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Development of Microfluidic Chip Technology for Spatial Three-Dimensional Liquid Chromatography

De Vos, Jelle; Themelis, Thomas; Amini, Ali; Eeltink, Sebastiaan

HTC-16

29-31 January 2020
Ghent, Belgium

16th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology

http://www.htc-16.com

FINAL PROGRAM

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Ken Broeckhoven, Brussel, BE

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16TH INTERNATIONAL SYMPOSIUM ON HYPHENATED TECHNIQUES IN CHROMATOGRAPHY AND SEPARATION TECHNOLOGY

HTC-16

SYMPOSIUM VENUE

Het Pand
Onderbergen 1
9000, Ghent, Belgium

ORGANIZING SECRETARIAT

Dienst Congres en Event
htc16@kuleuven.be
KU Leuven, Leuven, Belgium

WWW.HTC-16.COM

Dear Colleagues and Friends,

It is my great pleasure to welcome you at the 16th International Symposium on Hyphenated Techniques in Chromatography and Separation technology (HTC-16), that will take place at Conference Center '*Het Pand*' in Ghent (Belgium). HTC-16 is organized under the auspices of the Royal Flemish Chemical Society (KVCV) and the Royal Society of Chemistry (RSC).

The HTC-16 conference is the premier platform for state-of-the-art developments in separation technologies and hyphenated techniques. The conference encompasses 3 parallel sessions consisting of plenary lectures, keynote lectures, tutorials, oral and poster presentations. An entire parallel session is dedicated to young emerging scientists. The symposium also hosts an attractive technical exhibition where vendors present their newest instruments and developments, topped with technical seminars. I would like to thank our sponsors, in particular, Shimadzu, Thermo Fisher Scientific, and Agilent Technologies, and all other sponsors and exhibitors for their support of the conference.

The scientific program covers fundamental and practical aspects of liquid-phase and gas chromatography, including UHPLC-MS, 2D-LC, GC(×GC)-MS, SFC, *etc*. The RSC Separation Science Group sessions organized on Thursday and Friday focus on high-throughput analysis, sample preparation and introduction, and data complexity, mining, and curation.

To encourage scientific exchange and friendship building, the scientific program is topped off with a rich social program consisting of a Belgian Beer tasting event on Wednesday, the Symposium Dinner on Thursday evening, and a Farewell Drink.

On behalf of the entire organizing team of HTC-16, welcome to Ghent!

Sebastiaan Eeltink

Vrije Universiteit Brussel

HTC-16 Chairman

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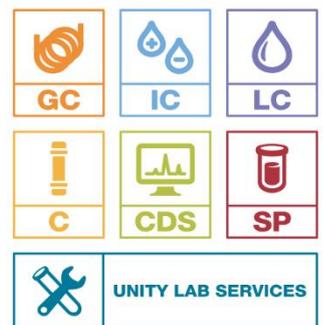
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GL Sciences is a comprehensive manufacturer of analytical devices and consumables. We manufacture and sell equipment, columns, fillers, and consumables.

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Interscience develops turnkey GC&MS solutions for industry and academia since 1975. Locations: Louvain-la-Neuve, Belgium, Breda, The Netherlands; Facilities: GC, GC/MS, GC/MSMS, GCxGC/TOFMS, GC-HR/AM MS, LC-GC, SIFT-MS, Headspace, P&T, thermal desorption, pyrolysis; Activities: Hardware development & integration, Method development & validation, Sample prep automation, Feasibility studies, Consulting, Training, Website: www.inter.science, www.is-x.com, www.sampleq.com

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SepSolve Analytical

SepSolve Analytical is a supplier of robust and affordable GC×GC and detector (FID and TOF MS) technology. We work with you to deliver a package of analytical instrumentation, accessories and methodology to solve your separation science problems with the unrivalled power of multi-dimensional gas chromatography. Our knowledge and expertise enable us to understand the key issues in any analytical challenge, and so advise you on the best package of instrumentation to deliver the results you're looking for.

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Based in Cambridgeshire, UK, Anthias Consulting Ltd are experts in the analytical sciences in the fields of Gas Chromatography (GC, GCxGC), Liquid Chromatography (HPLC, UHPLC, LCxLC), Mass Spectrometry (GC-MS, LC-MS, ICP-MS), and spectroscopy techniques (ICP-OES, IR, UV/Vis, AA) and all their related techniques. We provide a range of services in consulting, instrument training, demonstrations and applications, method development and optimisation and tec hnical authoring for analysts, instrument manufacturers and technology companies from small, independent laboratories and academic institutions to multi-national corporations.

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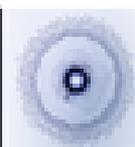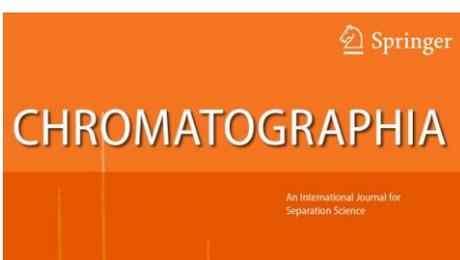
BELGIAN BEER TASTING SPONSORED BY THERMO FISHER SCIENTIFIC

The traditional Belgian Beer tasting event will take place on Wednesday 29 January 2020 from 18:30 in the poster room on the first floor of the conference center. Your voucher will be provided at the registration desk. Enjoy a choice of high-quality Belgian beers and selected snacks including Belgian Fries.

CONFERENCE DINNER

The HTC-16 Conference Dinner will be on Thursday 30 January 2020 in Monasterium Poortackere, Oude Houtlei 56, 9000 Ghent. Located in the "land (akker) in front of the gate (poort)" the Sint-Autbertus Beguinage was built in 1278. In the 19th century, a monastery and a new chapel were built. It is in this chapel that the conference dinner will take place.

The venue is about 300 m from the HTC-conference center "Het Pand" (5 min walking distance).

The dinner will start with an aperitif at 19:00, followed by a seated dinner at 20:00.

FAREWELL DRINK SPONSORED BY SHIMADZU

On Friday afternoon, 31 January 2020, HTC-16 will reach its end. Join together with your symposium friends for a last exchange of ideas and experiences while enjoying a Belgian beer or another drink. All participants and accompanying persons are kindly invited, no registration or voucher needed.

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2020 HTC INNOVATION AWARD

The HTC Innovation Award has been launched to celebrate the work of scientists who are innovatively evolving the field of hyphenated techniques. LCGC Europe will present the award at the event. Applications from separation scientists worldwide were welcomed and LCGC Europe readers were invited to nominate themselves or others in the course of 2019.

The winner has been selected by the HTC-16 Scientific Committee and the HTC-16 Industry Board based on the following criteria:

- o The winner has made an innovative contribution to the field of separation science by introducing new methodologies, new instrumentation, or new techniques in the field with a strong focus on applicability.
- o The winner is a scientist under 45 years of age.

The winner of the 2020 HTC Innovation Award will present his/her research at the HTC-16.

Previous HTC Innovation Award winner:

- o HTC-15 Cardiff (2018)
  Carolin HUHN, professor for *Effect-based Environmental Analysis* at the Chemistry Department, Institute for Physical and Theoretical Chemistry, **Eberhard Karls Universität Tübingen (Germany)**

HTC AWARD

The most innovative oral presentation given during the conference will receive the HTC Award, sponsored by Elsevier. The winner will be selected by the HTC-16 Scientific Committee and the HTC-16 Industry Board. The HTC award will be presented during the closing session of HTC-16 on Friday afternoon. Previous HTC-Award winners were:

- o Janusz Pawliszyn (1996)
- o N. Semenov (1998)
- o Heidi Goenaga-Infante (2000)
- o Aviv Amirav (2002)
- o Gert Desmet (2004)
- o Luigi Mondello (2006)
- o Robert Shellie (2008)
- o Oliver Trapp (2010)
- o Tuulia Hyötyläinen (2012)
- o Frank David (2014)
- o Paola Dugo (2016)

POSTER AWARDS

The three most innovative poster contributions will receive the HTC-poster award, sponsored by Analytical Science Advances (Wiley). To qualify for a poster award, you must have declared yourself a candidate upon submitting your abstract. Prof. Deirdre Cabooter will lead a jury consisting of a large number of recognized international experts on gas and liquid phase separations and hyphenated techniques.

BEST HTC TUBE

The best HTC TUBE video will receive an award sponsored by Chromatographia (Spinger). The jury will be composed of Dr. Achim Treumann (KBI Biopharma, BE), Prof. Hans-Gerd Janssen (Unilever, NL), and Prof. Andre De Villiers (Stellenbosch University, ZA).

CONGRESS VENUE

The conference will be held in '**Het Pand**', the culture and congress center of Ghent University

Het Pand
Onderbergen 1
9000 Ghent
Belgium
tel. +32 (0)9 264 83 05
https://www.ugent.be/het-pand/en

The historical city of Ghent is located 60 km from Brussels international airport that has connections to over 120 destinations in Europe and is directly accessible by train from the airport. The Brussels South airport (Charleroi) that accommodates many low-cost airlines is located at 80 km from Ghent.

HOW TO REACH THE VENUE

The conference venue is within walking distance of most hotels in the city of Ghent.

By Tram:
From the Ghent Sint-Pieters train station, take Tram 1 (every 6 min.) or 24 (every 20 min.) and exit at stop 'Korenmarkt'. Tickets are available at every stop at the ticket machine.

By Road:
Ghent is located near the intersection of the E40 (Brussels-Calais) and E17 (Antwerp-Lille) highways. Underground parking (Parking Sint-Michiels) is available across the street from the conference venue.

By Rail:
In addition to the connection to the airport, Ghent has direct rail links to many cities in Belgium such as Brussels, Antwerp and Liège that host international train stations with high speed train links to France, The Netherlands, Germany and the UK. Ghent station (Gent Sint-Pieters) is located near the edge of the city and is a 2.5 km walk from the conference venue, the historical city center and hotels.

By Plane:
Brussels Airport is located in Zaventem, 15 km from the center of Brussels and 60 km from the conference venue in Ghent. The airport has over 120 direct connections to European cities, 12 to the USA and Canada, and more than 20 in Asia. The train station is located conveniently at the lower floor of the airport. The Brussels South airport (Charleroi) is located at 80 km from Ghent and has a shuttle service every 30 min to the Brussels-Midi train station

OFFICIAL LANGUAGE

English is the official Symposium language. No translation will be provided.

OFFICIAL LANGUAGE SHORT COURSES AND SYMPOSIUM DATES

Short courses: Tuesday, January 28, 2020 from 09.00 to 17.00
Symposium: from Wednesday, January 29, 2020 at 09.00 to Friday January 31 at 17.00

REGISTRATION DESK OPENING HOURS

Tuesday January 28   08:00 – 09:00 (for short course only)
Tuesday January 28   16:00 – 18:00 (pre-registration for conference)
Wednesday January 29   08:00 – 18:00
Thursday January 30   08:00 – 18:00
Friday January 31   08:00 – 15:00

Badge and conference bag will be available at the HTC-16 registration desk. All participants and exhibitors have to wear the name badge in the conference area, visible at all times.

SCIENTIFIC SESSIONS

Tuesday January   09:00 – 17:00   Short courses

Wednesday January 29   09:00 – 18:30   Scientific sessions
                          From 18:30   Belgian beer tasting (tickets required)

Thursday January 30   08:30 – 18:00   Scientific sessions
                          From 19:00   Conference dinner (tickets required)

Friday January 31   09:00 – 16:15   Scientific sessions
                          From 16:20   Farewell Drink

EXHIBITION

The exhibition is an important component of the conference, so please take the time to thank the exhibitors for their generous support of the program by visiting the booths located at the ground floor and first floor.

Exhibition area opening hours:

Wednesday January 29	09:00 – 17:00
Thursday January 30	10:00 – 17:00
Friday January 31	10:00 – 14:30

Exhibitor pass and media pass only allow entry in the Exhibition Area and do not allow entry in the scientific session.

LUNCHES

Wednesday January 29	12:55 – 14:00
Thursday January 30	12:55 – 14:00
Friday January 31	12:55 – 14:00

COFFEE BREAKS

Wednesday January 29	10:45 – 11:15	15:55 – 17:00
Thursday January 30	10:45 – 11:15	15:55 – 17:00
Friday January 31	10:45 – 11:15	

INSTRUCTION FOR SPEAKERS

All presentations must be in PowerPoint 4:3 format and must be copied to the laptop in your session at the latest 20 min before the start of your session. Speakers are recommended to arrive at least 15 minutes before the start of the session to introduce their self to the session chairs. Speakers are to respect time limits for their talks as provided by their chairmen.

