologically active substances such as hormones and ß-agonists is prohibited in stock farming. A maximum residue limit has not been established due to the high toxicity represented by these substances to the human health. Low reporting levels in urine (1 µg L^{-1} or below) must be analytically achieved in order to successfully monitor the administration of the banned compounds. LC-MS/MS analysis, by making use of a triple quadrupole analyser, is the methodology of choice for confirmatory targeted analysis of veterinary residues because it offers high sensitivity and selectivity. However, for multi-class, multi-component analysis this approach requires extensive compound-dependent parameter optimization. Likewise, for confirmatory purposes, two SRM transitions are required which limits the number of compounds in a run, and for some analytes only non-specific transitions or just only one transition can be selected [1]. Full scan approaches using high resolution mass spectrometry (HRMS) can provide a rapid and sensitive wide-range screening of veterinary drugs overcoming the limitations of SRM analysis [2]. In this work, an ultra high performance liquid chromatography − HRMS (UHPLC−HRMS) methodology is proposed for the multi-residue screening of veterinary drugs in urine by making use of an Orbitrap analyser − Exactive™. A resolving power of 50,000 FWHM was selected while working at full scan mode in both positive and negative mode. Urine samples were extracted with acetonitrile followed by a cleanup with dispersive-SPE with PSA/C18 and centrifugation for the removal of matrix components. The final extract was re-dissolved using water-acetonitrile (95:5). A good chromatographic separation was achieved using an Accucore PFP column (100×2.1 mm, 2.6 µm) at 35°C. A high mass accuracy

Keywords: veterinary drugs, screening, urine, LC−HRMS, Orbitrap

P-33
SUPERSCREEN TETRA HS: A SUPERSENSITIVE ENZYME–RECEPTOR ASSAY FOR HIGH THROUGHPUT DETECTION OF TETRACYCLINES IN FOODSTUFFS

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Tetracyclines are broad-spectrum antibiotics, often used in veterinary practices to prevent and control some diseases and to increase animal's ponderal state. SuperScreen TETRA HS is a receptor assay based test kit for quantitative determination of tetracyclines in different matrices of animal origin. A DNA target sequence, immobilized onto a microtiter plate, and tetracyclines in standards/samples compete for binding to the receptor. The method allows the detection of a wide range of tetracyclines (tetracycline, oxytetracycline, chlortetracycline, doxytetracycline) with high sensitivity. The assay procedure is ELISA-like and the total assay time is 90 minutes. For all matrices, sample preparation is very simple and involves a single dilution step in buffer. Different tetracycline measuring ranges make the assay suitable for honey, muscle, meat drip (3.75−50 ppb) and raw milk (7.5–100 ppb). Kit specificity and sensitivity with the lower crossreacting molecule (oxytetracycline) have been assessed according to criteria of European Decision 657/2002: in all cases SuperScreen TETRA HS CCbeta are much lower than EU MRLs.

Keywords: Tetracyclines, receptor assay, screening

P-34
APPLICATION OF VERY HIGH PRESSURE NANO-LIQUID CHROMATOGRAPHY COUPLED TO TIME-OF-FLIGHT MASS SPECTROMETRY FOR VETERINARY DRUGS

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The newfangled analytical tools available for screening of small molecules such as veterinary drugs, hormones and pesticides are based on ultra performance liquid chromatography (UPLC) coupled to Time-of-Flight mass spectrometry (ToF−MS). This technique allows screening for a theoretically unlimited number of compounds and gives the possibilities to search, retrospective, for new emerging compounds. New emerging compounds can be for example detected in advance by a bioactivity and/or biorecognition assay. The volume of the extract is small

Keywords: nanoUPLC, sustainable chemistry, veterinary drugs, Time-of-flight

P-35
DATA WAREHOUSING IN RESIDUE AND CONTAMINANT ANALYSIS

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New techniques for chemical screening based on (multidimensional) chromatography with (high-resolution) mass spectrometry makes it possible to screen for a large number of different residues and contaminants in food and feed. The bottleneck of these techniques is the amount of data generated and the tools available for exploration of the data. These tools are often brand or instrument specific. Within RIKILT a generic software tool has been developed which is capable to reduce the size of data files (irrespective brand or vendor) without losing scientific mass spectrometric information. The software tool (metAlign) has been combined with in-house developed search software such as Search_LCMS and Search_GCMS. The tool Search_LCMS (or Search_GCMS) can be used to search a data file within seconds for a theoretical unlimited amount of compounds. This search tool uses a simple database consisting out of a comma separated value (CSV) data file which contains information about; compound name, mass, mass deviation tolerance, retention time and retention time deviation tolerance. These CSV files can be prepared in Microsoft Excel©. The development of these software tools and static databases are a first step to help analytical chemist who are facing a huge challenge when it comes to efficient verification of compliance of large numbers of residues and contaminants in multiple sample matrices. Preferably, the chemist is able to search at the same time for the occurrence of 'new' contaminants which are not yet regulated. The next step is to make these static local databases more dynamic and in such way that people from other institutes and companies can access these search databases. Currently, the static data is transferred to an online dynamic database which contains an accompanying web interface. Now every computer connected to the internet can access this web interface and analytical chemist are assisted in downloading the proper database and metAlign settings. Furthermore, extra information is available in the online database such as fragmentation data, isotopic pattern and linkage to compound specific information in the chemspider database. The developed approach will improve consistency of the results and also makes data processing more efficient.

Keywords: data warehousing, high resolution mass spectrometry, databases, metAlign

P-36
DETERMINATION OF AMINOGLYCOSIDES IN RAW COW'S MILK

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The aminoglycosides (AG) are a large group of broad-spectrum antibiotics of bacterial activity against some Gram-positive and many Gram-negative bacteria. Their residues in food of animal origin such as milk can be potential hazard for the human health as they may cause an increased resistance of microorganism strains. Maximum residual limits have been established by EU community and applied methodologies for AG analysis must provide high specificity and sensitivity. AGs characteristically contain two or more aminosugars linked by glycosidic bonds to an aminocyclitol component. Due to their polarity they exhibit extreme solubility in water. The method for the quantitative determination and confirmation of dehydrostreptomycin, streptomycin, kanamycin, neomycin and gentamicin is presented in this study. Sample of milk was defatted by centrifuging 4000 rpm/15 min/4°C and removing fat layer, proteins were removed by precipitation with 30% TCA solution, vortexing and centrifuging at 4000 rpm/10 min/4°C. The supernatant was adjusted to pH 7–8 by KOH solution. Mixture was again centrifuged for 3 min. and supernatant was applied to WCX SPE cartridge. After conditioning and washing the cartridge with methanol the elution with 2% acetic acid was performed. The eluent was evaporated, reconstituted with mobile phase and analysed by HPLC. For HPLC determination HILIC chromatographic columns (X-Bridge HILIC) in isocratic mode and with ELSD detection (2424 ELSD, Waters) on Alliance 2696 separation module (Waters) was performed. Mobile phase consisted of mixture 0.1% formic acid and acetonitrile. Parameters of ELSD detection were set as follows: N_2 pressure 50 psi, drift tube temperature 55°C. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) with positive electrospray ionization was used for confirmation of AG residues in milk. Data acquisition was carried out in selected reaction monitoring (SRM) mode, monitoring two SRM transitions for each aminoglycoside. Ions transitions monitored in SRM mode were m/z 584.2 -> 246.1 and 263.2 for dihydrostreptomycin; 582.2 -> 246.1 and 263.2 for streptomycin, 485.1 -> 205.1 and 163.1 for kanamycin; 615.2 -> 163.1 and 161.1 for neomycin and 478.2 -> 322.2 and 157.1 for gentamicin, respectively. In case of LC/MS/MS confirmation, the limit of detections were 0.07 µg/l for dihydrostreptomycin; 0.54 µg/l for streptomycin, 0.53 µg/l for kanamycin; 0.38 µg/l for neomycin and 0.41 µg/l for gentamicin, respectively. The method was validated according to the EU requirements.

Keywords: Aminoglykosides, Milk, HPLC

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P-37
SURVEY OF TETRACYCLINE ANTIBIOTICS IN FOODS, KOREA

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This study has been conducted to determine the residual amount of tetracycline antibiotics such as oxytetracycline(OTC), tetracycline(TC), chlortetracycline(CTC), doxycycline(DC) in stock farm products(beef, pork, chicken, eggs and milk) and marine products(flatfish, jacopever, eel and shrimp). Samples were purchased from the markets located in the major cities (Seoul, Busan, Incheon, Daegu, Daejeon, Gwangju, and Ulsan) in Korea. Tetracycline antibiotics were analyzed by HPLC-PDA according to the Korean Food Code. For the confirmation of detected antibiotics in samples, positive samples were analyzed by LC/MS/MS according to the Korean Food Code. All the monitoring data were satisfied with criteria of CODEX, which located within 70~120% recovery range, less than 20% of relative standard deviations. Tetracycline antibiotics are more prevalent in the marine products among 221 samples. Most frequent antibiotics detected in marine products were oxytetracycline (OTC), but their residue levels were below the MRL of 0.2mg/kg as the sum of 3 tetracyclines except doxycycline (DC). This study result provides useful information consecutive monitoring project to guarantee food safety.

Keywords: tetracycline, oxytetracycline, chlortetracycline, doxycycline, monitoring

P-38
ANTIBIOTIC RESIDUE CONTROL IN FRANCE: COLLABORATIVE STUDY FOR A MULTIRESIDUE TANDEM MASS SPECTROMETRIC METHOD USING SPIKED MUSCLE REFERENCE MATERIALS

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The control of antibiotic residues in France is primarily based on a microbiological screening followed by a post-screening step using a LC−MS/MS multiresidue method. Apart from this strategy, it must be stressed that some of the confirmatory methods are also implemented at the screening step in specific control plans. Different LC−DAD, LC−FLD or LC−MS/MS methods are then used for the confirmation of positive samples. If the microbiological screening is carried out in the different field laboratories, then the post-screening and the confirmation are still carried out at the NRL level. The post-screening LC−MS/MS method was validated by the NRL both for muscle and for milk matrices. The validation was performed according to the Decision 2002/657/EC and to an internal guideline dedicated to the validation of screening methods. This method allows to detect and to identify almost 60 analytes from the major antibiotic families: penicillins, cephalosporins, sulfonamides, macrolides, lincosamides, tetracyclines, quinolones and aminoglycosides. After a few years of experience at the NRL level, it was decided to transfer the method toward the 12 field laboratories equipped with triple quadrupole or Q-Trap instruments. As a follow-up of a training session at the NRL facilities, the different participants were supplied with the antibiotic standards in order to implement the method in their respective laboratory conditions taking into account their respective instruments purchased from different suppliers. A collaborative study was then organized to check the applicability of the method in laboratories and the performance achieved using different equipments. The method of extraction being very simple, the limiting factor was rather related to the sensitivity of the different MS/MS apparatuses. Five pork muscle materials spiked each one with 5 antibiotics at the MRL level or closed to the MRL level were sent to the participants. Analyses were asked to be carried out within a period of 6 weeks. A global qualitative analysis of the results was performed according to Mc Clure (1990) to assess the sensitivity, the specificity, the false positive rate and the false negative rate of the method. An assessment of the overall method performances was then operated per antibiotic for the whole set of participating laboratories and led to successfully confirm the results of the NRL in-house validation of the method. At last, a data assessment conducted per type of instruments proved that apparatuses of quite new generations were needed to detect correctly the different antibiotics. Results of this collaborative study will be presented. This method is now starting being applied this year in the French antibiotic residue control plan in meat.