ORAL COMMUNICATION SESSIONS

- o   Please arrive at your session at least 15 minutes before the start of the session to introduce yourself to the session chair and to submit your presentation.
- o   Kindly note that session chairs are under very strict instructions to keep their sessions on schedule. There are three sessions running in parallel with strict time constraints.
- o   Time allowed for presentation: 30 min for each keynote lecture or tutorial lecture, 20 min for each oral presentation, and 15 min for an "Young Emerging Scientist" lecture. This timing includes discussion, so please limit your presentation to allow time for question.

POSTER SESSIONS

Posters should be printed in portrait A0 format. Suitable materials to attach the posters to the board will be provided at the registration office, do not use any other material to attach your poster to avoid damage to the poster boards.

All posters will be on display during the entire conference. Poster have to be set up on Wednesday January 29 between 08:00 and 09:00 and removed on Friday before 14:30. If you don't remove your poster as mentioned above, the Organizing Secretariat will remove it (Organizing Secretariat assumes no responsibility of problems or damage).

"Posters will be organized according to topic:
- o DAT: Data complexity, mining and curation
- o FOOD: Advances in food analysis
- o FUN: Fundamentals
- o GC(xGC): Gas chromatography and GCxGC
- o HYP: Hyphenated approaches
- o MDLC: Multi-dimensional analysis
- o PHA: Advances in (bio)pharmaceutical analysis
- o SAM: Sample preparation and introduction
- o STA: Developments in stationary phases
- o SFC: Advances in supercritical fluid chromatography"

The best poster award winners will be announced on Friday, January 31, 2020 during the Closing Plenary Session at 16:00.

HTC TUBE

HTC-TUBE is a new initiative we will try for the first time. All you need is a video, no abstract or paper are required. The video lasts no more than 3 minutes and highlights your scientific results to a broad audience.

SPECIAL ISSUE PUBLICATION – ELSEVIER - JCA

All authors of both oral and poster presentations are kindly invited to submit manuscripts based on your presentation(s) at the HTC-16 meeting for publication in Journal of Chromatography A with the intention of publishing in a Special Issue that is dedicated to this symposium.

CONFERENCE WIFI

Free WIFI access is available throughout the conference venue. Please connect to guestHtc1 (password: mtngc7YL).

ONSITE REGISTRATION

Onsite registration can be made only by credit card.

Participant type	On-site
Academic /Industry	750 €
Student*	450 €
1-day registration	300 €
Accompanying person	300 €
Conference dinner**	80 €
Beer tasting**	30 €

* Student must provide an official proof of the full-time student status.
** This event has a maximum capacity.

Registration Fees (Academic, Industry and Student Conference) include:
- o Access to oral and poster sessions
- o Access to the exhibition hall
- o Coffee breaks and lunches on Wednesday, Thursday, and Friday
- o Farewell drink on Friday
- o Badge and conference bag

Accompanying Person Fee includes:
- o Coffee breaks and lunches on Wednesday, Thursday, and Friday
- o Farewell drink on Friday

GENERAL INFORMATION

The use of still or video cameras and cell phones is prohibited during oral sessions and in the poster and exhibition areas without the express consent of the poster presenter or exhibitor
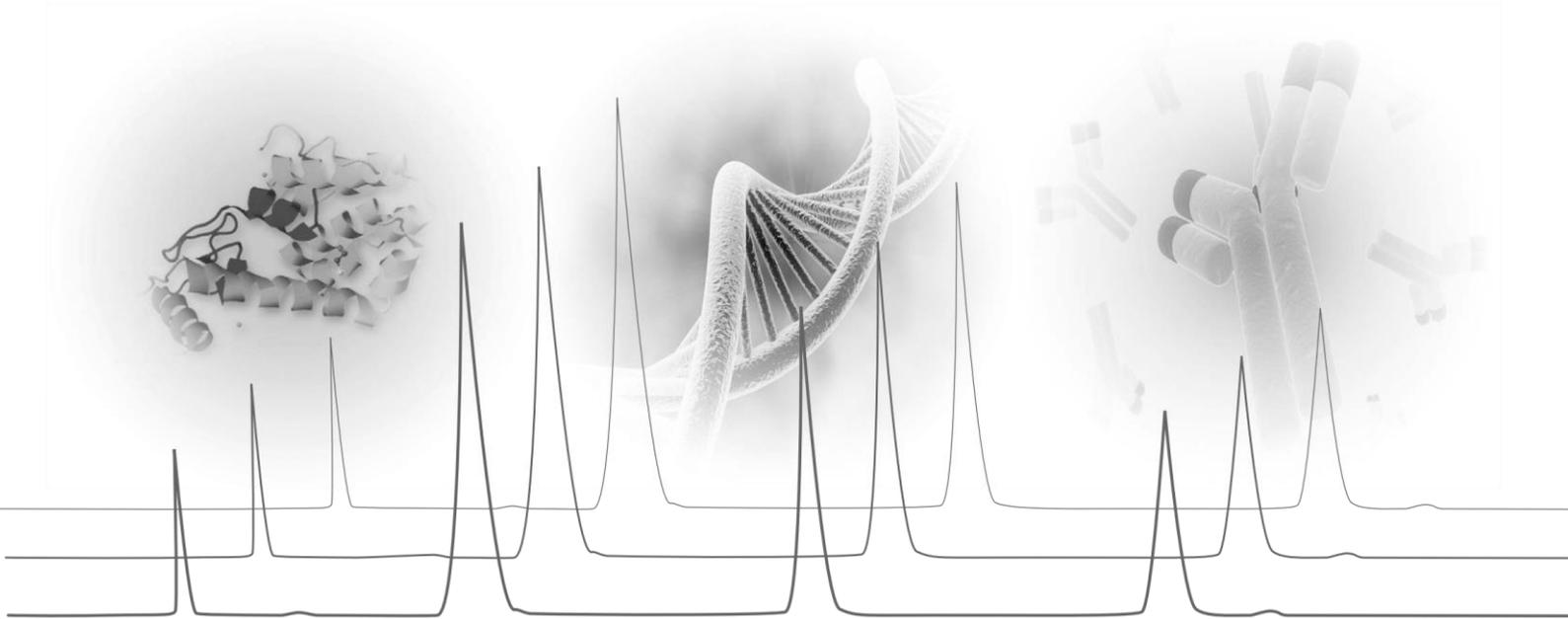
MEETING LOCATIONS

Oral presentations take place in the Refter lecture hall located at the ground floor, the Rector Vermeylen hall located at the second floor, and the Prior hall located at the 1st floor.

The exhibition of the platinum and gold sponsors are located in the Kapittel room at the ground floor (next to the main lecture hall). Silver and bronze sponsors are located at the Klooster corridor at the first floor next to the posters

Coffee/tea breaks and lunches take place next to the exhibitors.

FLOOR PLAN & EXHIBITORS
Ground floor: Platinum and Gold Sponsors
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- RIC-Gerstel
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- Thermo Fisher Scientific
- Waters
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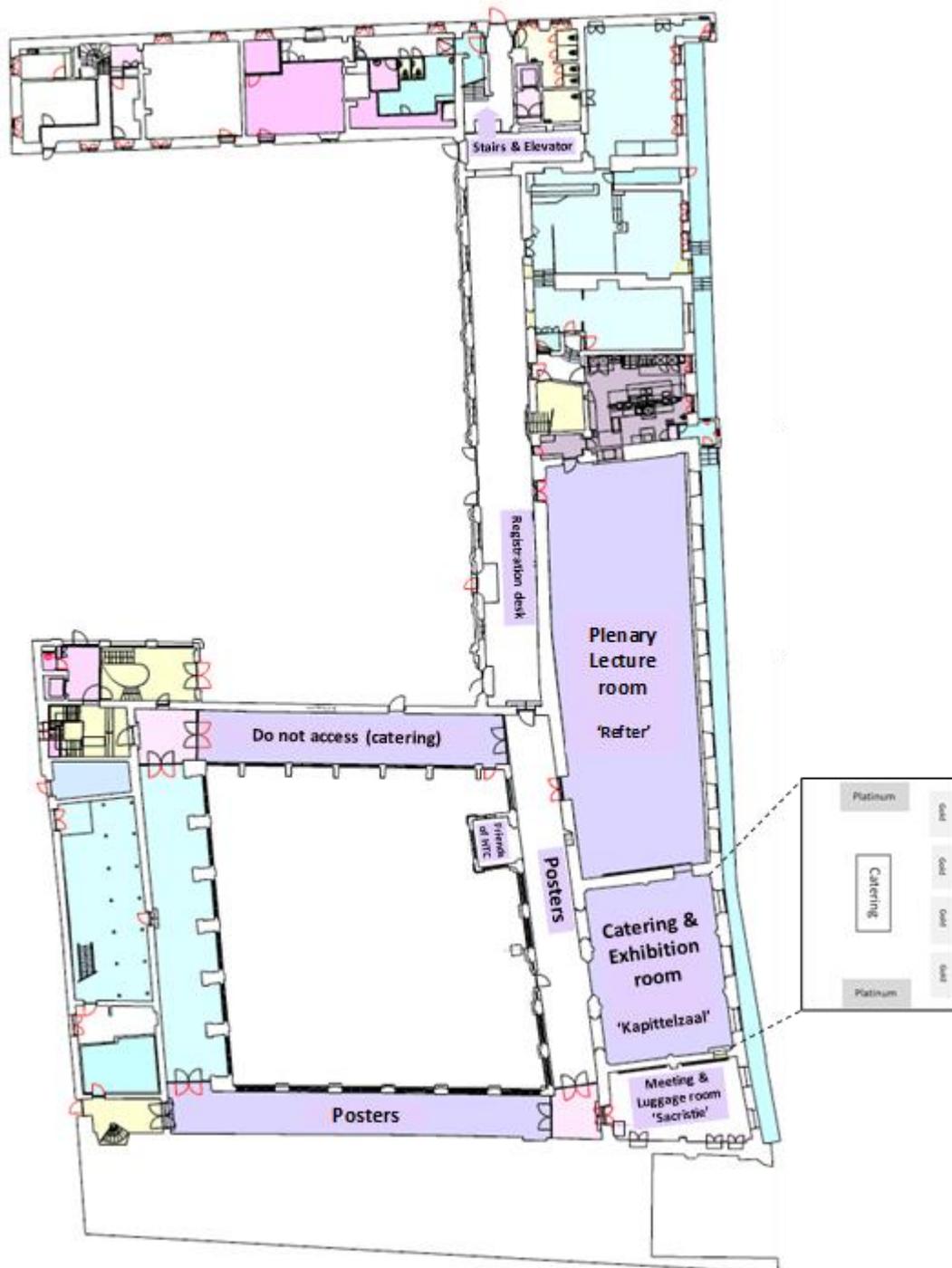
First floor: Silver and Bronze Sponsors

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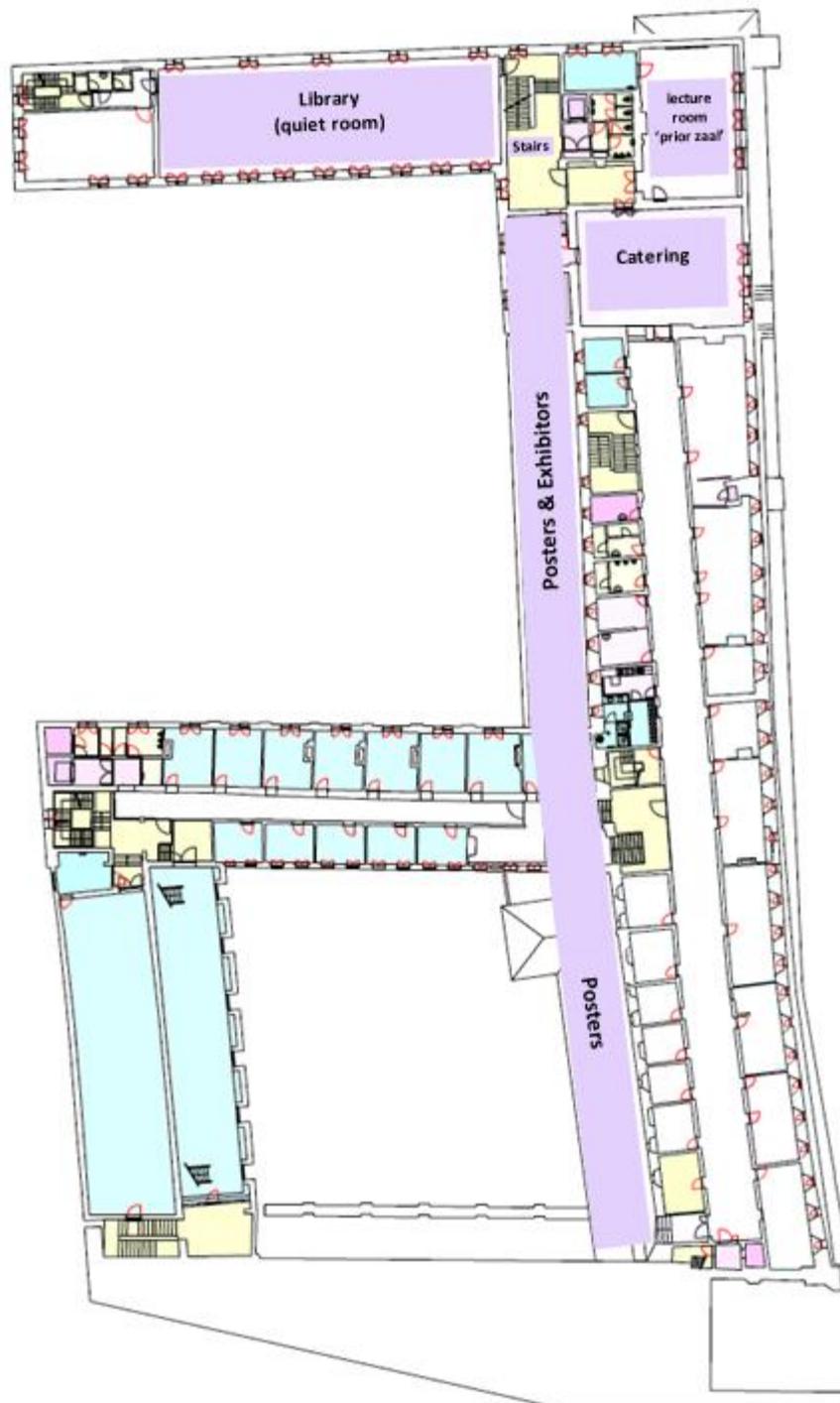
Second Floor: Lecture room 'Rector Vermeylen' and live-streaming room 'Oude Infirmerie'
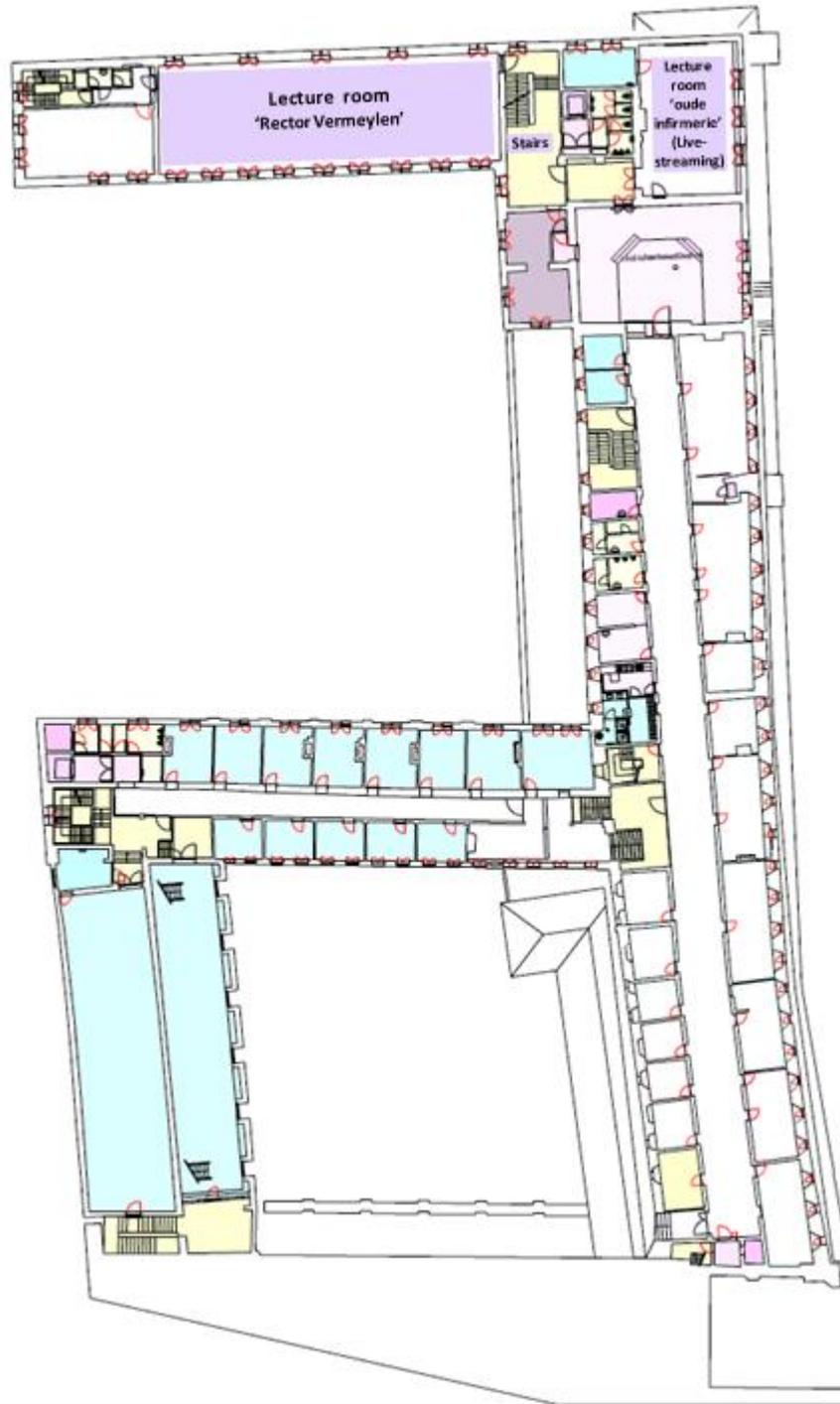
SHORT COURSE 1

MULTIDIMENSIONAL AND HYPHENATED TECHNIQUES IN LC AND GC
Tuesday January 28th 2020, 09:00 – 17:00
Speakers: Joeri Vercammen (Ghent University, BE), Dr. Dwight Stoll (Gustavus Adolphus College, USA), Dr. Davy Guillarme (University of Geneva, CH).

In the twenty years of its existence, GCxGC has evolved into a mature technique that enables comprehensive analysis of a complex samples in petrochemistry, food and environmental analysis. Nonetheless, the GCxGC footprint remains relatively small within the entire chromatography sphere. The goal of this training is to provide sufficient insight to convince those in doubt whilst at the same time provide new insights to its early adopters. Therefore, the following topics will be addressed: (a) Origins of GCxGC, (b) The concept of orthogonality, (c) Theory and basic principles, (d) Comparison of modulator technology, (e) Data handling challenges, (f) Recent applications, (g) Q&A. Each participant is encouraged to provide real life challenges he/she is confronted with during daily routine. These questions will be proactively incorporated into the training to make it even more relevant to everyone attending.

Two-dimensional liquid chromatography (2D-LC) is increasingly becoming recognized as a powerful and flexible analytical tool that can be used in a variety of applications areas, particularly when conventional one-dimensional (1D) separations are inadequate for some reason. Leveraging the potential of 2D-LC requires that users understand the underlying principles of the technique, and differences between 1D and 2D separations in method development. We will address several key concepts from a theoretical point of view, including undersampling, and the different types and modes of 2D separation (e.g., heartcutting and comprehensive separations). Then, we will use examples of contemporary separations of both small molecules (e.g., pharmaceutical impurity profiling) and larger biomolecules (e.g., peptides and proteins) to illustrate both what it is possible, and the steps in method development that are critical to successful separations. Finally, several practical topics will be discussed, including coupling to mass spectrometric detection, quantitation, and data visualization and analysis. We intend for the course to be practical in nature, with a solid theoretical foundation. We encourage attendees to bring any questions they have about separations they are currently doing, or plan to do, for discussion during the course.

SHORT COURSE 2

CANNABIS ANALYSIS

Tuesday January 28th 2020, 09:00 – 17:00

Speakers: Dr. Barbara Paccheti (EMMAC) Dr. Gesa Schad, Dr. Allegra Leghissa, Dr. Xaver Monninghoff (Schimadzu), Dr. Hansjörg Majer (Restek), Dr. Flavio Franchina (University of Liège, BE).

Due to recent changes in legislation all over Europe, the medicinal cannabis market continues to grow, and CBD infused edibles, food supplements and cosmetic products are enjoying an unprecedented surge in popularity. Accurate cannabis analysis is required in order to ensure product quality and consumer safety. Cannabinoids are the primary active components of cannabis and the target compounds for potency testing. Terpenes influence the homeopathic effect, and contaminants such as pesticide residues and microbial contamination could cause serious harm, especially to immuno-compromised medicinal cannabis users.

This short course will cover the basics of cannabis and the industry, as well as the state-of-the-art testing methods currently available. In the first part, the basics of cannabis analysis and background on cannabis industry will be discussed. The second part handles basic testing for potency, moisture and mycotoxines, followed by advanced testing for terpenes, pesticides and chemical residues. In the final part of the short course, the analysis of (S)VOC compounds in cannabis is discussed. Speakers from academia (Flavio A. Franchina, Université de Liège), industry (Barbara Pacchetti - EMMAC) and experts from instrument manufacturers (Gesa Schad , Allegra Leghissa and Xaver Mönninghoff – Shimadzu; Hansjörg Majer - Restek), will cover these different topics.

WEDNESDAY JANUARY 29, 2020

Room : Refter (ground floor)

09:00 **HTC-16 OPENING CEREMONY**
Session Chair: Sebastiaan Eeltink (Vrije Universiteit Brussel, BE) and John Langley (University of Southampton, UK)

09:15 **PL01 – UHPLC and prefractionation to increase metabolite identifications in metabolomics assays**
Robert Kennedy (University of Michigan, USA)

10:00 **PL02 – Nano- and capillary LC separations Using micro-pillar array columns for maximal efficiency and robustness**
Gert Desmet (Vrije Universiteit Brussel, BE)

10:45 **Coffee Break & Exhibition**

Parallel sessions

Room : Refter (ground floor)

BIOPHARMACEUTICAL ANALYSIS

Session Chair: Davy Guillarme (University of Geneva, CH)

11:15 **KL01 – Expanding the analytical portfolio for the characterization of protein biopharmaceuticals**
Koen Sandra (Research Institute Chromatography, BE)

11:45 **TU01 – HPLC analysis of biologicals in a cGMP environment**
Achim Treumann (KBI Biopharma, BE)

12:15 **OC01 – Enhanced peptide mapping and HCP analysis with Trapped Ion Mobility Spectrometry (TIMS) using a variety of LC flow regimes**
Stuart Pengelley (Bruker Daltonics, DE)

12.35 **OC02 – Polysorbate profiling and impurity quantitation in biotherapeutics formulations by HPLC with charged aerosol detection following on-line sample clean-up**
Frank Steiner (Thermo Fisher Scientific, DE)

Room : Rector Vermeylen (2nd floor)

GC-MS AND GC×GC

Session Chair: Hans-Gerd Janssen (Unilever, NL)

11:15 **KL02 – In-vitro characterization of lung inflammation mechanism**
Jean-François Focant (Université de Liège, BE)

11:45 **KL03 – Learning the lessons from multidimensional separations**
Tadeusz Gorecki (University of Waterloo, CA)

12:15 **OC03 – Problems and solutions related to calibration of thermal desorber – gas chromatography**
Kris Wolfs (KU Leuven, BE)

12.35 **OC04 – Extending the application range of a GC×GC high-resolution TOF-MS platform for fuel analysis by hyphenation to direct inlet thermal analysis techniques**
Ralf Zimmermann (Helmholtz Zentrum München GmbH/Univerity of Rostock, DE)

Room : Priorzaal (1st floor)

MAXIMIZING RESOLUTION

Session Chair: Hamed Eghbali (DOW, NL)

11:15 **TU02 - Maximising Resolution Through Hyphenation**
Tom Lynch (Tom Lynch Analytical Consultancy, UK)

11:45 **YES1 – How to further increase the peak capacity of sub-hour on-line LC x LC separations?**
Soraya Chapel (Université Claude Bernard Lyon, FR)

12:00 **YES2 – Resolving power of high-porosity nanostructured monolithic columns in liquid chromatography for proteomic applications**
José Luís Dores-Sousa (Vrije Universiteit Brussel, BE)

12.15 **YES3 – High potential of a three dimensional approach (CPC×SFC/HRMS) to analyse HPAH in vacuum gas oil**
Carole Reymond (IFP Energies Nouvelles, FR)

12:30 **FP01 – Assessment of operating conditions affecting the peak capacity of intact protein bioanalysis in HIC**
Raphael Ewonde Ewonde (Vrije Universiteit Brussels, BE)

12:35 **FP02 – The importance of sufficient chromatographic separation for identity confirmation**
Henk Gerritsen (Wageningen Food Safety Research, NL)

12:40 **FP03 – An experimental procedure for the in-depth evaluation of band broadening phenomena in capillary and nano-size columns**
Haibin Li (KU Leuven, BE)