Keywords: Antibiotics, residues, multiresidue method, LC-MS/MS, collaborative study

P-39
DEVELOPMENT OF A MOLECULARLY IMPRINTED POLYMER-MATRIX SOLID PHASE DISPERSION METHOD FOR SELECTIVE DETERMINATION OF B-ESTRADIOL AS ANABOLIC GROWTH PROMOTER IN GOAT MILK

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During the past decade, the residue of anabolic growth promoters in foods has been concerned issues for their impact of the endocrine disruptors on human health. Anabolic promoters were initially used as growth promoters, improving the efficacy of feeding conversion in animals through increasing in bone density, muscular mass and red cells. Administration of anabolic growth promoters in animals is now prohibited by the European Union because of their potential risk to human beings, however they are illegally used. β-estradiol (E2) is one of the natural estrogens used as growth promoters which may produce toxic and carcinogenic effects even at low levels. Therefore, accurate analysis of E2 in dairy samples is important for a reliable health risk assessment. However, complexity of these samples often affects the accuracy of E2 determination at trace level. This problem can be solved with the application of matrix solid-phase extraction method (MSPD). In this work we have developed a simple and effective sample treatment based on a combination of MSPD method with molecular imprinted polymer technology for simultaneous clean up and quantitative extraction of E2 in goat milk. Determination of E2 in milk samples was done by HPLC-DAD. Results have indicated that the use of an E2 molecularly imprinted polymer (E2-MIP) as dispersant sorbent in the MSPD method is a promising application for sample treatment in the selective determination of E2 in goat milk samples.

Keywords: MIP, MSPD, endocrine disruptors, HPLC-DAD

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P-40
MINIATURIZED ELISA FOR MONITORING ANTIBIOTIC RESIDUES IN MILK

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Streptomycin is the most widespread antibiotic in the aminoglycocides family. As such, the development of a rapid and sensitive method for the determination of trace amounts of streptomycin is an important issue for public health. Currently, there is a need for a high-throughput screening method with a broad-spectrum detection range. A sensitive sandwich enzyme-linked immunosorbent assay (ELISA) in 384 as well as 1536 well plate formats was developed and applied to detect streptomycin and kanamycin residues in milk. This work describes the optimization and miniaturization of assay using a chemiluminescence reaction. The analytical performance and detection limit for analysis of antibiotic residue was optimized in 384 well plates and further extended to 1536 well plates with a 10-fold reduction in assay volume in skimmed milk. Streptomycin/Kanamycin antiserum was coated on micro well plate. The analytes were quantified using horseradish peroxidase (HRP) as label. The established ELISA analyzed milk samples spiked with streptomycin at 10, 15, 50, 100, 150 and 200 ng/ml. The miniaturised ELISA was found highly sensitive for low level determination of analytes. The developed assay will facilitate high throughput analysis of antibiotic residues such as streptomycin and kanamycin in milk. The assay can be extended for multiresidue analysis in whole milk samples (3.5% fat) without much sample pretreatment.

Keywords: Streptomycin, Kanamycin, Milk, ELISA, Miniaturization

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P-41
DEVELOPMENT OF QUANTUM DOTS-BASED LATERAL FLOW IMMUNOASSAY FOR DETECTION OF CHLORAMPHENICOL IN MILK

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One of the important tasks for ensuring food safety is detection of veterinary drugs. Chloramphenicol (CAP) is an antibiotic intensively used in livestock and poultry farming for therapy of bacterial infections and due to this accumulated in foodstuffs of animal origin. Majority of countries have been established maximum residue levels of CAP for different foodstuffs. Instrumental methods (chromatography, ELISA, etc.) are generally used to detect drug residues. These methods are sensitive, but are not suitable for rapid screening. In contrast, lateral flow (immunochromatographic) tests are labor-efficient rapid assays. Commercial immunochromatographic strips are based on gold or latex particles. The aim of our investigation was to develop and apply at first time quantum dots (QDs)-based fluorescent strips for food control on the example of CAP control in milk. Invitrogen water-soluble QDs with emission peak at 625 nm were covalently coupled with anti-CAP monoclonal antibodies. To produce lateral flow strip, CAP-bovine serum albumin conjugate was immobilized onto MDI (India) nitrocellulose membrane as test line, and goat-anti-mouse antibody - as control line. The obtained QD–antibody conjugate was added onto a macroporous pad of the strip. Pure and CAP-spiked cow milk samples were diluted to 20% to eliminate the matrix effect. After their contact with the strip QD-labeled antibodies migrated along the membrane by capillary forces and competitively interact with native and conjugated CAP. The bounded QDs in test and control zones were detected under excitation by UV-light, thus, one or two red fluorescent lines could be seen. Limit of CAP revealing in milk by this assay with visual detection of test line disappearance is 5 ng/mL. In the case of using portative reader, the working range of quantitative assay is 0.25-2 ng/mL. The analysis time is 20 min. The storage of test strips during 6 months at RT does not lead to reliable change of their characteristics. The obtained results demonstrate efficiency of QDs in rapid tests application for dairy foodstuffs.

Keywords: chloramphenicol, milk, quantum dots, lateral flow assay

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P-42
CAN THE USE OF COCCIDIOSTATS IN POULTRY BREEDING LEAD TO RESIDUES IN VEGETABLES? AN EXPERIMENTAL STUDY

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Of the drugs approved for veterinary and agricultural purposes, antibiotics are among the substances with the highest usage. Coccidiostats account for a large fraction of these antibiotics. In poultry farming anticoccidial drugs are widely used as feed additives for the prevention and treatment of coccidiosis. According to the European Food Safety Authority the acceptable daily intake (ADI) levels are situated in the mid to low µg.kg bodyweight-1.day^{-1} for humans. Toxic effects can range from neurotoxic effects to maternal toxicity and foetotoxicity and to focal degeneration of skeletal muscles. The use of these compounds is strictly regulated and maximum residue limits (MRLs) have been set for these compounds in matrices such as eggs, milk and meat in order to minimize the intake. There is however another possible exposure route that has not been taken into account yet. Because coccidiostats, and veterinary medicines in general, are often poorly absorbed, manure from treated animals may contain high concentrations of coccidiostats. For instance, 98% of the administered dose of diclazuril is excreted within 10 days, of which the parent compound accounts for 85−95%. Experimental studies have shown that the uptake of veterinary medicines into plants from soil containing contaminated manure may occur. This gives rise to several questions regarding the impact on the environment, resistance problems and allergy and public health and issues. Moreover, this 'alternative' exposure route might necessitate the establishment of MRL values for vegetables or the adjustment of already existing MRLs for other matrices. Plant uptake of chemicals, especially of veterinary medicines, is a branch in residue analysis which is largely unexplored. To study the possible uptake of coccidiostats by vegetables, five groups of poultry received a 39 day three-phase feeding schedule with feed containing the maximum allowed level of a coccidiostat. Following coccidiostats were used in the animal trial: monensin, lasalocid A, salinomycin, diclazuril and nicarbazin/narasin and 1 control group. In a next step tomato, carrot, potato, zucchini and lettuce were grown on soil containing manure from the treated poultry. For each vegetable/treatment combination at least 4 vegetables were harvested and after freeze-drying analyzed with a validated LC-MS/MS method. This presentation will give an overview of the obtained results of this experimental study.

Keywords: coccidiostats, LC-MS/MS, plant, uptake, residue, veterinary

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SCREENING OF ANTIBIOTIC RESIDUES IN MEAT USING LC–HIGH RESOLUTION MASS SPECTROMETRY

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During the ten past years, many analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have been developed for multi-residue screening purpose using MRM mode. More recently, new screening approaches using high resolution mass spectrometry (HRMS) have been reported for screening of residual compounds, using equipments like time-of-flight (TOF) or orbital trap mass detectors (Orbitrap). These instruments allow a full-scan acquisition of all signals obtained from the ionisation source, without preselecting any compounds. Here we describe the ability of LC-HRMS for screening of veterinary drugs belonging to the main classes of antibiotics: penicillins, cephalosporins, sulphonamides, macrolides, tetracyclines aminoglucosides and quinolones in meat. Extractions protocols were developed in order to reach sensitivity suitable for the target screening concentration based on regulatory maximum residue limit (MRL) set in European Union (EU).Compounds were successfully identified in spiked samples by their accurate mass and LC retention times from the full-scan mass data acquired using LTQ-Orbitrap mass spectrometer at resolving power 60.000 FWHM. An automatic process of the data allows a practical and rapid identification of compound. The methods characteristics were evaluated: specificity, sensitivity, limits of detections was determined for each compound. The applicability of the method was tested by analysing several naturally incurred muscle tissue samples.

Keywords: Antibiotics, residues, high resolution mass spectrometry, screening, orbitrap.

P-44
DETERMINATION OF TWENTY ANTICOCCIDIALS IN EGG AND MUSCLE BY UPLC–MS/MS

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A UPLC–MS/MS method was developed for the confirmatory analysis of 16 anticoccidials (arprinocid, clopidol, decoquinate, diaveridine, diclazuril, ethopabate, halofuginone, imidocarb, lasalocid, laidlomycin, maduramycin, monensin, narasin, nicarbazin, robenidine and salinomycin) and screening analysis for a further 3 anticoccidials (toltrazuril, toltrazuril sulphoxide and toltrazuril sulphone) in egg. Samples were extracted with acetonitrile without clean-up and extracts were analysed by UPLC-MS/MS using Acquity BEH C8 column chemistry. The method was validated according to Commission Decision 2002/657/EC. The validation includes the determination of linearity, within laboratory repeatability/reproducibility, decision limit (CCα) and detection capability (CCβ). A total of 50 samples can be analysed in a single day using the assay. The method has been extensively evaluated through application to real test samples and proficiency material.