12:45 **FP04 – HPLC as a PAT tool featuring ballistic separations and direct process sampling**
Przemek Stasica (GSK, UK and University of Leeds, UK)

12:55 **Lunch Break & Exhibition and Posters (even numbers)**

13:30 **Vendor Seminar: Shimadzu - Room: Refter (ground floor)**

Parallel sessions

Room : Refter (ground floor)

HIGH-RESOLUTION PROFILING

Session Chair: Erwin Kaal (DSM Biotechnology Center, NL)

14:15 **KL04 – Chiral separations and hyphenated techniques**
Michael Lammerhofer (University of Tuebingen, DE)

14:45 **KL05 – Miniaturized selective extraction devices for trace analysis in complex samples**
Valerie Pichon (ESPCI Paris, FR)

15:15 **OC05 – Multidimensional performance assessment of micro pillar array column chromatography combined to ion mobility-mass spectrometry for proteomics research**
Marianne Fillet (University de Liege, BE)

15:35 **OC06 – Targeted metabolomics of the brain – the quest for sensitivity, selectivity and throughput**
Ann van Eeckhaut (Vrije Universiteit Brussel, BE)

Room : Rector Vermeylen (2nd floor)

METHOD DEVELOPMENT

Session Chair: Peter Van Broeck (Janssen Pharmaceutica, BE)

14:15 **KL06 – Multi-dimensional chromatographic approaches to characterize protein biopharmaceuticals**
Davy Guillarme (University of Geneva, CH)

14:45 **KL07 – Impact of structural similarity on accuracy of retention time prediction: Theory and applications**
Roman Szucs (Pfizer R&D, UK and Commenius University, Bratislava, SK)

15:15 **OC07 – Implementing principles of Analytical Quality by Design (AQbD) for method development in quality control environment**
Cedric Hubert (Université de Liège, BE)

15.35 **OC08 – Development and qualification of a heartcutting 2D-LC method for determination of light-induced degradants of pediatric drug product delivered with liquid and soft foods**
Hans Thys (Janssen Pharmaceutica, BE)

Room : Priorzaal (1st floor)

SYSTEM DESIGN AND NOVEL COLUMN TECHNOLOGIES

Session Chair: Peter Schoenmakers (University of Amsterdam, NL)

14:15 **TU03 – Extra-column broadening in modern UHPLC instrumentation**
Ken Broeckhoven (Vrije Universiteit Brussel, BE)

14:45 **YES4 – Characterization of proteins with in-situ synthesis of miniaturized monolithic Immobilized Enzymatic Reactors coupled on-line with nano-liquid chromatography**
Stan Perchepied (ESPCI Paris, FR)

15:00 **YES5 – Nanofibrous sorbents: Promising newcomers in on-line biological sample handling**
Hedvika Raabova (Charles University, CZ)

15:15 **YES6 – Assessing effects of instrument configuration on dispersion and retention characteristics for UHPLC method development**
Zhuoheng Zhou (Vrije Universiteit Brussel, BE)

15:30 **FP05 – Development of an improved protocol for the measurement of molecular diffusion coefficients of biopharmaceuticals**
Donatela Sadriaj (University of Leuven, BE)

15:35 **FP06 – Design and evaluation of flow distributors for radially elongated hexagonal pillar arrays column using CFD modeling**
Farideh Haghighi (Alzahra University, IR)

15:40 **FP07 – Numerical and Experimental Investigation of Sample Loss and Dispersion Occurring in Sample Loops Used in 2D-LC**
Ali Moussa (Vrije Universiteit Brussel, BE)

15:45 **FP08 – Determination of the diffusion coefficients in SFC for a wide variety of samples and conditions**
Vincent Pepermans (Vrije Universiteit Brussel, BE)

15:55 Coffee Break & Exhibition and Posters (odd numbers)

Parallel sessions

Room : Refter (ground floor)

ADVANCES IN INDUSTRY

Session Chair: Joeri Vercammen (Interscience and Ghent University, BE)

17:00   **KL08 – Trends in analytics in pharma industry**
Peter Van Broeck (Janssen Pharma, BE)

17:30   **Industry Pitch - Driving proteomic nanoLC-MS to new crossroads, are we heading towards extra sensitive or increased throughput applications?**
Robert van Ling (Pharmafluidics, BE)

17:40   **Industry Pitch – Experience the power of having a GC in your laptop: free modeling software to simulate GC separations**
Jaap de Zeeuw (Restek, NL)

17.50   **Industry Pitch – New and innovative solutions for BioLC**
Daniel Eßer (YMC, DE)

18:00   **Industry Pitch – Characterization of new MS-compatible mixed-mode RP/AX HPLC columns**
Thomas Walter (Waters, USA)

18:10   **Industry Pitch – Gaining insights into the complex chemistry of cannabis aroma**
Laura McGregor (SepSolve Analytical, UK)

Room : Rector Vermeylen (2nd floor)

NEW TRENDS IN LC-MS

Session Chair: Michael Lammerhofer (University of Tuebingen, DE)

17:00   **KL09 – Utilising the power and selectivity of supercritical fluid chromatography and mass spectrometry to detect and quantify polymeric materials across a range diverse applications**
John Langley (University of Southampton, UK)

17:30   **OC09 – Advanced mass spectrometry in biomarker discovery for emerging contaminants**
Adrian Covaci (University of Antwerp, BE)

17:50   **OC10 – Development of IP-LC-MSMS methodology to monitor Tau phosphorylation around T217 in human CSF as biomarker read-out in clinical study samples of Alzheimer diseased patients**
Sebastiaan Bijttebier (Janssen Pharmaceutica, BE)

18.10   **OC11 – Exploring the application of liquid chromatography-ion mobility-mass spectrometry for targeted and untargeted metabolomics**
John Walsby-Tickle (Oxford University, UK)

Room : Priorzaal (1st floor)

ANALYTICAL TECHNOLOGIES IN LIFE SCIENCE RESEARCH

Session Chair: Valerie Pichon (ESPCI Paris, FR)

17:00   **TU04 – High throughput comprehensive lipid/protein composition of lipoproteins in normal and dyslipidemic patients**
John R. Barr (Centers for Disease Control and Prevention, USA)

17:30   **YES7 – HILIC-MS/MS analysis of histamine and its main metabolites in human urine samples in the search of novel biomarkers for irritable bowel syndrome**
Maxim Nelis (KULeuven, BE)

17:45   **YES8 – Approaches from relative to absolute quantification in untargeted lipidomics by surrogate calibration**
Bernhard Drotleff (University of Tuebingen, DE)

18:00   **YES9 – Colorectal cancer: biomarkers and effect size**
Nicolas Di Giovanni (University of Liège, BE)

18:15   **YES10 – Mimicking the blood-brain barrier by a biomimetic platform based on comprehensive two- dimensional liquid chromatography for drug diffusion studies**
Giacomo Russo (Ghent University, BE)

18:30 Poster presentations and Belgian Beer Tasting Event (Sponsored by Thermo Fisher Scientific)

THURSDAY JANUARY 30, 2020

Room : Refter (ground floor)

PLENARY RSC SEPARATION SCIENCE GROUP SESSION

Session Chair: John Langley (University of Southampton, UK)

08:30 **PL03 – What are the challenges faced by the separation scientists in environmental analysis today?**
Leon Barron (King's College London, UK)

09:15 **KL10 – Dealing with Earth's most complex mixtures**
Nicholle Bell (University of Edinburgh, UK)

Parallel sessions

Room : Refter (ground floor)

FOOD ANALYSIS

Session Chair: Andre de Villiers (Stellenbosch University, SA)

09:45 **KL11 – The importance of analytical chemistry and molecular knowledge in developing consumer-preferred food products**
Gerd Janssen (Unilever Research, NL)

10.15 **KL12 – Determination of enzyme activity using time resolved (LC-) Ion Mobility- Mass Spectrometry**
Erwin Kaal (DSM Biotechnology Center, NL)

Room : Rector Vermeylen (2nd floor)

HIGH-RESOLUTION TECHNIQUES

Session Chair: František Švec (Charles University, CZ)

09:45 **TU05 – Method development and optimization in analytical SFC**
Caroline West (University of Orleans, FR)

10:15 **KL13 – A closer study of ion-pair chromatographic separations of new therapeutic oligonucleotides**
Torgny Fornsted Karlstad University, SE)

Room : Priorzaal (1st floor)

SOFTWARE ADVANCES FOR MULTIDIMENSIONAL LC/GC

Session Chair: Jean-François Focant (Université de Liège, BE)

09:45 **TU06 – Think Bayesian: old and new solutions for massive chromatographic data-analysis**
Gabriel Vivo-Truyols (Tecnometrix, ES)

10:15 **YES11 – Establishing an unique open-source benchmark dataset for the comprehensive evaluation of GC×GC software**
Benedikt Weggler (University of Liège, BE)

10:30 **YES12 – Unlocking automated method development: new peak-tracking algorithm for very fast data analysis of (LC×)LC-MS and (GC×)GC-MS data**
Stef Molenaar (University of Amsterdam, NL)

10:45 **Coffee Break, Exhibition & Posters (odd numbers)**

Parallel sessions

Room : Refter (ground floor)

RSC SEPARATION SCIENCE GROUP: SAMPLE PREPARATION AND INTRODUCTION
Session Chairs: Graham Mills (University of Portsmouth, UK) / Scott Fletcher (Hall Analytical, UK)

11:15   **KL14 – Recent advances in sample preparation and sample introduction techniques: a view from Pharma**
Rob Vreeken (Maastricht University, NL)

11:45   **OC12 – The use of experimental design and automation for pharmaceutical solid oral dosage form sample preparation**
Paul Ferguson (AstraZeneca, UK)

12:15   **OC13 – QuEChERS: a versatile tool for monitoring strategies in life science, environmental and manufacturing industries**
Ruth Godfrey (Swansea University, UK)

12.35   **OC14 –Comparison of OMCL methods for N-Nitrosamine impurities in Sartans- The ongoing challenges and wider issues**
Mark Harrison (AstraZeneca, UK)

Room : Rector Vermeylen (2nd floor)

INNOVATIONS AND APPLICATION IN INDUSTRY
Session Chair: Achim Treumann (KBI Biopharma, BE)

11:15   **KL15 – Infrared ion spectroscopy (IR-IS): a promising tool for metabolite identification**
Filip Cuyckens (Janssen Pharmaceutica, BE)

11:45   **KL16 – Molecular weight distribution characterization of reactive higher ethyleneamines using size-exclusion chromatography**
Hamed Eghbali (DOW, NL)

12:15   **OC15 – Is chromatography still at the heart of the future lab?**
Thorsten Teutenberg (Institut für Energie- und Umwelttechnik e. V., DE)

12.35   **OC16 – Automated preparation and analysis of haloacetic acids in water by GC/MS**
Mathias Vanlancker (Interscience, BE)

Room : Priorzaal (1st floor)

HYPHENATION AND MODULATION
Session Chair: Paola Dugo (University of Messina, IT)

11:15   **TU07 – Advances in two-dimensional liquid chromatography**
Bob Pirok (University of Amsterdam, NL)

11:45   **YES13 – Enhancing sensitivity in 2D-LC by hyphenating temperature-responsive phases with reversed phase liquid chromatography**
Kristina Wicht (Ghent University, BE)

12:00   **YeS14 – Developing active-modulation interfaces for hyphenation of light-induced-degradation reactors with LC separations**
Mimi den Uijl (University of Amsterdam, NL)

12.15   **YES15 – Application of evolutionary algorithms to optimise one- and two-dimensional gradient chromatographic separations**
Bram Huygens (Vrije Universiteit Brussel, BE)

12:30   **FP09 – Development of optimization strategies for heart-cut two-dimensional liquid chromatography**
Denice van Herwerden (University of Amsterdam, NL)

12:35   **FP10 – A comparative study of UniSpray and Electrospray for the ionization of neuropeptides in LC-MS/MS**
Jana Bongaerts (Vrije Universiteit Brussel, BE)

12:40   **FP11 – Deciphering the complex distributions of cellulose ethers by 2D-LC**
Tijmen Bos (Vrije Universiteit Amsterdam, NL)

12:45   **FP12 – Optimization of an untargeted LC-MS method for metabolite profiling of restricted-volume plasma samples**
Karen Segers (Vrije Universiteit Brussel, BE)

12:55   Lunch Break, Exhibition & Posters
13:30   Vendor Seminar by: **Agilent Technologies** - **Room: Refter (ground floor)**

Parallel sessions

Room : Refter (ground floor)

RSC SEPARATION SCIENCE GROUP: HIGH-THROUGHPUT ANALYSIS

Session Chair: Bob Boughtflower (University of Edinburgh, UK and GSK, UK)

14:15 — **KL17 – HTA and the clinical world**
Lewis Couchman (Analytical Services International, UK)

14:45 — **KL18 – The robot in your lab; Friend or Foe?**
Kathy Ridgway (Anatune, UK)

15:15 — **OC17 – Will bioanalysis surrender to the robotic army?**
Arundhuti Sen (GSK, UK)

15:35 — **OC18 – Capillary electrophoresis: speed and selectivity for high-throughput analysis**
Gordon Ross (Agilent Technologies, UK)

Room : Rector Vermeylen (2ⁿᵈ floor)

LC×LC(×LC) AND COUPLED COLUMNS

Session Chair: Frank Steiner (Thermo Fisher Scientific, DE)

14:15 — **KL19 – Advantages and limitations of HILIC in the second dimension of comprehensive two-dimensional liquid chromatographic separations: A kinetic evaluation**
Andre de Villiers (Stellenbosch University, SA)

14:45 — **TU08 – Approaches towards method development in two-dimensional HPLC**
Monika Dittmann (Agilent Technologies, DE)

15:15 — **OC19 – Development of Microfluidic Chip Technology for Spatial Three-Dimensional Liquid Chromatography**
Jelle De Vos (Vrije Universiteit Brussel, BE)

15.35 — **OC20 – From batch to continuous processing: purification of a bioactive peptide by means of Multicolumn Countercurrent Solvent Gradient Purification**
Martina Catani University of Ferrara, IT)

Room : Priorzaal (1ˢᵗ floor)

GAS CHROMATOGRAPHY AND DATA ANALYSIS

Session Chairs: Gabriel Vivo-Truyols (Tecnometrix, ES)

14:15 — **TU09 – Exotic fragrances of Namibia: Application of current fragrance analysis trends**
Stefan Louw (University of Namibia, NA)

14:45 — **YES16 – Development of an untargeted and targeted multi-class method for cannabis products**
Flavio Franchina (Université de Liège, BE)

15:00 — **YES17 – European lacquer in Context. Strategies to find THM-GC/MS resin biomarkers and application on historical lacquered objects**
Louise Decq (KIK-IRPA, BE)

15:15 — **YES18 – Optimization of untargeted screening workflow for the characterization of lung fluid samples**
Pierre-Huges Stefanuto (Université de Liège, BE)

15:30 — **FP13 – Revealing the reactivity of isomers of bio-oils by GC coupled to FTIC resonance mass spectrometry**
Diana Catalina Palacio Lozano (University of Warwick, UK)

15:35 — **FP14 – Multivariate Calibration of Chromatographic Fingerprints to Predict Antioxidant Potential in Argan kernels**
Mourad Kharbach (Vrije Universiteit Brussel, BE)

15:40 — **FP15 – KairosMS: A new tool for the processing of hyphenated ultrahigh resolution mass spectrometry data**
Hugh Jones (University of Warwick, UK)

15:45 — **FP16 – Mass spectrometry with operation at constant ultrahigh resolution (OCULAR)**
Latifa AlOstad (University of Warwick, UK)

15:55 **Coffee Break & Exhibition and Posters (even numbers)**

Room : Refter (ground floor)

HTC TUBE

Session Chairs: Frederic Lynen (Ghent University, BE) / Joeri Vercammen (Ghent University, BE)

17:00 To be announced

19:00 **Aperitif** at the Monasterium PoortAckere
20:00 **HTC-16 Conference Dinner**

FRIDAY JANUARY 31, 2020

Room : Refter (ground floor)

HTC INNOVATION AWARD LECTURE AND DISCUSSION SESSION

Session Chairs: Alasdair Matheson (LC-GC) and Deirdre Cabooter (KU Leuven, BE)

09:00   **KL20 – Winner to be announced at the HTC-16 conference**

Parallel sessions

Room : Refter (ground floor)

HYPENATED TECHNIQUES

Session Chair: Ken Broeckhoven (Vrije Universiteit Brussel, BE)

09:45   **KL21 – Membrane-assisted solvent- and sorbent-phase microextraction of difficult liquid matrices**
Hian Kee Lee (National University of Singapore, SG)

10:15   **KL22 – High-resolution two- (and three-) dimensional liquid chromatography**
Peter Schoenmakers (University of Amsterdam, NL)

Room : Rector Vermeylen (2nd floor)

COLUMN TECHNOLOGY

Session Chair: John Langley (University of Southampton, UK)

09:45   **KL23 – Porous polymer monolithic structures in hyphenated chromatographic techniques**
Frantisek Svec (Charles University, CZ)

10:15   **KL24 –Shedding light on novel zwitterionic-teicoplanin chiral stationary phases for liquid chromatography: from fundamentals to innovative applications**
Alberto Cavazzini (University of Ferrara, IT)

Room : Priorzaal (1st floor)

COUPLING TO MASS SPECTROMETRY

Session Chair: Koen Sandra (RIC, BE)

09:45   **YES19 – Detecting STAMPs of Microfluidic Separations by SERS and MALDI-MS**
Pascal Breuer (University of Amsterdam, NL)

10:00   **YES20 – Unlocking the composition of gasoline gum content by application of GC-MS and UHPSFC-MS**
Andreas Panagiotopoulos (University of Southampton, UK)

10.15   **YES21 – UHPSFC-MS to unravel complex PEG derivatives used in pharmaceutical formulation**
Sergio Cancho Gonzalez (University of Southampton, UK)

10:30   **YES22 – Development and implementation of multi-dimensional LC-MS setup for a faster and more effective characterization of bio-therapeutic products**
Julien Camperi (Genentech, USA)

10:45 Coffee Break & Exhibition and Posters

Room : Refter (ground floor)

RSC SEPARATION SCIENCE GROUP: DATA COMPLEXITY, MINING, AND CURATION

Session Chairs: Sam Whitmarsh (BP, UK) / Arun Sen (GSK, UK)

11:15 **KL25 – The devil's in the detail of our data processing**
David Kilgour (Nottingham Trent University, UK)

11:45 **KN26 – Data analytics: the journey from complexity to clarity**
Camilla Liscio (Anatune, UK)

12:15 **OC22 – Extracting value from untargeted e-cigarette aerosol analysis: a metabolomics approach**
Michal Brokl (BAT, UK)

12.35 **OC23 – Non-targeted analysis of organic contaminants in complex environmental matrices – Applications of metabolomics data workflow**
Caroline Gauchotte-Lindsay (University of Glasgow, UK)

Room : Rector Vermeylen (2nd floor)

ADVANCES IN MULTI-DIMENSIONAL SEPARATIONS

Session Chairs: Rudy Senten (Royal Flemish Chemical Society, Belgium)

11:15 **KL27 – Recent progress on the simulation of second dimension separations for 2D-LC, with application to biomolecule separations**
Dwight Stoll (Gustavus Adolphus College, USA)

11:45 **KL28 – Exploiting comprehensive two-dimensional liquid chromatography (LC×LC) for the determination of bioactive compounds in natural products**
Paola Dugo (University of Messina, IT)

12:15 **OC24 – Automated flexibility to implement two-dimensional and multiple LC-MS methods in a single instrument setup**
Giorgia Greco (Thermo Fisher Scientific, DE)

12.35 **OC25 – Approaches towards multidimensional LC within the biopharmaceutical industry**
Isabelle François (BE)

Room : Priorzaal (1st floor)

NEW ANALYTICAL METHODOLOGIES

Session Chairs: Jelle De Vos (Vrije Universiteit Brussel, BE)

11:15 **TU10 – Basic principles of analytical method validation**
Erwin Adams (KU Leuven BE)

11:45 **YES23 – Characterization of the functionality-type × molecular weight distribution of complex polyesters using NPLC×SEC**
Gino Groeneveld (University of Amsterdam, NL)

12:15 **YES24 – Analysis of base oil mixtures with automated machine learning**
Samuel Ellick (University of Bristol, UK)

12.30 **YES25 –Intelligent invertebrate toxicology (iNVERTOX): Linking metabolomics to behavioural changes in a freshwater invertebrate**
Thomas H. Miller (King's College London, UK)

12.30 **YES26 – Monolithic molecularly imprinted polymer and nano-liquid chromatography for on-line miniaturized trace analysis in biological fluids**
Thomas Bouvarel (ESPCI, FR)

12:55 Lunch Break & Exhibition

13:30 Vendor Seminar by: **Agilent Technologies** - Room: Refter (ground floor)

Room : Refter (ground floor)

HTC-16 CLOSING SESSION

Session Chairs: Frederic Lynen (Ghent University, BE) and Sebastiaan Eeltink (Vrije Universiteit Brussel, BE)

14:30 **PL04 – Synthesis and functionalisation of nanostructured porous polymer materials for analytical applications**
Emily Hilder (University of South Australia, AU)

15:15 **PL05 – Reflections on half a century of research in capillary gas chromatography**
Pat Sandra (RIC, BE)

16:00 Poster Awards and HTC Award

16:15 Closure

16:20 Farewell Drink sponsored by **Shimadzu**

VENDOR SESSIONS

Wednesday, January 29 at 13:30 – Shimadzu
Title: *Carotenoids and Apocarotenoids Determination by SFE/ SFC Triple Quadrupole Mass Spectrometry*
Presenter: Prof. Paola Dugo, Food Chemistry, University of Messina, Italy.

Thursday, January 30 at 13:30 – Agilent Technologies
Title: *Mass Analysis Designed for Everyone*
Presenter: Lilla Guricza, PhD Application Scientist, Agilent Technologies, Waldbronn, Germany.

Friday, January 31 at 13:30 – Agilent Technologies
Title: *Applying GCxGC to botanical flavour profiling in gin using SPME and Smart Connected GC - Using Self Aware systems to increase productivity*
Presenters: Onno Kwast, Application Chemist, JSB Netherlands and Remko Van Loen, GC & GC/MS Product Specialist Agilent Technologies.

PL-01

UHPLC and Prefractionation to Increase Metabolite Identifications in Metabolomics Assays

Robert Kennedy
University of Michigan, United States of America

Metabolomics and lipidomics assays by LC-MS/MS can produce thousands of detected features. Achieving identification of compounds however has lagged the ability to detect such compounds. In this work, we explore two chromatography-centric approaches for increasing the compound identifications possible. In the first approach we explore use of long columns operated at 2400 bar and above for resolving complex mixtures and the effect of such separations on compound identifications. We find that increasing peak capacity afforded by columns that are 50 cm long packed with 1.7 um particles results in substantial increases in compounds identifications made by database matching for lipid extracts of plasma. Even higher peak capacity, up to 10,000, by coupling a prefractionation HILIC column (off-line) to a reversed-phase UHPLC columns yields a further, but less dramatic increase in lipid identifications. Our second approach is motivated by the hypothesis that low signals arising from insufficient concentration results in mass spectra of insufficient quality to yield good identification. We explore use of substantial preconcentration on columns along with prefractionation. A variety of strategies are used, but we find it is possible to increase positive identifications by over 3-fold compared to single dimension UHPLC. Both approaches will be described for analysis of pooled plasma samples.

PL-02

Nano- and Capillary LC Separations Using Micro-Pillar Array Columns for Maximal Efficiency and Robustness

Gert Desmet[1], Jeff Op de Beeck[2], Geert Van Raemdonck[2], Kurt Van Mol[2], Bo Claerebout[1], Natalie Van Landuyt[2], Paul Jacob[2]

[1]Department of Chemical Engineering, Vrije Universiteit Brussel, Brussels, Belgium;
[2]PharmaFluidics NV Technologiepark-Zwijnaarde 3, 9052 Gent, Belgium

As an alternative to the conventional packed bed nano LC columns that are frequently used in bottom-up proteomics research, a breakthrough in column manufacturing recently led to the introduction of silicon-based micromachined nano LC chip columns known as micro pillar array columns (µPAC™). These columns have an inherent high permeability and an unprecedented low 'on-column' dispersion due to the perfect order of the separation bed .Due to the perfect order, the peak dispersion originating from heterogeneous flow paths in the separation bed is eliminated (no A-term contributions). Sample components remain therefore much more concentrated during separation resulting in unprecedented separation performance. The freestanding nature of the pillars also leads to much lower backpressure allowing a high operational flow rate flexibility with exceptional peak capacities. In addition, the monolithic nature of the beds makes them extremely rugged and guarantees an unprecedented longevity of the columns. Moreover, it also guarantees run-to-run time repeatability that is about an order of magnitude better than packed bed columns, thus facilitating and accelerating protein identification.

After the introduction of a 200 cm long column, which is ideally suited to perform comprehensive proteome research, a 50 cm long µPAC™ column is now available which can be used in a more routine research setting. With an internal volume of 3 µL, this column is perfectly suited to perform high throughput analyses with shorter gradient solvent times (30, 60 and 90 minute gradients) and it can be used over a wide range of flow rates, between 100 and 2000 nL/min. Recently performed experiments with 500 ng of HeLa cell digest indicate that an increase in protein identifications up to 50% and a gain of 70% in peptide identifications can be achieved when comparing the 50 cm µPAC™ column to the current state-of-the-art packed bed columns. In the recent months, we have also been developing a column that is deeper and wider to meet the requirements of capillary LC flow rate. New data on these columns will be presented at the conference.