Keywords: anticoccidial residue, UPLC–MS/MS, egg. poultry muscle

P-45
SIMULTANEOUS DETERMINATION OF 5 AMINOGLYCOSIDE RESIDUES IN FOODS OF ANIMAL ORIGIN BY UPLC–MS/MS

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The aminoglycosides are widely used in veterinary medicine and animal husbandry particularly for treatment of bacterial infections. But improper administration without observing the withdrawal time for treated animals can result in antibiotic residues in foods of animal origin and development of antibiotic resistance in bacteria has long been attributed to the overuse of antimicrobials in human medicine. With an increase in the consumption of animal food products in recent years, the extensive monitoring of these residues is required for food safety. In this study, we have performed the monitoring of 5 aminoglycosides (gentamicin C1, C1a, C2 and C2a, neomycin, streptomycin, dihydrostreptomycin and spectinomycin) on 40 bovine muscles, 37 swine muscles, 22 chicken muscles, 13 milks, 20 carps, 16 trouts, 13 halibuts, and 16 jacopever. The test portion is extracted by sonication with 2% trichloroacetic acid and 10mM phosphate buffer. The extract was adjusted to pH 7.5 and applied to the combination of two SPE catridges (Oasis HLB and WCX catridge). The eluted was analyzed by UPLC-MS/MS and method was linearly calibrated from 50 to 1000 ng/ml and correlation coefficient of calibration curve was 0.999. The recovery ranged form 67.3% to 82.7% and the limit of detection and quantification were 3~10 ng/ml and 9~30 ng/ml, respectively. The results were achieved in 177 samples purchased form the major cities in South Korea and 4 samples yielded a positive screening result.

Keywords: aminoglycosides, antibiotic residue, UPLC-MS/MS, residue analysis

P-46
VALIDATION OF A HIGH SENSITIVITY ELISA KIT FOR A BROAD RANGE SULFONAMIDES DETECTION IN FOOD AND FEED

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In residue control, screening technologies are powerful tools which provide rapid responses in the analysis of many samples when conventional analytical methods are too costly or cumbersome. For a first screening in food and feed, the enzyme-linked immunosorbent assay (ELISA) technique is widely used. However in ELISA screening of veterinary drugs one of the most frequent drawbacks is the limited antibody cross-reactivity and the consequent difficulty to detect a broad range of compounds belonging to a certain drug group. For sulfonamides, for example, the majority of commercially available ELISA kits is specific to one or to few substances and therefore they are not suitable for the simultaneous detection of all required sulfonamides at the levels of interest in food (meat, milk, honey and eggs) and feed. As a consequence, until now, the Italian official laboratories have been forced to use chromatographic methods to screen this important drug class. Recently an ELISA kit was developed by TECNA S.r.l. (Trieste, Italy), I'screen Sulfa, reporting a broad range of cross-reactivities towards the mostly employed sulfonamides. This work describes the validation of this ELISA screening in samples (feed, honey and muscle) collected within the official monitoring plans. The validation approach has followed the general criteria of Commission Decision 2002/657/EC and the experimental instructions reported in the EU CRL Guidelines. In order to assess the effective sulfonamide absence, twenty samples of each matrix were previously analysed applying the routine confirmatory method based on high performance liquid chromatography with UV detection (HPLC-DAD). In ELISA experiments, the manufacturer protocol was applied for sample preparation of muscles; however new protocols were introduced for honey and feed. The considered sulfonamides were: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline and sulfathiazole. Firstly all these sulfonamides were spiked in representative muscle, honey and feed samples in order to determine the relative crossreactivities of each analyte in each matrix considering the sample treatment as well. Consequently, during method validation, a low cross-reacting sulfonamide was chosen for spiking the twenty blank samples. Surprisingly, some of the HPLC-DAD negative samples were identified as being suspect by the ELISA screening; therefore they were re-analysed with an even more sensitive technique i.e. liquid chromatography coupled to tandem mass spectrometry. Traces of sulfonamides were confirmed in all cases. The estimated detection capabilities (CCβ) were at least 10, 5 and 2000 µg/kg in muscle, honey and feed, respectively. Since these values were established with the lowest cross-reactant sulfonamide within those included in the scope of the method, all the others were detectable at lower concentrations.

Keywords: sulfonamides, ELISA, muscle, honey, feed

P-47
OCCURRENCE OF UNAVOIDABLE CARRY-OVER OF COCCIDIOSTATS IN FEED

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Coccidiosis, an intestinal plasmodium infection, is a major infectious disease in poultry and rabbits. Eleven different coccidiostats are licensed in the EU for the prevention of coccidiosis in these animal species. According to their chemical nature and main biological activity, these compounds can be grouped as ionophoric (monensin, lasalocid sodium, salinomycin, narasin, maduramicin and semduramicin) or non ionophoric (robenidine, decoquinate, nicarbazin, diclazuril and halofuginone) substances. They are used as feed additives, mixed upon request into the compounded feed. As feed companies may produce a broad range of feed in the same production line, carry-over of coccidiostats may occur as a consequence of the transfer from a batch where coccidiostats are used as authorized feed additives to a batch of non target feed (i.e. feed intended for animal species or categories not provided for in additive authorization). This cross-contamination can induce adverse health effects in non-target animals due to a specific sensitivity of mammalian species as compared to poultry. Residue formation in edible tissues of non-target species may result in unexpected human exposure through the consumption of animal products. To protect the animal health and assure a negligible risk to consumers, Directive 2009/8/EC has established maximum contents for unavoidable coccidiostats carry over in non target feed. A survey was carried out to investigate the coccidiostats occurrence in feed. One-hundred samples were analyzed as part of an official feed inspection in the period 2010–2011. The samples included 62 poultry, 23 cattle, 8 pig feed samples and 8 feed samples for other animal species (rabbits, sheep, goats, horses and trouts). In our knowledge no surveillance results were reported monitoring concentrations up to a few parts per billion for all authorized coccidiostats. Surprisingly the results highlighted a very high rate of feed containing at least one residue (64%). In addition 8.9 and 2.0 percent of the tested samples were simultaneously contaminated by four and five coccidiostats, respectively. The substances most frequently found were: monensin (44%), followed by lasalocid (30%) and robenidine (20%). Halofuginone was not detected in any of the cases. Considering the animal species and category, six feed samples (6.0%) were not compliant: four samples exceeded the maximum contents fixed in Directive 2009/8/EC for monensin and one sample for salinomycin and one more for diclazuril. The determined levels were generally lower than 1 mg/kg, however in some cases higher concentrations were detected.

Keywords: Survey, carry-over, coccidiostats, Directive 2009/8/EC, animal feed

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DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF ELEVEN COCCIDIOSTATS IN FEED USING LIQUID CHROMATOGRAPHY / TANDEM MASS SPECTROMETRY

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Coccidiostats are mainly used in poultry production to prevent coccidiosis, a parasitic disease of the intestinal tract caused by unicellular organisms. Currently eleven substances are authorised as feed additives in accordance with Regulation 2003/1831/EC on additives for use in animal nutrition and their authorisations lay down specific conditions for use such as the target animal species for which the additives are intended. Recently Directive 2009/8/EC has established maximum contents for unavoidable coccidiostats carry over in non target feed, i.e. in feed for which the use of coccidiostats is not authorised. These limits have been fixed considering 1% and 3% of the maximum contents permitted in target feed for each substance and, therefore, they range from 0.010 mg/kg for diclazuril to 3.75 mg/kg for lasalocid and monensin sodium. As a result of this legislation there is a need for reliable multiresidue procedures to help enforce it. A sensitive method for the eleven authorised coccidiostats (decoquinate, diclazuril, halofuginone, lasalocid, maduramycin, monensin, narasin, nicarbazin, robenidine, salinomycin and semduramicin) was here developed using liquid-chromatography-tandem mass spectrometry (LC−MS/MS). After the addition of four internal standards, feed samples were extracted with ethanol and purified using SPE Silica cartridges. The dry residues were redissolved in methanol and injected onto the LC−MS/MS system. Separation was performed on a Synergi Fusion column (150 mm × 2.0 mm, 4 µm) by gradient elution with flow rate of 0.25 mL/min. The mobile phases were acetonitrile and water both containing 0.1% formic acid. The analytes were ionized in positive or negative electrospray mode and at least two ions each were chosen for multiple reaction monitoring (MRM). The method was successfully validated over three days. Taking into account the different limits set in Directive 2009/8/EC even for the same substance depending on the animal species and categories for which the product is intended, eight progressive validation levels were investigated covering all maximum foreseen contents: 0.0032, 0.010, 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg/kg. The method sensitivity allows quantification and confirmation of the coccidiostats at a 0.5% carry over level or lower. Validation criteria of trueness, precision (repeatability and intra-laboratory reproducibility), along with measurement uncertainty were calculated for all analytes.

Keywords: LC−MS/MS, Coccidiostats, Directive 2009/8/EC, animal feed

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MONITORING OF TETRACYCLINES IN MEAT AT THE LEVEL REQUIRED BY THE RUSSIAN FEDERATION

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Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and doxycycline (DOX) are tetracycline antibiotics which are commonly used in human and veterinary medicine. They are broad-spectrum antibiotics which are authorized to be used in food-producing animals. However, meat intended for human consumption is not allowed to contain antibiotic residues. Therefore the national residue control programme is carried out annually in accordance with both national and European Union (EU) legislation. Typically analysed matrices are pork, broiler, turkey, fish, wild boars, egg, milk and honey The need to monitor tetracyclines at residue levels lower than set in the EU legislation arose from differences in MRL-values in the EU and the Russian Federation. A maximum residue limit (MRL) for tetracyclines set by the EU is 100 µg/kg while MRL set by Russia is ten times lower i.e. 10 µg/kg. If the analytical method does not meet the Russian federation criteria an export ban from Finland to the Russian Federation may result. In this study we developed and validated a HPLC−MS/MS method to determine tetracycline residues in edible tissues at the levels required by the Russian Federation. Tetracyclines were extracted with EDTA-McIlvaine buffer followed by fat removal with hexane-dichloromethane (1:3, *v/v*). Trichloroacetic acid (1%) was added to the extract to precipitate proteins. Solid-phase extraction clean-up was performed on a Bond-Elut C18 after centrifugation and paper filtration of sample extract. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C18 (3.5 µm, 150 × 2.1 mm) reversed phase column with a gradient elution using water-acetonitrile in formic acid (0.2%). The mass spectral analysis was operated on a Micromass Quattro Micro triple quadrupole mass spectrometer using a positive ion MS/MS mode. The identification and confirmation of tetracyclines measured by LC−MS/MS was achieved on the basis of the differences in retention time and in the ion ratio between two product ions. Tetracyclines were validated at concentrations of 5, 10 and 15 µg/kg i.e. 0.05. 0.1 and 0.15 × MRL (EU) using matrix-matched calibration curve on the range 5–100 µg/kg. Validation was performed according to the Commission Decision 2002/657/EC. Validation parameters like repeatability, within-laboratory reproducibility, recovery, decision limit (CCα) and detection capability (CCβ) as well as positive identification and quantitation of tetracyclines at low level will be presented.