KL-01

Expanding the analytical portfolio for the characterization of protein biopharmaceuticals

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Protein biopharmaceuticals, such as monoclonal antibodies, are on the rise! These recombinantly produced therapeutic macromolecules currently account for 25% of the total pharmaceutical market and today monoclonal antibodies are considered the fastest growing class of therapeutics. Their success is driven by their efficacy in disease areas with a high unmet medical need such as oncology and autoimmune diseases.

With an immense therapeutic potential comes an equally great structural complexity. Indeed, protein biopharmaceuticals are large and heterogeneous, and their in-depth characterization is highly demanding towards analytics. This challenge has triggered the creativity of analytical scientists and resulted in many innovative tools for studying these ever more complex products.

In this presentation, hot topics like multidimensional liquid chromatography, micropillar array columns, instrument and column inertness and native mass spectrometry will be touched upon and its use in biopharmaceutical analysis will be illustrated with real life examples from the presenter's laboratory.

TU-01

HPLC analysis of biologicals in a cGMP environment
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HPLC with a variety of different detectors is a standard tool in the pharmaceutical industry. In this tutorial I would like to introduce participants to the principles of the workings of an analytical laboratory in a cGMP environment. We will address the following questions: What is cGMP? Why is it useful? How does performing an experiment in a cGMP environment differ from a research environment e.g. in an academic laboratory? What are particular challenges?

OC-01

Enhanced peptide mapping and HCP analysis with Trapped Ion Mobility Spectrometry (TIMS) using a variety of LC flow regimes

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Bottom-up mass spectrometry approaches have already been established for peptide mapping and Host Cell Protein (HCP) analysis, but the requirement for full coverage in a short timeframe and high sensitivity still present a significant challenge. PASEF (parallel accumulation and serial fragmentation) scans are uniquely enabled by Trapped Ion Mobility Spectrometry (TIMS) and significantly increase both acquisition speed and depth of sample coverage. Data will be presented showing how peptide mapping and HCP analysis can benefit from such an approach, coupled with analytical- and nano-flow HPLC systems.

OC-02

Polysorbate profiling and impurity quantitation in biotherapeutics formulations by HPLC with charged aerosol detection following on-line sample clean-up

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Polysorbate is an excipient that acts as emulsifier in most biopharmaceutical formulations. It is a complex mixture and also includes process impurities. Moreover, it degrades or causes degradation of other formulation components over time and these degradants can be of concern for human health. The molecules have no chromophore and so polysorbate is typically detected by mass spectrometry or, more commonly in QC, evaporative light scattering detection (ELSD) or charged aerosol detection (CAD).

HPLC analysis of polysorbate in protein-containing therapeutic formulations normally requires separation of the protein and polysorbate prior to the excipient analysis. There are different ways to address this, ranging from off-line sample clean-up to fully automated or hyphenated methods for direct injection of the formulation. The latter can incorporate a range from on-line SPE clean-up approaches to multi-heartcut 2D-LC workflows for more in-depth formulation characterization. In contrast to these information rich analytical processes, single-peak elution of polysorbate after pre-fractionation is also described, which eliminates any information on the composition of the emulsifier and its impurities.

We will focus on the on-line SPE approach that initially separates mAb and polysorbate by ion exchange and then analyzes polysorbate by gradient reversed phase chromatography. Undiluted mAb formulations of 100 mg/mL protein, 90 mg/mL sucrose, and 0.05 to 0.5 mg/mL polysorbate 80 or 20 (also known as Tween 80 or 20) were injected into the UHPLC system. Peak areas detected by CAD were used for quantification. A dual pump delivered both analytical and inverse gradients for compensation to normalize the detector response. The CAD's uniform response behavior allowed quantification of the polysorbate components. This is relevant because of the absence of calibration standards for individual components, in particular the impurities and degradants. The results revealed differences in polyester and polyol levels due to changes over time and different polysorbate purity grades. An acetonitrile/isopropanol mixture showed the best balance of elution strength, necessary to ensure elution of the polyesters, and CAD response. Finally, sample carry-over was successfully minimized.

KL-02

In vitro characterization of lung inflammation mechanisms

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Exhaled breath analysis has a high potential for early non-invasive diagnosis of lung conditions. Most of lung diseases involve a certain level of inflammation. The characterization of the ongoing inflammation processes is crucial to define proper medication. The inflammation processes are associated with oxidative stress, which yields to the conversion of chemical from the membranes (as polyunsaturated fatty acids) into volatile compounds secreted by the lungs [1]. The understanding of the metabolic pathways involved in volatile markers production could open new therapeutic routes for inflammatory diseases.

In this study, the lung inflammation was simulated in vitro. A549 epithelial cells, originally isolated from human alveoli, were cultured with and without oxidative agents (from chemical or biological origins) as part of a discovery study for lung inflammation mechanisms characterization. The cell culture volatile organic compounds (VOCs) were extracted by solid phase micro-extraction (SPME) and analyzed by comprehensive two-dimensional gas chromatography hyphenated to time-of-flight mass spectrometers (GC×GC-TOFMS). The complete analytical workflow was optimized using central composite design model. Univariate and multivariate feature selection approaches, i.e. Fisher Ratio and Random Forest, were then used to compare the volatile profile of the epithelial cells in different inflammatory conditions and to identify specific inflammatory markers.

According to the type of inflammation induced, significantly different VOCs were produced by the epithelial cells. For both chemical and biological stresses, an increased production of hydrocarbons, aromatics and alcohols was observed. However, more than 50 % of the specific VOCs were produced only after a biological stress. Based on this output, cross-comparison with metabolic pathways databases (e.g. KEGG) was performed. This work is setting up the basis of a multimodal and biomedical project on lung inflammation characterization. The future implementation of multi-omics screening could reveal new information on the complete molecular mechanisms involved in lung inflammation episodes.

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KL-03

Learning the lessons from multidimensional separations

Tadeusz Górecki[1], Alshymaa A. Aly[1], Hei-Yin Chow[1], Magriet Muller[2], Andre de Villiers[2], Bob W.J. Pirok[3], Peter Schoenmakers[3]

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Comprehensive two-dimensional chromatographic separations are one of the most exciting recent developments in separation science. Comprehensive two-dimensional gas (GC×GC) and liquid chromatography (LC×LC) provide peak capacities and selectivities which are impossible to accomplish using conventional one-dimensional separations. Both techniques share the same principle: the effluent from the first dimension column is divided into small fractions which are injected periodically into the second dimension column for additional separation. Interestingly, though, both techniques have been developing practically independently of each other. Fraction collection and re-injection is handled in GC×GC by a special interface called a modulator. Many different modulator designs have been developed over the years, each one with its own set of strengths and weaknesses. Columns used in the two dimensions of GC×GC are never fully orthogonal because analyte volatility always plays a significant role. Since it is extremely unlikely that both very strongly and very weakly retained analytes be present in a single fraction, the separation in the second dimension of GC×GC can be carried out under practically isothermal conditions with good results. Transfer of the analytes between the two dimensions in LC×LC is typically accomplished using a simple interface consisting of two sample loops and a multiport valve. On the other hand, fully orthogonal separation mechanisms are used in the two dimensions in most applications, which requires the separation in the second dimension to be carried out using gradient elution. We believe that both techniques can benefit from each other if these traditional approaches are modified. In GC×GC, a significant improvement in peak capacity can be achieved by using temperature programming in the second dimension. In LC×LC, good orthogonality and high peak capacity can be achieved using the same separation mechanisms in both dimensions with parallel gradients. This has been confirmed both theoretically and using an on-line system. The talk will explore the lessons we can learn from both techniques and show how the best features of one method can be transferred to the other.

<u>OC-03</u>

Problems and solutions related to calibration of thermal desorber – gas chromatography

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Thermal desorption (TD) combined with gas chromatography (GC) was originally developed for offline air monitoring. In the mean time, more and more applications arose in the pharmaceutical field where conventional approaches like headspace – GC are not usable. A nice example is the determination of residual solvents in solid drug carriers like mesoporous silica, gelatine and albumin [1,2].

For the latter applications, proper reference introduction prior to calibration is not so evident. Indeed, the analysis of solid samples using liquid reference solutions is problematic since the procedures for calibration and sample analysis are not the same. In practice, often offline liquid calibration (OLC) is performed and results are accepted, even if they are inaccurate since assumptions in terms of complete adsorption and desorption are not fulfilled.

Although the issues related to the common OLC technique are known among users, no in-depth investigation about possible causes has been carried out. Such research has been realised here and explanations for the observed phenomena are given so that a better insight has been achieved and possible solutions could be elaborated.

With a TD tube containing only quartz filters, a relative loss of more than 80% was noticed for some solvents due to tube manipulation processes. Enclosing a bed of mesoporous silica as alternative sorbent limited the losses to about 25% when samples were immediately analysed, and even better results were obtained when tubes were stored for several hours so that proper adsorption could take place. An additional sweep gas during injection boosted the transfer of analytes with recoveries above 95%. However, one could still not be sure that complete desorption had been accomplished. So, an additional injector was installed on the apparatus to allow direct injection of reference compounds in the primary desorption gas stream of the TD, which is referred to as inline liquid calibration (ILC). This sorbent free, independent calibration tool avoids the drawbacks of other approaches [3].

References:

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OC-04

Extending the Application Range of a GC×GC High-Resolution TOF-MS platform for Fuel Analysis by Hyphenation to Direct Inlet Thermal Analysis Techniques

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Comprehensive GC with MS detection (GC×GC-MS) is established in the field of petroleomics for the detailed analysis of e.g. diesel fuel. However, matrices in the intermediate region to non-volatility, such as marine heavy fuel oils (HFO) cannot be targeted completely by GC. Here we present a new approach for the investigation of high-boiling complex materials, by hyphenation of thermo-analytical techniques (in addition to GC×GC) to a high-resolution-TOFMS platform. HFOs with vastly different chemical properties were investigated by a GCxGC-multi-reflection TOF-MS System (GC×GC-HRMS). Peak region- and MS-spectral filters were applied to group components into compound classes. However, it was not possible to completely correlate the molecular information derived from the GC×GC-HRMS results to the chemical and physical bulk properties, due to discrimination of the non-volatile compounds. In order to target also non-volatile parts of the samples, a thermal balance was coupled to the same MS-platform (TGA-HRMS). Samples were heated (40°C to 1000°C) under N2 and evolving gases were transferred to the HR-TOF-MS, while the online measurement of the sample-mass gave direct quantitative information. Although, TGA-HRMS lacks the separation power of two-dimensional chromatography, it showed to be a valuable complementary technique to investigate the whole boiling range of HFOs. From 40°C to 400°C volatile substances evaporate according to their atmospheric boiling point, while high-boiling components are decomposed in the pyrolysis phase between 400°C and 600 °C. Exact masses of detected gases could be used to chemically discriminate the HFO-samples and reveal molecular patterns, which were in agreement to the GCxGC-results. Moreover, also the non-volatile components could be analyzed, which led to better correlation to elemental analysis compared to GC×GC-HRMS. As a third separation technique we used a direct inlet probe (DIP-HRMS) for thermal analysis in the ion source vacuum. This avoids thermal decomposition of high-boiling substances. In contrast to TGA-HRMS, the samples were heated from 40°C to 400°C directly in the ion source. Due to the reduced pressure (10-6 mbar), the volatility of sample constituents is greatly increased, which enabled mostly destruction-free volatilization of heavy fuel oils up to an atmospheric equivalent boiling point of 750°C.

The combined application of three complementary separation techniques (GCxGC, TGA and DIP) before mass-spectrometric detection, showed to be an efficient approach for the comprehensive chemical description highly complex petrochemical materials with high-boiling constituents. Since all techniques were combined on one MS-platform, derived datasets could be easily integrated and compared.

TU-02

Maximising Resolution Through Hyphenation

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Many analytical practitioners believe improving resolution in chromatography can only be achieved by improving the resolving power of the chromatographic separation - either by optimising the stationary phase performance, the mobile phase conditions, or the temperature programme for single column methods. Some methods do require complete resolution of all the components but for many applications the complete resolution is not required and it is the improved resolution of class type species or individual components of interest which is the ultimate goal of the analysis. In chromatographic methods this can be achieved by the optimum combination and selection of a range of other technical levers including: - sample preparation, selective sampling, sample introduction, multidimensional separations, detector selection and selective data capture and analysis.

This tutorial will discuss how these different technical levers can be applied to resolve the components of interest using real examples from industry.

YES-1

How to further increase the peak capacity of sub-hour on-line LC x LC separations?

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In the past decades, on-line comprehensive two-dimensional liquid chromatography (LC x LC) has proved to be a powerful technique for pushing the limits of the separation power. In the quest for higher peak capacities, on-line LC x LC has thus emerged as an attractive technique for the characterization of increasingly complex samples. In on-line LC x LC, the peak capacity is theoretically the product of the peak capacities in both dimensions. As a result, it can be significantly increased compared to 1D-LC. However the gain in peak capacity decreases as the analysis time decreases. For a separation of peptides in 30 min, the gain in RPLC x RPLC was found to be only of a factor of 3 compared to RPLC1.

In the present work we will critically compare several possible approaches for further increasing the peak capacity in case of sub-hour on-line LC x LC separations. These approaches include: (i) maximizing the degree of orthogonality with HILIC x RPLC instead of RPLC x RPLC, (ii) using very high temperature (> 100°C) in the second dimension, and (iii) using shift gradients instead of full gradients. Despite increasing the retention space coverage, a major issue of the first approach is the important solvent strength mismatch in the second dimension between the injection solvent and the mobile phase. This may lead to severe injection effects in the second dimension (band broadening, peak distortion, and breakthrough phenomenon). The possible strategies to limit these effects will be discussed. The second approach consists in elevating the column temperature in order to increase the flow-rate, and hence the peak capacity in the second dimension. The advantages and limits in RPLC x RPLC will be clearly highlighted. Finally continuous shift gradients aims at increasing the retention space coverage when similar retention mechanisms are considered as in RPLC x RPLC. However, their use may limit the focusing effect at the column inlet, thereby lessening the effect on peak capacity.

These approaches will be discussed through different applications including the separation of a tryptic digest of proteins, the separation of an aqueous extract of bio-oil, and the separation of an environmental sample.

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YES-2

Resolving power of high-porosity nanostructured monolithic columns in liquid chromatography for proteomic applications

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Particle-packed columns are the first choice as stationary phases to be used in LC for peptide and protein analysis due to its excellent loadability and separation characteristics. Rigid polymer-monolithic stationary phases have emerged as attractive alternative for packed-bed columns. This is due to ease of preparation but also the freedom in structure engineering defining kinetic performance limits and the wide range of surface chemistries available. By fine-tuning the macropore and microglobule size on multiple length scales, the chromatographic performance of current state-of-the-art packed columns can be surpassed. To achieve high resolving power within a short analysis time, monolithic entities in the submicron range need to be synthesized, with macropores ranging between 100 – 500 nm (for fast analysis) or 500 nm – 1 μm (for high-efficiency separations).

The limitations and possibilities that exist regarding the effect of structural inhomogeneity on chromatographic dispersion of high-permeability monolithic materials will be a central point of discussion of this contribution. Aiming at high-resolution separation of biomolecules, high-porosity poly(styrene-co-divinylbenzene) monolithic materials featuring nano-sized macropore and globule sizes were developed. The thermodynamic and kinetic properties of the reaction were systematically tuned by varying the porogen ratio, crosslinker density, initiator content, and temperature. Attempts in decreasing the globule and macropore size below a certain threshold led to a point where structural inhomogeneity (A-term) became significant. Optimized polymer monolithic entities yielding separation impedance values <1000 were achieved. High-resolution separations of intact protein were achieved, considering the impact of the gradient volume on the overall performance of these columns. While minimizing the extra-column contribution to band broadening, application for high-throughput analysis is also demonstrated, showing 5 runs of ballistic separation of 6 proteins in a minute (total cycle time of 12 seconds).

YES-3

High potential of a three dimensional approach (CPC×SFC/HRMS) to analyse HPAH in vacuum gas oil

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Conversion of heavy crude oils in petroleum fuels is a topical energy issue, making enhancement of the existing processes performance very challenging for refiners. Hydrocracking (HCK) is one of the main route to transform vacuum gas oils (VGO) to lighter valuable products. These high boiling point (350-550°C) distillates contain problematical heavy polycyclic hydrocarbons (HPAH) that are known to be responsible for undesirable effects on catalyst activity and tend to accumulate in the process reactor all along the HCK reaction. Thus, it is crucial to monitor accumulation of these hydrocarbons and to understand their formation mechanisms. The molecular composition of VGOs introduced in the reactor significantly differs from the HCK products one as native VGOs contain more aromatic, more alkylated and more sulphur-containing HPAH than their products. In literature some works have already proposed analytical methods to quantify HPAH in hydrocracked products [1]. Nevertheless nowadays no methodology allows to clearly identify and to determine the amount of HPAH in the VGO feedstocks.

In response to the complexity of such samples, two multidimensional separation methods have been developed in hyphenation to a Fourier Transform ion cyclotron resonance mass spectrometer equipped with an atmospheric pressure photo-ionisation source, (+)APPI/FT-ICR/MS. In each case first dimension corresponds to centrifugal partition chromatography (CPC) which has been proved to be a relevant way to separate molecules according to their alkylation stage. For the second dimension, non-aqueous reversed phase chromatography (NARP) has been compared to supercritical fluid chromatography (SFC). 2D cartographies of several samples have been plotted as CPC×NARP and CPC×SFC maps. Finally CPC×SFC hyphenated to high-resolution mass spectrometry was the most judicious configuration to get a well-organised chromatogram providing separation according to alkyl substitutions on one hand and to the number of aromatic rings on the other hand. Quantification of HPAH in VGOs thanks to this method has been demonstrated using pyrene-d10 to evaluate the matrix effect occurring in (+)APPI for several industrial samples.

The aim of this presentation is to show the high potential of this innovative hyphenation. The method development, in particular the choice of the two dimensions of separation will be discussed as well as the mechanism of retention and the hyphenation with high resolution mass spectrometry. Application of the optimised conditions to different industrial feedstocks will illustrate the actual input of a three-dimensional (CPC, SFC and HRMS) methodology.

References:

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KL-04

Chiral separations and hyphenated techniques

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Over decades chiral separation technologies have developed into seemingly mature analytical techniques. A large number of chiral stationary phases are nowadays commercially available and allow separation of all kind of enantiomers or stereoisomers. Chiral stationary phases (CSPs) based on polysaccharide selectors are dominating the field, followed by glycopeptide antibiotics CSPs. A variety of alternatives exist if these columns fail. Industry has established extended automated screening programs, both by HPLC but also SFC, to figure out the most suitable chiral column for specific application problems. High throughput is a serious demand in those column screens because in initial drug discovery a large number of potential drug entities must be screened. To cope with this demand, most of the successful selector chemistries have meanwhile been transferred from standard 5 μm fully porous particle (FPP) technologies to superficially porous particle (SPP) or sub-2μm FPP particle technologies. While enantioselectivity was mostly maintained, the new modern particle technologies allow much faster separations. Especially SPP technologies seems to be advantageous and chiral separations at sub-second speed have been illustrated.

Subminute separations are of course a great benefit not only in high-throughput screening but also for enantioselective 2D-LC. Fast chiral separations with such modern columns can be coupled to non-enantioselective RPLC separations in the first dimension in order to separate complex mixtures of enantiomers by comprehensive RP×chiral or specific difficult to separate mixtures of stereoisomers by multiple heart cut RP-chiral separation systems. Such a setup has shown applicability for enantiomer separations of the entire set of proteinogenic amino acids and some isobaric/isomeric congeners in the context of non-ribosomal peptides and therapeutic peptides, respectively. We have established such a multiple heart cut enantioselective RP-chiral separation system with SPP type chiral column based on quinine carbamate selector. Twenty five (25) amino acids have been enantioresolved in a run time of about 100 min. Meanwhile the run time could be reduced to 60 min. Also comprehensive chiral×chiral 2DLC setup has been established. It gives very particular elution pattern of amino acid enantiomers which may allow straightforward data processing.

If such analysis is performed in presence of real matrix, hyphenation of such separation systems to mass spectrometry is mandatory. Enantioselective matrix effects, however, may complicate the MS analysis and lead to inaccurate data. To overcome this problem, a technology for the straightforward synthesis of 13C-stable isotope labelled D-amino acid internal standards has been developed. It can be used in enantioselective metabolomics based on high-resolution mass spectrometry hyphenated to one or two-dimensional liquid chromatography.

KL-05

Miniaturized selective extraction devices for trace analysis in complex samples

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The evolution of the instrumentation in terms of separation and detection allowed a real improvement of the sensitivity and the analysis time. However, the analysis of ultra-traces from biological fluids requires often a step of purification and of preconcentration before the chromatographic analysis. Therefore, extraction sorbents based on a molecular recognition mechanism appear as powerful tools for the selective extraction of a target molecule and of its structural analogs to obtain more reliable and sensitive quantitative analysis in biological fluids.

Immunosorbents based on the use of specific antibodies of the molecule(s) of interest, oligosorbents based on the use of aptamers (i.e. oligonucleotides of a specific sequence able to bind a given molecule with the same affinity as antibodies) and molecularly imprinted polymers, which synthesis leads to the formation of specific cavities mimicking the recognition site of the antibodies, constitute powerful tools to improve the selectivity of the extraction procedure. Indeed, these tools allow the removal of matrix components during the sample treatment thus avoiding the risk of matrix effects largely encountered in LC/MS analysis. They strongly contribute to an enhancement of the reliability and of the sensitivity of the final analytical method.

After having described the principle, the advantages and the limits of these different extraction tools, this presentation will focus on the description of approaches to miniaturize these tools as their on-line coupling with nanoLC. Several applications to real samples will be provided to illustrate the huge potential of these tools applied to 50-150 nL complex samples.

OC-05

Multidimensional performance assessment of micro pillar array column chromatography combined to ion mobility-mass spectrometry for proteomics research

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To understand the complex nature of organisms and decipher specific modulation of molecular pathways induced by diseases, identification and functional investigation of the proteome are needed. However, the major limitation of proteomic studies remains the complexity of biological structures and physiological processes, rendering the path of exploration paved with various difficulties and pitfalls. Moreover, the proteomes of mammalian cells, tissues and body fluids are complex and display a wide dynamic range of protein concentrations. To overcome these issues, effective sample preparation, state-of-the-art mass spectrometry instrumentation and extensive data processing and data analysis are required.

In this presentation, the interest of using nanofluidics separation systems coupled to ion-mobility (IM) mass spectrometry (MS) will be discussed in the context of global proteomic analysis. We will compare the ability of two acquisition modes (DDA vs DIA) obtained by IM-Q-TOF using liquid chromatographic (LC) systems to decipher complex proteomics samples. Among the nanoLC systems tested, micro pillar arrays columns (µPAC) are recent nanoflow liquid chromatographic systems featuring highly ordered pillars containing an outer porous shell grafted with C18 groups. µPAC was compared to two other nanoflow systems and a state-of-the-art ultra high performance liquid chromatography (UHPLC) system. Performances in the four dimensions of information (LC, IM, MS and intensity) were calculated to assess the multidimensional efficiency of each tested system. Overall, µPAC combined to IM prove to enhance the proteome coverage by identifying two times more peptides than other tested nanoflow LCs and ten times more than classical UHPLC system.

OC-06

Targeted metabolomics of the brain – the quest for sensitivity, selectivity and throughput

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In vivo determination of brain mediators plays an important role in providing insight in how the brain functions. Microdialysis is a well-established sampling technique that allows collection of the chemical messengers in conscious freely moving rodents in healthy and pathological conditions. The selected analytical technique to quantify these mediators should have high sensitivity as microdialysis results in low sample volumes (microliter range) with chemical messengers present in picomolar to micromolar concentrations. Moreover, temporal resolution can be crucial to obtain real-time information regarding the targeted messengers. As the extracellular fluid is a complex mixture of numerous messengers and metabolites, selectivity of the method is also an important feature. Furthermore, there is a high demand for faster analysis to increase sample throughput since many samples per animal are generated.

For the quantification of monoamine neurotransmitters, the HPLC-electrochemical detection method available in the lab was transferred to a UHPLC system. The optimized method resulted in a ± 3-fold increase in sensitivity and throughput, allowing the measurement of these monoamines in smaller sample volumes or in brain areas with lower basal concentrations. Using the optimized method, we demonstrated that local administration of the ghrelin receptor agonist MK0677 into the ventral tegmental area significantly increased dopamine release in nucleus accumbens, medial prefrontal cortex and amygdala of C57BL/6J mice.Recently, it has been demonstrated that astrocytes, the major type of glial cells in the central nervous system, are also active elements in the brain. Astrocytes sense and integrate synaptic activity and, depending on intracellular Ca^{2+} levels, release gliotransmitters that have feedback actions on neurons. Chemogenetic modulation is used to study which gliotransmitters are released upon astrocytic activation. However, the temporal resolution obtained with microdialysis did not allow to see changes in glutamate and D-serine concentrations. For this purpose, biosensor experiments for measuring glutamate are now performed in collaboration with John Lowry (Manooth University; Ireland).Quantification of neuropeptide mediators is even more demanding, since their extracellular levels are in the low picomolar range and the relative recovery of these compounds during microdialysis is limited. The quantification of neuropeptides in dialysates necessitates the use of nano/capillary UHPLC-MS/MS methods and all parameters need to be optimized to achieve maximal sensitivity. Previously, we have developed strategies to reduce peptide adsorption during sample preparation and LC analysis. Now, we focus on the increase in sensitivity in the mass spectrometer by the addition of supercharging agents and/or the use of an alternative ionization source Unispray. Furthermore, we are comparing cerebral open flow microperfusion with standard microdialysis methods.