Keywords: tetracyclines, meat, LC−MS/MS

P-50
MS AND MS TANDEM PERFORMANCE IN PROFICIENCY TESTING FOR VETERINARY DRUGS RESIDUES IN FOOD

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The interlaboratory ring tests are excellent tools for the Quality External Verification of the laboratory performances. An analysis of a collection of interlaboratory data results could be a good source of information about a certain analytical method. After several years organizing proficiency testing for veterinary drug residues in food, Progetto Trieste, has considerable information about the several methods used by international laboratories from this sector, particularly with regard to the percentage of satisfactory results for each method.
The most widespread detection method for the confirmation of positive results in veterinary drug residues analysis is the Mass Spectrometry.
Results: The analytical trial under study was the analysis of beta-agonists in liver. Data came from seven ring test which took places within 2002 and 2010. In the first two ring test, samples analysed were negative. There were used two different detector methods: Diode Array Detector (DAD) and Mass Spectrometer Detector (MS). 95% of data were obtained with MS. Any laboratory provided false positive results. Therefore, the specificity was 100%. The number of results reported to Progetto Trieste for positive samples was 80, from which 77 were obtained using MS detector.
Progetto Trieste assigned to each results a Z-score value (z). The mean of |z| of the 80 results is 0.55, if we clear the data by cancelling the results from other detection methods than MS, the mean become 0.53. Regarding data obtained with detector MS, the satisfactory data (|z| < 2) ratio is 87%, not satisfactory data (|z| > 3) ratio is 3,9 % and there are a 9.1 % of not evaluated data because no quantification was reported. There were 2 outliers (outliers ratio is 2.6%). From the 77 results with MS detector, 10 were obtained with MS, 65 with MS Tandem (MS/MS) and 2 with MS/MS/MS. The respective |z| mean are 1.13, 0.45 e 0.29. The two date of MS/MS/MS were considered unrepresentative.In the group of data obtained with MS/MS there were several chromatographic separation methods used: HPLC, LC and UPLC. In the following table are reported the main data found about these methods:

method	n	|z| mean	%|z|<0,5
GS	4	0.69	25
HPLC	51	0.44	45
LC	9	0.55	44
UPLC	1	0,1	not applicable

Inside the HPLC/MS/MS data it has been elaborate the mean of |z| regarding the chromatographic column used. Further information is available about the mean of |z| regarding sample preparation and other aspects of the analytical method. Furthermore, it could be remark that there were not a overestimation or underestimation trend with MS, since the negative z values are the 54% and positive values are the 46%.
Conclusions: MS detector is confirmed as an excellent detector for the analysis of beta-agonists in liver with an specificity of 100% and |z|<2. The best performances, in Z-score terms, for this analysis were obtained with the combination of MS detector with HPLC separation method.

Keywords: drug, tissue, Z-score

P-51
LC-MS/MS FAST ANALYSIS OF ANDROGENIC STEROIDS IN URINE USING POROSHELL 12-EC C18 COLUMN

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Liquid chromatography tandem mass spectrometry method was developed and validated to detect eight androgenic steroids: trenbolone, nortestosterone, boldenone, methylboldenone, testosterone, methyltestosterone, 17α-1-testosterone and17β-1-testosterone in bovine urine. The sample preparation before LC-MS/MS analysis involved an enzymatic hydrolysis with glucuronidase AS-HP, isolation of free hormones from urine on C18 SPE column and purification of the extract using liquid-liquid extraction with n-pentane. For chromatographic separation of steroids, the Poroshell 120-EC C18 column (150 × 2.1 mm, 2.7 µm) was used, the mobile phase consisted of methanol and water (7+3, V1+V2) in isocratic mode was operated. Total column flow was 150 µl min^{-1} and the column was maintained at a constant temperature of 400°C. Mass spectrometric measurement was achieved using API 4000 triple quadrupole (QqQ) instrument with a TurboIon-Spray source operating in positive electrospray ionization mode. Depending on the target compound, two or three diagnostic signals (multiple reaction monitoring transitions –MRM) were monitored. The procedure was validated according to the Decision 2002/657/EC. The recovery ranged from 76 to 126% for all examined compounds. The reapetability was below 20% and reproducibility did not exceed the limit 25%. The linearity was good for all analytes in the whole range of tested concentrations, as proved by the correlation coefficients greater than 0.98. The decision limit (CCα) ranged from 0.11 µg L^{-1} for 17β-nortestosterone to 0.78 µg L^{-1} for 17α-trenbolone, whereas the detection capability (CCβ) from 0.18 µg L^{-1} µg/l to 1.1 µg L^{-1} respectively. The obtained values are lower than the recommended concentations (RC) for the compouns tested. Application of innovative Poroshell column allowed for very good chromatographic separation of steroids at much shorter time of analysis.

Keywords: androgenic steroids, liquid chromatography, mass spectrometry

P-52
SCREENING AND CONFIRMATORY GC–MS METHODS FOR THE DETECTION OF TRENBOLONE IN BOVINE URINE

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Trenbolone acetate (TBA) is a synthetic steroid with strong anabolic properties. In animals, TBA alone or in combination with 17ß-estradiol, is used to improve weight gain and feed efficiency. TBA is administered by subcutaneous implantation in the ear. The dosage of TBA varies with manufacturer of the implant, ranging between 40 and 300 mg per animal. The metabolism of TBA appears complex and species dependent. TBA upon entering the circulatory system is rapidly hydrolyzed to its active free form, 17β-trenbolone (TBOH). In the bovine species, the 17β-epimer is the major metabolite occurring in muscle, the 17α-epimer is the major metabolite occurring in the excreta, bile and liver. Elimination in the urine occurs following conjugation, predominately to glucuronic acid. Sensitive and selective methods were developed for the screening (GC-MS) and confirmatory analysis (GC−MS/MS) of 17α-trenbolone in bovine urine. To a filtered urine sample, 10 ng internal standard of 17β-TBOH-d_3 was added (corresponding 1 µg L^{-1}). In the first stage of the analysis, the enzymatic hydrolysis of trenbolone metabolites with glucuronidase AS-HP in acetate buffer (pH 5.2) solution was carried out. Free compounds were extracted from urine with diethyl ether. For the purification of the sample extract, liquid-liquid extraction with sodium carbonate buffer and water were performed and next, solid phase extractions with C18 and NH2 columns were applied. The purified, evaporated extract was subjected to derivatisation reaction. For trenbolone, two derivatisation steps were used: first with MSTFA/I2 solution and second with MSTFA only. The screening analysis were performed using GC 6890 N gas chromatograph interfaced to an Agilent MSD 5973 detector, the confirmatory studies were carried out on an Agilent MS/MS detector. The separation of the analyte on HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) was conducted. The methods have been validated according to the Commission Decision 2002/657/EC. The CC_α and CC_β values are based on the detection of the most abundant ion and the values: 0.28 µg L^{-1} and 0.48 µg L^{-1} for screening and 0.21 µg L^{-1} and 0.36 µg L^{-1} for confirmatory methods were obtained respectively. The repeatability and within laboratory reproducibility for the validation level 1 µg L^{-1} were lower than 25% for both detection techniques. The recovery of 17α-trenbolone was over 85%.

Keywords: gas chromatography, mass spectrometry, trenbolone

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DEVELOPMENT AND VALIDATION OF A MULTICLASS MULTIRESIDUE U–HPLC–HR–ORBITRAP–MS METHOD FOR THE QUANTITATIVE SCREENING OF VETERINARY DRUG RESIDUES IN MEAT

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Veterinary medicines, comprising several classes of compounds, are extensively used in animal husbandry for various reasons. Upon administration, the residues of these drugs appear in multiple matrices derived from the treated animal (i.e. milk, edible tissues and urine). To control in a swift and efficient manner whether possible veterinary drugs do not exceed the legally implemented Maximum Residue Limits (MRLs), multiclass multiresidue screening methods enabling the detection of a large amount of veterinary medicines are desirable. Therefore, during this study a benchtop Exactive™ high resolution and high mass accuracy Orbitrap mass spectrometer coupled to an ultra-high performance liquid chromatography system was employed. The meat extraction procedure, based on Peters et al. (2009) consisted of a liquid/liquid extraction with a mixture of acetonitrile and water (6:4, v/v), followed by a solid phase extraction procedure (Strata™ X 60 mg/3 mL, Phenomenex). In total 56 veterinary drugs, belonging to different classes were incorporated in the method, which in turn was validated according to Commission Decision 2002/657/EC. This developed quantitative screening method obtained for all compounds a R^2 > 0.98. For all veterinary drugs, except for the class of Non-Steroidal Anti-Inflammatory Drugs (NSAID's) where proper internal standard appeared to be missing, the required performance characteristics (detection capability, precision, selectivity, specificity and ruggedness) were reached. For example, the within-laboratory reproducibility of all compounds, except for the NSAID's, was below 15% or 20% depending on the mass fraction, respectively > or < than 100 µg/kg. Additionally, the decision limits and recoveries have been calculated as the case for confirmatory methods. If it was not for the criterion of the identification points, which cannot be obtained when relying on HR-Orbitrap-MS technology. During following experiments, proper internal standards for the NSAID class will be incorporated in the developed method to reach the defined criteria (2002/657/EC). As for the near future, it has been suggested that the European Commission would adapt the current legislation of residue analysis for allowing the use of full-scan HR-MS detection at 50.000 FWHM or more for screening and identification purposes. For this, the number of identification points earned with HR-MS full-scan analysis (≥ 50.000 FWHM) should be augmented to 3 or 4, depending on the fact if a permitted limit is established or not.

Keywords: Veterinary drugs, Orbitrap mass spectrometer, U-HPLC, meat

P-54
STABILITY OF THYREOSTATIC DRUGS, IN PARTICULAR THIOURACIL IN BOVINE AND PORCINE URINE

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The knowledge of the stability of a certain analyte in a matrix is of high importance as it may support anomalous findings obtained during re-analysis of non-compliant samples for purposes of arbitration analysis. Even more, it is imperative for the robustness of samples and their analytical results in time. Therefore, this study aimed at determining the stability of thyreostatic drugs, in particular of thiouracil (TU), in urine. This analyte has in the last years drawn the attention because of the paradox surrounding its exogenous and/or endogenous status [1]. Initial studies showed that thyreostats in urine are highly unstable during freeze-thaw cycles, due to matrix effects. Also, at room-temperature significant losses were observed. These observations initiated studies into possible conservation approaches. Incurred and spiked (50−100 µg/L) urines were analysed for the presence of TU during a period of 6 months. In addition, the effect of pre-treatment (pH = 1 and 0.1 M EDTA) of the samples was investigated. To this end, the clean-up and LC-MS/MS method described by Pinel et al. (2005), has been utilised [2]. All incurred urines were upon sampling divided into two aliquots, one remained unaltered, the second acidified and supplemented with EDTA. The outcome of this study indicated a significant difference, both for incurred as well as for spiked urines, between the unaltered and acidified aliquots. Acidifying urine led to higher signals and prolonged detection. Thus in the future, for legislative and research purposes pre-treatment of urines is advisable.

[1] Pinel et al. Food Addit. Contam. 2006, 23, 974.
[2] Pinel et al. J. Chromatogr. A, 2005, 1085, 247.

Keywords: Thyreostatic drugs, Stability, Urine, UPLC, Mass spectrometry

P-55
IDENTIFICATION OF 'UNKNOWN' MICROBIAL GROWTH INHIBITORS IN ANIMAL FEED BY LC–TOF–MS WITH ACCURATE MASS DATABASE SEARCHING

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Microbial growth inhibition tests are widely used as a screening approach for the detection of antibiotics in animal feed. Animal feed samples, which show inhibition in the microbial test, are measured with group specific LC-MS methods to identify the active compound that is responsible for the positive result. However, in some cases the active compound cannot be identified with the targeted LC-MS methods and another approach is necessary to identify the 'unknown' microbiological active compound. In this study an alternative approach is developed and tested to identify possible unknown active compounds in feed samples. The approach is based on four steps viz. 1) the sample preparation and extraction, 2) fractionation of the sample extract, 3) identify which fraction shows microbial growth inhibition followed by step 4) LC-ToF-MS analyses to identify the unknown compound by using accurate mass database searching. In order to detect unknown antibiotics with a wide variety of physical properties, a generic sample preparation method is necessary to extract the compounds and to clean and concentrate the primary extract. As a result the primary extract is split up in two parts and one part is purified with reversed-phase and the second part with weak-cation-exchange SPE. Reversed-phase SPE is used as a generic purification step whereas weak-cation-exchange SPE is specifically used for the concentration of aminoglycosides and structure related compounds. Afterwards the SPE extracts are pooled for fractionation. Furthermore, the introduction of the fractionation step is chosen to focus the search of 'unknowns' on specific fractions showing microbial activity and not on the whole extract. Finally the data obtained by LC-ToF-MS are checked against a large accurate mass database of relevant and existing compounds downloaded from the Pubchem website. This database contains the trivial and IUPAC names, elemental compositions and log P values of about 50,000 compounds from the categories Toxicology, Pharmacology and Environmentals. The accurate mass and isotopic ratio were calculated from the elemental composition and are used to identify the unknown active compound. The developed method was tested with a set of 'known' antibiotics like apramycin, neomycin, oxytetracyclin and lincomycin in animal feed. From the results it was concluded that this approach is applicable for the use of samples containing 'unknown' growth inhibitors.

Keywords: Antibiotics, Solid Phase Extraction, Fractionation, unknown inhibition, microbial test

Acknowledgement: This project was financially supported by the Dutch Ministry of Economic affairs, Agriculture and Innovation

P-56
THE ANALYSIS OF HONEY FOR THE PRESENCE OF CHLORAMPHENICOL USING IMMUNOAFFINITY COLUMS

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The analysis of honey for the presence of chloramphenicol using immunoaffinity columns J. Mackie & C. Milligan, R-Biopharm Rhône Ltd Chloramphenicol is a broad spectrum antibiotic that is used in veterinary practice against both gram-positive and gram-negative bacteria. However, in some countries it is used to promote animal growth and to treat sick animals as a result of poor hygiene conditions on farms. Due to the toxicity of chloramphenicol and resistance to this antibiotic, it is no longer used as a first line agent. In most countries the drug is banned for use in food producing animals. Products with residue levels above the recommended levels are condemned and denied entry to the food chain. Surveillance and testing of antibiotics has increased leading to the need for a rapid, easy to perform and inexpensive test, capable of meeting legislative requirements. Analysis of antibiotics can often be problematic due to the very small levels present so method sensitivity and sample preparation are particularly important. The use of EASI-EXTRACT® CHLORAMPHENICOL immunoaffinity columns over comes these issues as they offer an easy way to extract, purify and selectively concentrate chloramphenicol from a wide range of food and feed commodities, including honey, royal jelly, bee pollen, milk and shrimp, allowing optimum detection by HPLC or LC-MS/MS. The method was validated in-house using EASI-EXTRACT® CHLORAMPHENICOL to test various honey samples. Recovery data ranged from 83−100% (RSD 3.8−10.3%) when used in conjunction with LC-MS/MS, while recovery data ranged from 79−91% (RSD 2.3−4.5%) for HPLC analysis. EASI-EXTRACT® CHLORAMPHENICOL offer improved clean-up and concentration of chloramphenicol from the sample providing cleaner LC−MS/MS and HPLC chromatography.

Keywords: Chloramphenicol, Antibiotic, Clean up, Honey, Immunoaffinity Columns

P-57
DETERMINATION OF SULFONAMIDES AND ANTIBIOTICS IN FOOD OF ANIMAL ORIGIN AND FEEDSTUFFS BY LC–MS

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Antibiotics and sulfonamides are used in the prevention and treatment of diseases of all types and categories of animals. Improper use of antibiotics and sulfonamides, their uncontrolled sales, non-compliance of prescribed dose and withdrawal period − the time it takes to be used antibiotic or sulfonamide is excreted from the body, leading to the presence of residues of antibiotics and sulfonamides in foodstuffs of animal origin for human and animal consumption. In SP Laboratory, determination of sulfonamides (Sulfapyridine, Sulfathiazole, Sulfadimidin, Sulfadimethoxine and Sulfaquinoxaline) and antibiotics (Penicillin G potassium salt, Erythromycin, Chloramphenicol, Bacitracin, Tetracycline hydrochloride, Oxytetracycline hydrohloride, Chlortetracycline hydrochloride and Tylosin-phosphate) in food sample of animal origin and feedstuffs was done by the UltiMate 3000 Rapid Separation LC system with Surveyor MSQ plus Mass Detector (Dionex, USA). Separation and identification of sulfonamides and antibiotics was done with analytical column Acclaim PolarAdvantage C16 (Dionex, USA). Mobile phase was 0.5% formic acid in acetonitrile and 0.5% formic acid in water, pH 2.4, flowing under isocratic elution. Flow rate was 0.2 ml/min. The homogenized sample was initially extracted in a buffered aqueous/1% acetic acid acetonitrile system with an extraction and partitioning step after the addition of salts. Finally, the sample was cleaned up using dispersive solid-phase extraction (dispersive-SPE). The final extracts were analyzed by the LC ESI−MS operating in positive and negative SIM mode (Single Ion Monitoring) which is more sensitive and selective determination. In the ESI source, high purity nitrogen was employed as the nebulizer. The ESI probe temperature was set at 400°C and the needle potential at 3kV. Positive and negative ion modes full scans data acquisitions were made over the *m/z* 200−1000 range. Validation parameters for the antibiotics and sulfonamides: range of calibration curve 0.5-5µg/ml, range of method 0.02−2ppm, recovery 87−112%, repeatibility 3.91−7.47%, reproduction 2.76−4.39%, precision 6.11−7.43%, uncertainty of measurement 11.9−14.6%, limit of quantification (LoQ) 20 ng/g. The Serbian legislation has been defined maximum concentration of sulfonamides in foods of animal origin which is 100 ng/g while in feedstuffs they are not allowed as well as antibiotics. During 2011, in SP Laboratory analyzed antibiotics and sulfonamides in more than 1000 selected samples (honey, milk, meat and their products and feedstuffs), which were collected from the Serbian market, and content of antibiotics and sulfonamides in selected samples were under the limit of quantification of method, 20 ng/g.

Keywords: food, antibiotics, sulfonamides, LC/MS

P-58
THE DETECTION OF COCCIDIOSTATS IN FOOD SAMPLES BY LCMSMS

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Novel Aspect On line solid phase extraction used to speed up coccidiostats detection in LCMSMS analysis. Introduction Coccidiostats are antiprotozoal agents that act upon Coccidia parasites. In the food industry they are used to treat infections in cattle and chicken and as such meat, chicken, egg and milk are regularly tested for these pharmaceutical compounds. Recently maximum levels for these compounds were set by the EU in Commission Regulation [(EC) 124/2009, L40, 7−11]. This work shows where LC/MS/MS can be used to detect coccidiostats including Narasin, Diclazuril Monensin used in the food industry. Methods In this work milk was used as an example matrix. Samples were extracted with Acetonitrile and concentrated and purified using solid phase extraction. Extracts were then analyzed by reversed-phase HPLC using a Shimadzu UFLC System and a conventional C8 column and also a small particle C8 column. Mass spectrometry analyses were performed on an ABSCIEX mass spectrometer using the Turbo V™ source in negative or positive ion electrospray mode depending on the Coccidiostats. Off line and on line approaches to solid phase extraction are compared. Preliminary Data Initial data shows that all coccidiostats tested can be detected below the maximum residue limit in food. Small particle size columns in combination with on line solid phase extraction have been shown to provide quicker analyses times with lower limits of detection compared to the traditional off line solid phase extraction approach.

Keywords: coccidiostats, LCMSMS analysis, matrix milk

P-59
IMPROVEMENT TO THE EXISTING TETRASENSOR AND EXTENSION OF SCOPE TO FEED, URINE AND THERMALLY PROCESSED MEAT MATRICES

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Tetrasensor is a competitive receptor-based lateral flow dipstick assay developed by Unisensor and detecting many tetracycline compounds at least at MRL values in different matrices such as milk, honey and raw animal tissues. Within WP2b of Conffidence EU-project, detection of tetracycline family residues with Tetrasensor was improved and extended to 3 additional matrices : urine, feed and heat processed meat. In order to fit with these matrices, new sample processing was developed and reagents were adapted to improve the test line signal. This dipstick-based assay allows the detection of tetracycline compounds at low levels of detection in each matrix (<25 µg/kg in tissue; < 50 ng/ml in urine ; <200 µg/kg in feeds) in less than 20 minutes. Accurate performances of this Tetracycline screening assay have been confirmed by in-lab validation studies (FERA). In conclusion, we have improved our generic Tetracycline-dipstick assay. This updated format allows detection of the most Tetracyclines in a large range of matrices including raw animal tissues, processed meat, feed, urine, honey, egg, water and seafood.

Keywords: Tetracyclines, Dipstick, Meat, Feed, Urine

Acknowledgement: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° KBBE-211326.

P-60
TRACE ANALYSIS OF FUMAGILLIN IN HONEY BY ULTRA–HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–ORBITRAP MASS SPECTROMETRY

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Nosema ceranae is a worldwide spread microsporidium, which is considered to be one of the main honey bee pathogens. The treatment of infectious diseases in food-producing animals is an essential aspect of veterinary medicine which, consequently, creates a need for the control of veterinary drug residues in food products. Fumagillin obtained from Aspergillus fumigatus is one of the few drugs known to be active against microsporidia. In this study, we have developed a rapid method for the analysis of fumagillin in honey. For the isolation of fumagillin residues, a QuEChERS extraction procedure was used, followed by ultra-high performance liquid chromatography–orbitrap mass spectrometry (UHPLC–orbitrapMS). The average analyte recoveries reached 92% and 83% at levels of 0.1 and 0.01 mg/kg, respectively, with the repeatability expressed as relative standard deviation (RSD) <15%. The lowest calibration level was 0.005 mg/kg. The developed method has been applied to the analysis of fumagillin residues in honey samples collected from veterinary treated beehives, infected by Nosema ceranae and fed with the technical product containing fumagillin.

Keywords: Honey, Fumagillin, Mass spectrometry, Residue analysis

Acknowledgement: This work was financially supported by the projects QH72144 and MSM6046137305.

P-61
A SURVEY OF TOTAL AMITRAZ RESIDUES IN HONEY PRODUCED IN SLOVENIA

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Amitraz is a veterinary medicinal product used to control the mite Varroa jacobsoni destructor, which is a wide spread parasite that affect honey beehives. The molecule of amitraz is unstable and easily hydrolysed to 2,4-dimethylaniline (2,4-DMA) and other products which contain DMA moiety (2,4-dimethylphenylformamide and N-(2,4-dimethylphenyl)-N'-methylformamidine). In the year 2011 a survey of 130 randomly sampled honey from Slovenia was carried out. The exceeded values of total amitraz residues according to European Union regulations were investigated. The analytical method was acid hydrolysis followed by the alkaline hydrolysis of amitraz and its metabolites to 2,4 - DMA. The extraction with organic solvent (n-hexane) and derivatization with heptafluorobutyric anhidride (HFBA) were followed. The active substance was quantified using gas chromatography with electron capture detection (GC-ECD) and the positive findings were confirmed by gas chromatography with mass spectrometry detection (GC-MS). The results of survey show that no sample among 130 exceeded the maximal residue limit (MRL) which is 0.2 mg/kg for total amitraz residues in honey. We confirmed the positive findings at 9 samples (7%), where the values ranged between 0.01 and 003 mg/kg. The majority of honey samples (121 samples or 93%) had the total amitraz content below the limit of quantification (LOQ) which is 0.01 mg/kg.

Keywords: honey, quality, amitraz, metabolites

P-62
RAPID DETECTION OF (LEUCO)MALACHITE GREEN IN FISH: A COMPARATIVE STUDY BETWEEN ANTIBODY, APTAMER AND RECEPTOR MG-BINDERS

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Malachite Green (MG) is a synthetic dye that is sometimes illegally used in aquaculture as antifungal, antimicrobial and antiparasitic agent. Due to its (mutagenic and carcinogenic) toxic effects on human health, this dye has been banned in animal product for human consumption in Europe. The European Commission has established a Minimum Required Performance Limit (MRPL) for the analysis of MG and its metabolite Leucomalachite Green (LMG) at 2 ppb. With aim to develop a rapid dipstick-based assay for the detection of MG/LMG in fish, we have characterized and compared 3 different types of anti-MG/LMG binding molecules including a polyclonal antibody, a RNA aptamer and a biological receptor. The best reagent has therefore been chosen and implemented to a lateral flow device format developed to detect MG/LMG at ppb level in fish tissue.

Keywords: MALACHITE GREEN, DIPSTICK, FISH

Acknowledgement: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° KBBE-211326.

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APPENDIX

H-53
PYRROLIZIDINE ALKALOIDS IN ANIMAL FEED – A SURVEY CONDUCTED IN THE NETHERLANDS

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Pyrrolizidine alkaloids (PAs) are secondary plant metabolites present in many plants belonging to the Asteraceae, Boraginaceae and Fabaceae families. They act in the plant as chemical defense compounds against herbivore attack. Unfortunately, they are also toxic for mammals and humans, causing hepatic veno-occlusive disease, liver cirrhosis and ultimately death. Particularly notorious are PAs present in ragwort and groundsel species (Senecio), as well as those present in various borage, heliotropium and crotolaria species, which are held responsible for hepatic disease in horses and cows and causing livestock losses worldwide. PAs can be found in nature in a very wide array of structures (over 600 PAs are known), presenting a considerable analytical challenge. In 2007, the European Food Safety Authority (EFSA) published its scientific opinion on PAs in animal feed (EFSA Journal, 447: 1 (2007)). It was concluded that analytical methods for the detection and quantification of PAs in animal feed were largely lacking and that there was a need for monitoring and survey data. A (semi)quantitative LC-MS/MS method was originally developed for the analysis of PAs present in ragwort species (Dutch survey PAs in animal forage, 2009, http://edepot.wur.nl/135952). The method has been expanded to comprise 70 PAs (tertiary bases and N-oxides of monoester, diester and macrocyclic diester structures) covering the major PA producing plant families. The method has been used for the analysis of 351 animal feed samples collected in 2006-2010. Most samples collected were forages and roughages (252 samples) but also samples of oil seeds (56) and samples of herbal feed additives (32) were included. In 47% of the samples analysed, PAs were detected above the limit of detection (4.5 µg/kg). Substantial amounts of PAs were found in forage and roughage samples, on average 272 µg/kg with a maximum of 22,750 µg/kg for a sample of hay. Macrocyclic diester PAs typical for Senecio species accounted for approximately 85% of the total PA content in forage and roughage samples, while mono and diesters (Boraginaceae type) accounted for the remaining 15%. Mono and diester PAs were the prominent type found in the oil seed samples (mostly soya), accounting for approximately 64%. Macrocyclic PAs accounted for 33% and PAs of the Monocrotolaria type for the remaining 3%. The average PA content found in oil seeds was only 13 µg/kg. Substantial amounts were found in the group of herbal feed additives, with a mean concentration of 316 µg/kg and a maximum of 2252 µg/kg. Mono and diester PAs typical for Heliotropium species were the most common (70%); Boraginaceae PAs and Senecio PAs accounted for 22 and 8%, respectively. It can be concluded that PAs are quite often present in animal feed, sometimes in considerable quantities. It cannot be excluded that prolonged consumption of these highly contaminated feeds can lead to negative health effects in sensitive species such as horses.

Keywords: pyrrolizidine alkaloids, animal feed, LC-MS/MS, survey

I-9
CHARACTERIZING INORGANIC NANOPARTICLES IN FOOD BY ELECTRON MICROSCOPY

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Characterization of the nanoparticles (NPs) within the food matrices is a challenging issue. There is not much information available concerning the behaviour of NPs within the food matrices. Most analytical methods dealing with NPs require major alteration or complete removal of food matrix prior to testing, hence potentially affecting characteristics of NPs. This poster presents an overview of electron microscopy methods which can be applied for characterization of NPs within the food matrix.

Keywords: Nanoparticles, Electron Microscopy, Detection, Characterization

Acknowledgement: The authors of the poster would like to acknowledge Ian Morrison for Clairscope™ imaging work and Ping Luo for crucial advice. Special thanks to staff, students and directory of JEOL Nanocentre for technical support.
The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245162.

LM-1
RAPID ANALYSIS OF PESTICIDES IN DIFFERENT FOOD MATRICES USING A DIRECT SAMPLING ANALYSIS (DSA) SOURCE

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Pesticide measurements are of interest to both environmental and food regulatory agencies. Fast screening with little or no sample preparation is ideal to provide economical turnaround of an increasing sample workload. In this work, we have developed a rapid method for determination of pesticides in different food matrices such as orange juice, orange peel and olive oil using a new direct sampling analysis ambient desorption source (DSA) and TOF Mass spectrometer The DSA (Direct Sampling Analysis) ionization source utilizes field free ± APCI. Field-free APCI is similar to APCI in that both techniques use a corona discharge needle as an ionization source. However, with the DSA field-free ionization source, the corona needle is fixed in position and shielded within the probe. This places the needle in the optimal position within the vaporized stream for the ionization process and decouples the needles corona field from the ion entrance field. Consequently, it is very easy to optimize the DSA source, as no mechanical adjustments of the needle or probe position are required, and the electrical field around the ion entrance to the MS is unaffected by the corona field. The DSA source was interfaced to a PerkinElmer Axion™ 2 TOF mass spectrometer and data were processed using AxION Solo™ target analysis software. The TOF MS provided accurate mass measurement of the pesticides with less than 2 ppm mass accuracy, enough to provide molecular formula confirmation. DSA source parameters such as nitrogen gas flow rate, source temperature, capillary entrance voltage, sample position were optimized to provide maximum sensitivity. For measurement of pesticides in liquid matrices such as fruit juice and olive oil, the sample was pipetted onto a novel steel mesh sample accessory for measurement. The measurement of pesticides on solid surfaces such as an orange peel was accomplished by surface swabbing of the fruit surface with polyurethane foam. The measurement of pesticides in food matrices was performed with no sample preparation. The detection limits of various pesticides were 3 ppm or better and analysis time was less than 30 seconds. Expansion of the work to additional hazardous and nutritional chemicals will be explored.

Keywords: Pesticides, TOF, Instrumentation, Analysis

LM-2
AN ELISA TEST FOR THE DETECTION OF NIFURSOL RESIDUE IN CHICKEN MUSCLE AND SHIRMP TISSUE

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Nifursol (3,5-Dinitro-N'-(5-nitrofurfurylidene)salicylohydrazide) is an antibiotic, mainly used as a feed additive in the prevention of histomoniasis in chickens and turkeys. Histomoniasis is potentially fatal disease caused by the protozoan Histomonas meleagridis. An ELISA test, named AgraQuant Nifursol, has been developed to detect 3,5-dinitrosalicylic acid hydrazide (DNSH), the marker residue of nifursol metabolites. The test is performed as a solid phase direct competitive ELISA using a horseradish peroxidase conjugate as the competing, measurable entity. The DNSH from chicken muscle or shrimp tissue is derivatised with nitrobenzoic aldehyde. After extraction, the derivatized DNSH compound is then mixed with enzyme conjugate in the antibody coated microwells. After incubation at room temperature for 30 minutes, the microwells are washed and enzyme substrate is added and allowed to incubate for an additional 15 minutes. Stop solution is then added and the intensity of the resulting yellow colour is measured optically with a microplate reader at 450 nm with a differential filter of 630 nm. The test has limit of detections of 0.41 µg kg^{-1} and 0.24 µg kg^{-1} on chicken muscle and shrimp tissue, respectively. Results obtained from internal studies assessing accelerated stability, accuracy, precision and cross reactivity determined the test to be accurate, precise, sensitive and effective for measuring nifursol residue in chicken muscle and shrimp tissue.

Keywords: ELISA, Nifursol, DNSH, Chicken muscle, Shrimp tissue

LM-3
3-MCPD-ESTERS ANALYSIS IN EDIBLE OILS AND FATS USING LARGE VOLUME INJECTION AND COMPREHENSIVE GC×GC-TOF MS

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Recently there has been considerable interest in the formation of 3-chloropropane-1,2-diol (3-MCPD)-fatty acid esters during edible-oil processing. In model systems the 3-MCPD-esters have been shown to yield free 3-MCPD as a result of lipase activity. Because of the suspected toxicity of the free 3-MCPD, new methods for the analysis of its fatty acid esters have recently been developed. The current analytical methods used for the analysis of 3-MCPD esters in edible oils and fats actually measure the total 3-MCPD content of the oil or fat after hydrolysis. The procedures consist of many subsequent steps starting with the hydrolysis, removal of the fatty acids (as their FAMEs), extraction of the free 3-MCPD with salting out, derivatisation with phenylboronic acid, preconcentration by solvent evaporation and finally GC-MS analysis. Deuterium labelled 3-MCPD-d5 or esters thereof, are used as internal standards. Potential problems in this procedure are degradation of the 3-MCPDs during (alkaline) hydrolysis resulting in high detection limits, and the formation of additional 3-MCPDs if chloride salts are used in the salting out extraction. Our new work here shows the use of comprehensive GC×GC–ToF MS with large volume injection for faster, more reliable and more sensitive 3-MCPD analysis in oils and fats. Sample aliquots up to 25 µl are injected. As a result, the salting out step can be excluded from the sample preparation. The two benefits are low detection limits are obtained even at low extraction recoveries and thus the side reaction with the chloride can be eliminated. A further advantage is the final preconcentration step could be skipped making the method faster and reducing the manual sample handling. A clear advantage of the use of comprehensive GC×GC–ToF MS is the substantially improved resolution of the GC separation leading to the elimination of interferences even at very low 3-MCPD levels. Also higher system stability is achieved due to the open-source design of the Time-of-Flight Mass Spectrometer.

Keywords: 3-MCPD, GC×GC–TOFMS,Comprehensive Gas Chromatography, Time-of-Flight Mass Spectrometry

LM-4
ANALYSIS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN COMPLEX MATRICES BY GAS CHROMATOGRAPHY WITH HIGH RESOLUTION-TIME-OF-FLIGHT MASS SPECTROMETRY (GC-HRTOFMS)

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in a vast number of household consumer products including furniture, upholstery, electrical equipment, electronic devices, and textiles. These compounds can enter the environment through manufacturing process emissions, off-gassing from various products, recycling wastes, and leaching from waste disposal sites. PBDEs have been detected in air, sediments, surface water, fish, and other marine animals. These compounds have been widely used since the 1970s and there is growing concern about their persistence in the environment and their tendency to bioaccumulate in the food chain. Various sample matrices, including sewage sludge, food products, and human breast milk were analyzed by gas chromatography High Resolution Time-of-Flight Mass Spectrometry (GC–HRTOFMS) using novel multi-reflecting TOF technology. This data highlights the advantages of GC–HRTOFMS for the analysis of PBDEs in extremely complex matrices. The ability of HRTOFMS to selectively extract masses at high resolution virtually eliminates background noise allowing both successful screening and trace level detection to be achieved.

Keywords: GC-HRTOFMS, Multi Reflecting, PBDE, High-Resolution Time-of-Flight Mass Spectrometry, Gas Chromatography

LM-5
ANALYSIS OF POLYCHLORINATED BIPHENYLS (PCBS) IN FISH OIL SUPPLEMENTS BY GAS CHROMATOGRAPHY WITH HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY (GC-HRTOFMS)

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Health associations recommend eating two servings of fish, especially fish with high fat content, such as tuna and salmon, per week. This recommendation is due to the presence of omega-3 fatty acids in high fat content fish species. Studies have shown that omega-3 fatty acids have many health benefits. These include decreased risk of cardiac dysrhythmia, decreased triglyceride levels and slowed growth rate of atherosclerotic plaque. Unfortunately, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) which are known to bioaccumulate in the food chain are also present in these same high fat content marine species. Because of such environmental contaminants, the FDA recommends limiting the consumption of predatory fish that reside at the top of the food chain. Fish oil supplements from various suppliers were analyzed by gas chromatography and High Resolution Time-of- Flight Mass Spectrometry (GC–HRTOFMS) using novel multi-reflecting TOF technology. This data highlights the advantages of GC–HRTOFMS for the analysis of PCBs in extremely complex biological matrices. The ability of HRTOFMS to selectively extract masses at high resolution virtually eliminated background interferences attributed to the matrix which allowed both successful screening and trace level detection to be achieved.

Keywords: GC-HRTOFMS, Multi Reflecting, PCB, High-Resolution Time-of-Flight Mass Spectrometry, Gas Chromatography

LM-6
EASY ENZYMATIC TESTS FOR FOOD ALLERGENS DETECTION

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The SEDIUM RD Company and Food Research Institute Prague developed the semi-quantitative Easy Enzymatic Test (EET) for detection of the food allergens in raw materials as well as production lines for allergen free products, including swab. The principle of well-known and verified classical ELISA method is applied. This Easy Enzymatic Test for the detection of food allergens is based on the reaction of specific antibodies with allergenic protein in extracts from various foods. All necessary reagents are ready to use in reaction vials that form the complete set of EET. The set is designed for the analysis of five or ten different samples. The result of the analysis is interpreted in the form of: a negative sample (does not contain allergenic protein); a positive sample (contains allergenic protein). No personal laboratory skills are needed. Samples are extracted in the pre-filled extraction buffer and after sedimentation they could be tested directly. The positive and negative samples are analyzed simultaneously so that the objective comparison of true sample and the correctness of the assay performance are under control. The EET sets for gluten, milk, egg and peanut detection are currently available.

Keywords: allergen, allergen detection, ELISA, food allergen

LM-7
ELISA KIT FOR THE DETERMINATION OF PEANUT PROTEIN

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A new high sensitive Peanut ELISA kit has been developed by the SEDIUM RD Company and Food Research Institute Prague for quantitative determination of peanuts proteins. Peanut ELISA kit is designed for detection of the peanut proteins in raw materials as well as production lines for allergen free products. This sandwich assay is based on own rabbit polyclonal antibodies where crude extract from roasted peanut was used for immunization doses. The kit does not produce any false positive results or cross-reactivity with broad range of food matrix including cereals, nuts and legumes. The method of sample extraction was tested and in-house validation of the kit was performed to determine the important assay parameters as the analytical sensitivity, functional sensitivity, repeatability and recovery. This testing also included the stress test of individual kit components. The number of model samples and commercial food samples was analyzed by the Peanut ELISA kit.

Keywords: peanut, food allergen, allergen detection, ELISA

LM-8
MONITORING ANTI-IMPOTENCE DRUGS AND ITS ANALOGUES IN FOODS

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Illegal compounds such as drugs and its synthetic analogues have been detected in foods until a recent date in Korea. Especially, unknown compounds that have the modified chemical structure of anti-impotence drugs such sildenafil, vardenafil, and tadalafil were frequently detected in various foodstuffs. Illegal compounds that have modified chemical structure of the drugs have been used to avoid the government inspection. The adulteration of foods with drug analogues is potentially dangerous for human health because it is not proved their safety at all. In order to ensure food safety, we investigated the actual condition of the suspected samples and monitored about 51 domestic retail foods. Two simultaneous analytical methods were established using HPLC/PDA and confirmed with LC/MS for 31 analogues and 5 anti-impotence drugs. Anti-impotence drugs and its analogues were detected in 13 items. Tadalafil was detected with range of 4,139–65,315 mg/kg in 8 items. Sildenafil with range of 3,621–394,438 mg/kg in 6 items. Anti-impotence drugs analogues such as octylnortadalafil and demethylsildenafil was detected with range of 1,174–214,094 mg/kg in 4 items. Hydroxythiohomosildenafil was detected 14,717mg/kg in one items

Keywords: Illegal compounds, Anti-impotence drugs and its analogues, HPLC-PDA, LC/MS

Acknowledgement: This research was supported by Grant Number 11161kfda151 from the KFDA in 2011.

LM-9
ANALYSIS OF GLYCOSYLATED TERPENS IN LIQUEROUS MUSCATEL WINES BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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In Portugal, Setúbal and Douro regions have significant productions of fortified wines made with Moscatel grape varieties: these wines are appreciated by consumers due to the pleasant fragrance and sweet taste. Terpene compounds may occur in free form but the non-volatile glycosylated terpens have higher concentrations and are precursors of the free forms after enzymatic or acid hydrolysis. Analyses were carried out with a LC-DAD coupled with a tandem mass spectrometer type Triple Quadrupole (TQ) (Four Micro™ Micromass®, Waters®) equipped with an electrospray source (ESI) that was used in negative mode (range m/z 100 to 2000). For detection of these compounds we sought to chromatographic peaks whose mass spectra contained ions with values of m/z that could correspond to the quasi-molecular ion (M-1) and its adduct with the formic acid existing in the eluent (M+45). Six chromatographic peaks corresponded to compounds characterized by the ions *m/z* 447 (and 493) and were identified as conjugates of terpenols with arabinosylglucoside or apiosylglucoside. Five chromatographic peaks characterized by ions *m/z* 461 (and 507) may correspond to conjugates of terpenols with ramnosylglucose (rutinose). Alternatively, the ions m/z 461 (and 507) could also correspond to the conjugate of geranic (or neric) acid with arabinosylglucose or apiosylglucose. - The compound characterized by the ions *m/z* 475 (and 521) may correspond to the ramnosylglucoside of geranic (or neric) acid and was not previously reported. The monoglucosides of terpenols *m/z* 315 (and 361) and terpenol oxides *m/z* 331 (and 377) occur at lower retention times but detection and identification is more difficult because their concentrations are far lower. Some of these compounds could also be detected in extracts of grape skins.

Antonella Nasi et al., Identification of free and bound volatile compounds as typicalness and authenticity markers of non-aromatic grapes and wines through a combined use of mass spectrometric techniques, Food Chemistry, 110 (2008) 762–768.

Keywords: Wine, terpens, LC-MS/MS

Acknowledgement: Fundação para a Ciencia e Tecnologia (FCT): REDE/1518/REM/2005

LM-10
OPTIMIZATION OF GAS CHROMATOGRAPHY ION-TRAP TANDEM MASS SPECTROMETRY PARAMETERS FOR THE DETERMINATION OF LOW LEVELS OF PESTICIDES

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Full scan GC-MS analysis is commonly used for the screening of samples for compliance to maximum residue limits, as this technique provides both quantitative as well as library-matching-based qualitative information. But analysis in full scan mode often fails to provide the desired level of sensitivity and selectivity and suffers from unacceptably high matrix interferences [1,2]. To overcome these problems, gas chromatography-tandem mass spectrometry (GC-MS/MS) appears as a powerful technique because of its capability to exclude spectral interferences by separating coeluting compounds on the basis of compound-specific target-oriented multiple reaction monitoring (MRM) transitions [3].
A multiclass analysis method was validated and optimized for monitoring low level of pesticides in order to apply high-resolution of a gas chromatography with ion-trap and tandem mass spectrometry. The selected class of pesticide residues are atrazine desethyl atrazine, simazine, vinclozolin, alachlor, fenpropathrin, λ-cyhalothrin, permethrin, β-cyfluthrin, cypermethrin and fenvalerate. In this work, the influence of some analytical parameters on pesticide signal response is explored. Six IT-MS operating parameters, including isolation time (IT), excitation voltage (EV), excitation time (ET), factor q, ion source temperature (Tion source) and isolation mass window (IMW) were adjusted in order to optimize the instrument analytical performance. We are take the default parameters of the equipment as a factor q = 0.45, IT=12 ms, ET=15 ms, Tion source=250°C, EV=1 and IMW=1 were then modify one by one. The adjustment of all parameters substantially increased the sensitivity of IT-MS in the MS/MS mode. The results obtained show that four parameters (ET=5, EV=0.2, Factor Q=0.45 and IMW=4) had the strongest influence in signal response and in limits of detection.

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Keywords: Pesticides, Gas chromatography-tandem mass spectrometry

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LM-11
PESTICIDES RESIDUES IN STRAWBERRIES GROWN USING INTEGRATED PEST MANAGEMENT AND ORGANIC FARMING IN 2009–2010

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This work aims to determine differences in terms of pesticide residues content in Portuguese strawberries grown using different agriculture practices. Pesticides are among the most widely used chemicals in the world. Because of the widespread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets. Scientists do not yet have a clear understanding of the health effects of these pesticide residues. The QuEChERS sample preparation method was conducted and shown to have good performance for multiclass pesticides extraction in strawberries [1,2] . The screening of 40 pesticides residues was performed by gas chromatography-tandem mass spectrometry (GC-MS/MS). In quantitative validation, acceptable performances were achieved with recoveries 70–120 % and <20 % RSD for 37 pesticides. The limits of detection were in range of 0.3–23 µg/kg. The method was applied to analyze strawberry samples from organic and integrated pest management (IPM) practices harvested in 2009–2011. The results showed the presence of lindane (gamma-HCH) above the MRL in strawberries grown using farming practice and fludioxonil, cyprodinil, iprodione and fluazifop-p-butyl using integrated pest management below the MRL.

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[2] V.C. Fernandes, V.F. Domingues, N. Mateus, C. Delerue-Matos, Journal of Agricultural and Food Chemistry 59 (2011) 7582.

Keywords: Pesticides, GC-MS/MS, strawberries, organic farming

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LM-12
DETERMINATION OF PESTICIDES RESIDUES IN LETTUCES USING QUECHERS

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Nowadays, the assessment of pesticides in food is a major concern for scientists because of health problems to humans. In this work we followed a methodology based on an extraction and cleanup by QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe method). The initial extraction step we used 50 mL plastic centrifuge tubes containing 6 g anhydrous magnesium sulfate (MgSO4), 1.5 g sodium acetate (NaOAc) and for the cleanup of lettuce extracts we used 2 mL mini-centrifuge tubes containing 150 mg anhydrous MgSO4, 50 mg primary secondary amine (PSA) sorbent, 50 mg C18 (EC) and 50 mg graphitized carbon black (GCB). The chromatographic analysis was carried out in a Thermo Scientific Trace GC Ultra, with an ion trap mass spectrophotometer Polaris Q, MSn (n>5) detector. The lettuce samples were contaminated with the prepared mixtures of multiclass pesticides in the concentration range of 0.005 mg/kg - 0.300 mg/kg. The determination coefficients (R2) were greater than 0.99, indicating a good linearity of analytical curve and the limits of detection (LOD) were calculated by instrumental data. Matrix effects are known to be problematic in pesticide residue analysis. Therefore matrix standard calibration solutions were prepared by adding known amounts of the analytes to a residue-free sample to compensate matrix-induced chromatographic response enhancement observed for certain pesticides. Nevertheless the signal large decrease or even its disappearance in the analysis of some pesticides was observed. The lettuces samples were purchased from 4 major markets and 2 individual vegetables sellers in Esposende (Region of Minho, northwest of Portugal). The samples from major markets were portuguese lettuce samples from Estremadura, Trás-os-Montes e Alto Douro, Beira Litoral, Ribatejo and Alentejo.

Keywords: Lettuce, pesticides, QuEChERS, gas chromatography and mass spectrometry

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LM-13
CHARACTERIZATION OF WINE CIDER VINEGAR FERMENTATION REGARDING THE BIOMASS, VOLATILE AND SEMI-VOLATILE COMPOUNDS EVOLUTION

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A wine cider vinegar fermentation was realized in a pilot plant acetator, Chansard model, of 300L by using the acetic acid bacteria, Acetobacter senegalenisis. The aim of the present work was to analyze the biomass evolution during the fermentation process, to measure the production and consumption of the major organic acids as well as the other products of fermentation and to identify and quantify(%of total peak area) the volatile compounds formed. During the vinegar fermentation, samples were taken at various elapsed fermentation times in order to evaluate the changes of the volatile and semi-volatile fingerprints appeared during the elaboration. The evaluation of the sugars and organic acids formed, was followed by using the traditional high performance liquid chromatography (HPLC) technique, by using an ion exchange column: Supelcogel C-610H type (300mm × 7.8mm) and a pre-column Supelguard H type (5cm x 4.6mm) (Supelco). The linearity of the 10 analyzed compounds (glucose, fructose, ethanol, malic, succinic, lactic, formic, acetic, propionic and butiric acid) were over 0.99 of R_c in concentration between 0.125–4 μg/ml. The volatile compounds were analyzed at different fermentation times (2, 48, 60, 87 and 101 hours) by using a headspace-solid phase microextraction (HS/SPME) coupled with a gas chromatography and mass spectrometry technique (GC-MS) and a HS/GC–MS technique. Comparing the two gas chromatographic methods, significant differences have been found regarding the number of volatile compounds found and the major volatile compounds identified for each sample. The resulting data has been analyzed by the Principal Component Analysis (PCA) using a XLSTAT software version 2011.4.02 (Addinsoft 1995–2011). The Principal Component Analysis (PCA) was realized by using the volatile compounds that were identified in the gaseous phase from the analyzed samples and was used in order to have a complete representation of the main volatiles that can distinguish the vinegar analyzed samples.

Keywords: Vinegar fermentation, HPLC, HS/SPME-GC-MS, HS/GC-MS, PCA

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LM-14
PERFLUOROALKYL SUBSTANCES IN FRUITS, VEGETABLES AND DRY FOOD ITEMS COLLECTED IN FOUR EUROPEAN COUNTRIES; PERFOOD

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Since 1950, perfluoroalkyl substances and polyfluoroalkyl substances (PFASs) have been widely used in a broad range of industrial and commercial applications. As a consequence of the widespread use of PFASs and their resulting emissions, these substances have been detected in the environment, wildlife and humans (Buck et al., 2011). The relative significance of various humane exposure pathways is still not fully understood but it is clear that food appears to be one of the major contributors. Nevertheless, data on levels of PFASs in the human diet and drinking water are still rather scarce (D'Hollander et al., 2010). The small amount available literature is limited to individual national sample campaigns which makes comparison between different countries difficult. In addition, the extraction procedures, analytical details and quality control are deficient in most of the papers that were published before 2008. In the EU project PERFOOD, standardized selection of food items, sampling procedures and analytical methods as well as evaluation strategies were applied, enabling a unique assessment of the occurrence of PFASs in European food as well as the identification of major sources of PFAS exposure via food. In the 1st sample campaign of PERFOOD, food items were selected in respect to their average consumption amounts typical in four European main regions (East, West, North and South). During the sampling campaign in spring-summer 2010 more than 800 raw food items were purchased, homogenized and after pooling analyzed in selected laboratories. This study will present the results of the PFASs levels in fruit, vegetables and a variety of dry food items sampled in Czech Republic, Italy, Norway and Belgium. Target analytes were 4 perfluorosulfonates (C4, C6, C8 and C10) and 11 perfluorocarboxylates (C3–C14). Three different types of extraction procedures were used for fruit, vegetables and dry items. Analysis was performed using an UPLC coupled to a MS/MS. In general, the PFAS levels in all the food items were low (pg/g range) with the exception of some samples from Belgium which reached levels up to 1–2 ng/g. Short chain PFCA were more abundant compared to the longer chains. PFSAs had a lower detection frequency compared to the PFCAs. Highest PFASs levels (sum of PFSAs and PFCAs) were found in Belgian lettuce and strawberries (2 ng/g). The average sum PFASs levels were highest in Belgium and Norway, followed by Italy and Czech Republic. The higher levels found in Belgian could possibly be explained by the presence of a perfluorochemical manufacturing plant. To conclude the analyzed dietary items will not be the main contributors to the intake of PFCs through our diet. However, these data showed that these food items could be potential sources of exposure, especially if the fruit/vegetables origins from locations in the vicinity of point sources.

Keywords: PFASs, PFSAs, PFACs, food, Europe

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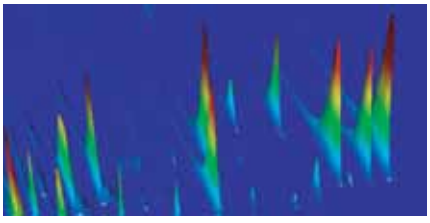


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