KL-06

Multi-dimensional chromatographic approaches to characterize protein biopharmaceuticals

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The characterization of therapeutic monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), is a tremendous challenge to state-of-the-art analytical technologies. Indeed, subtle changes in these large (> 150 kDa) molecules can have profound effects on efficacy and pharmacokinetic properties, thus it is important to have the ability to rapidly and accurately assess changes in the distribution of different isoforms (e.g., glycosylation, oxidation, deamidation, lysine truncation…) of such biomolecules.

Today, the most widely used analytical approaches for therapeutic protein characterization are liquid chromatography (LC) and mass spectrometry (MS), probably due to the remarkable developments of these strategies in the past few years, enabling a new level of performance. However, some chromatographic methods require tedious and time-consuming procedures, especially when the separation cannot be directly coupled with MS detection (e.g., because of the presence of non-volatile salts required for some separation modes).

The aim of this presentation will be to review the possibilities and trends of multidimensional LC and LC-MS, which provides rich opportunities to increase both the efficiency of the characterization process, and the value of the information gained from these analyses. During this presentation, various combinations of chromatographic dimensions will be explored for the deep characterization of biopharmaceuticals at the intact protein and middle-up levels of analysis.

KL-07

Impact of structural similarity on accuracy of retention time prediction: Theory and applications

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Impact of structural similarity of training sets on accuracy of prediction of chromatographic retention will be assessed. Comparison of multiple sources of molecular descriptors and machine learning algorithms will be provided together with practical applications from pharmaceutical drug development.

OC-07

Implementing principles of Analytical Quality by Design (AQbD) for method development in quality control environment

Cedric Hubert [a], Riccardo Deidda [a], Amandine Dispas [a,b], Thomas Van Laethem [a], Xuan Zhao [a], Pierre Lebrun [a,c] and Philippe Hubert [a]

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Analytical method performances have to be specified by the analyst trough the definition of the "Analytical Target Profile (ATP)", as proposed by the regulatory bodies. In the specific context of the pharmaceutical industry, regulatory authorities have recently imposed the assessment and management of risk throughout the entire product lifecycle. This includes the analytical procedure and consequently its own lifecycle.

The development step of an analytical method is still largely addressed using a "Changing One Separate Factor a Time (COST)" approach (also known as the "Quality-by-Testing (QbT)" approach). This strategy can lead to a suitable method for assessing the risk of routine use, even where the experimental domain is not examined. However, in order to consider an experimental domain rather than a set of specific experimental conditions during the development phase, a multivariate approach must be considered: the "Analytical Quality-by-Design (AQbD)" strategy. This strategy allows the definition of a "Design Space (DS)" by means of design of experiments (DoE). This DS, computed considering critical method parameters (CMP) associated to critical method attributes (CMA), allows the analyst to focus on the main objective of an analytical method: obtaining reliable results using a robust method.

Working in the context of analytical procedure, the validation step is a major part of the analytical method lifecycle. Indeed, the objective of analytical method validation is to demonstrate that this method is suited for quantifying the target analytes with an established and suitable level of accuracy, as defined by the "ATP". This is sometimes called the "fit-for-future-purpose" concept. The decision regarding the validity of a method based on prediction can be achieved by using the "β-expectation tolerance interval" (accuracy profile). The capability of this approach to manage the quantitative part of the analytical procedure is nowadays largely illustrated in scientific literatures.

Considering the assessment and management of risk throughout the analytical lifecycle, a global strategy allowing the unification of the development and validation phases in a single step could be considered as an improvement allowing the management of global analytical risk (i.e., for both qualitative and quantitative part of the analytical method). Indeed, such strategy could propose a validation of an entire experimental domain by means of the accuracy profile rather than a single set of specific experimental conditions. With this strategy, the DS will be no longer the place where qualitative performances are obtained, but also the space where quantitative performances of the analytical procedure are assessed and managed.

OC-08

Development and qualification of a heartcutting 2D-LC method for determination of light-induced degradants of pediatric drug product delivered with liquid and soft foods

Hans Thys, Ann De Nef, Willy Peys, An Van Cleempoel, Jean-Paul Boon, Kai Chen, Mario Hellings

Janssen Pharmaceutica, Belgium

Many commercial drug product dosage forms such as granules, pellets, powders, or tablets are labeled with additional instructions about the optional use of soft foods or liquids for better palatability and swallowability of certain targeted groups of patients (e.g., pediatric, geriatric). It is preferred and, in most cases, required to develop age-appropriate formulations during pediatric drug development, the development however often proves to be too complex. The use of a liquid such as water, milk and/or soft food as vehicles may be a better or even the only option for delivering the drug substance to the targeted patient population. Meanwhile, the use of liquids and/or soft foods as drug vehicles must be proven not to alter performance of the drug product and to be compatible and suitable for use in the targeted patient populations.

In the drug development of one of the pediatric drugs by Janssen Pharmaceutica, two degradants were found during the delivery of tablets with water. After characterization and investigation, the two degradants were believed to be induced and generated by room light due to the high sensitivity of drug substance. Soft foods, e.g. apple juice, banana and yoghurt, were used to replace water as vehicles of tablets, allowing more protection against light for the drug substance. A liquid chromatographic method was developed to determine the two light-induced degradants whereas a major challenge popped up with detection of the small peaks of degradants in the complex matrix of soft foods.

A heartcutting 2D-LC was then used to develop a method which allowed better separation of the degradants on the 2nd dimension with elimination of the interference of matrix from soft foods. The method was further optimized in term of sample preparation, injection volume, organic modifier and gradient etc. The developed method was to be qualified, where the specificity, accuracy, precision and robustness of determination of the two light-induced degradants can be ensured. With this application of 2D-LC in method development, the use of soft foods as vehicles was proven with no alter performance of this light-sensitive pediatric drug. The safety as well as the palatability in drug delivery of the patients was guaranteed.

TU-03

Extra-column broadening in modern UHPLC instrumentation
Ken Broeckhoven
Vrije Universiteit Brussel, Belgium

With the introduction of sub-2µm (superficially) porous particle around 15 years ago, significant improvement in separation speed and resolution became possible. These small particles allowed the use of shorter columns and the use of higher flow rates and were often packed in smaller ID column to avoid thermal effects (viscous heating), reduce solvent consumption and improve compatibility with MS (lower flow rates). These high performance small volume column are however very sensitive the dispersion occurring in the rest of the fluidic path of the instrument such as injector, mobile phase preheater, connection tubing and the detector cell or interface to e.g. MS. It was therefore not possible to use these narrow bore column on 'standard' HPLC instrumentation, as they required the use of so-called ultra-high performance instrumentation (UHPLC) that not only have a higher maximum operating pressure, but also have reduced extra-column dispersion.

After introducing the importance and consequences of extra-column dispersion, this tutorial will highlight the different contributions to extra-column broadening occurring in modern UHPLC instrumentation. Methodologies and the pro- and cons to estimate the amplitude of extra-column dispersion are critically reviewed and some easy hands on test to reduce and optimize your system configuration are provided.

YES-4

Characterization of proteins with in situ synthesis of miniaturized monolithic Immobilized Enzymatic Reactors coupled on-line with nano-liquid chromatography

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The characterization of proteins is mainly achieved by bottom-up approach that consists in an enzymatic digestion followed by analysis of the resulting peptides with reversed phase liquid chromatography coupled to tandem mass spectrometry (RPLC-MS/MS). In a complementary way, glycoproteins can also be studied with enzymatic digestions involving a glycosidase (e.g. PNGase F). Those digestions are usually carried out in solution or in gel. Yet, the experimental procedure is time consuming and tedious. To solve these issues, an alternative digestion mode using immobilized enzymes can be used. Still, the overall cost of analysis can easily rise up when expensive enzymes (e.g. PNGase F) are used. Ergo, the aim of this study is to develop miniaturized Immobilized Enzyme Reactors (IMERs) inside 100 μm i.d. capillaries.

Firstly, organic monoliths were in situ synthesized in 100 μm i.d. capillaries. They were characterized with SEM imaging and by measuring their permeability (Darcy's law) allowing evaluation of the macroporosity. As proof of concept, the monoliths were functionalized with covalent immobilization of either trypsin or pepsin after addition of a spacer arm and the resulting grafting yields were determined. These IMERs were next used for the on-line digestion and analysis of a model protein, Cytochrome C, allowing the evaluation of their performances. The obtained results were compared to previous ones obtained with IMERs functionalized with the same enzymes but in situ synthesized with organosilanes by a sol-gel approach, also in 100 μm i.d capillaries.

The monolith with the best performances will be further immobilized with PNGase F for characterization of the glycosylation profile of a glycoprotein.

YES-5

Nanofibrous sorbents: Promising newcomers in on-line biological sample handling

Hedvika Raabova[1], Lucie Chocholousova Havlikova[1], Jakub Erben[2], Jiri Chvojka[2], Frantisek Svec[1], Dalibor Satinsky[1]

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Nanofibrous polymers were tested as a new type of sorbent for extraction of low molecular analytes from protein-containing matrixes such as bovine serum albumin, bovine milk, and human serum. An extraction cartridge was filled with the nanofibrous sorbent and inserted in the 2-D chromatographic system. During the extraction in the first dimension, the analytes were retained on the sorbent while the protein ballast substances were washed off. After the column switch, the captured analytes were eluted and separated in the second dimension using analytical column.

The first study concerned a composite material made of polycaprolactone nanofibers coated on microfibrous scaffold. The protein-removing ability was tested using bovine serum albumin. We confirmed that the proteins were removed within one minute using water as the mobile phase. Then, two matrixes, bovine milk and human serum, were spiked with parabens as model analytes and directly injected in the extraction cartridge. The extraction efficiency of our nano/microfibrous sorbent was calculated as an analyte recovery. We observed recovery 78-118% for parabens in serum and 86-103% for the same compounds in milk under optimized extraction conditions. No interfering peaks were present in chromatograms. No macroscopic or microscopic changes of the composite material were observed even after 300 analysis.

Our second study led to a method for direct extraction of non-steroidal anti-inflammatory drugs from human serum. The validated method was successfully applied for diclofenac determination in real patient serum.

All experiments demonstrated great potential of the nanofibrous polymers as efficient sorbent for purification and simultaneous retention of low molecular analytes from protein-rich matrixes. Our sorbents possess high extraction efficiency, reusability, and long-term stability. Their coupling with column-switching chromatography system enable reduction in the time and laboriousness of analysis of biological samples.

The current research is focused on testing polyamide nanofibers functionalized with ionic groups. We are designing them for the extraction of beta-lactam antibiotics from bovine milk.

YES-6

Evaluation of system performance in ultra-high-pressure operation mode

Zhuoheng Zhou[1], Mauro De Pra[2], Frank Steiner[2], Gert Desmet[1], Sebastiaan Eeltink[1]

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The current state-of-the-art UHPLC technology comprises instrumentation allowing to operate columns packed with 1.5 um core-shell particles at operating pressures up to 1500 bar. To effectively utilize the potential of these columns, yielding peak volumes ranging between 0.5 and 10 uL, extra-column dispersion contributions need to be minimized.

In the current study, the instrument configuration of a UHPLC system with 1500 bar pressure capabilities was systematically altered and its effects on separation efficiency and retention were assessed in both the isocratic and gradient modes. Key factors affecting extra-column dispersion were investigated and the optimal system configuration with respect to extra-column dispersion and flow resistance is discussed for isocratic and gradient LC mode. Furthermore, pressure-induced retention effects have been evaluated, which affect method development and HPLC to UHPLC method-transfer strategies.

KL-08

Trends in analytics in Pharma Industry
Peter Van Broeck
Janssen Pharma, Belgium

As any other industry, the Pharma Industry is continuously in (r)evolution. In a globalized world, business and research models must follow the trends of the society. The external environment is changing. At patient level, healthcare becomes more personalised, with more empowerment, with higher expectations for quality of life and years of life saved. At technological level, new solutions are being developed by many different organisations, with a strong focus on data generation, digitalisation and real-world-evidence. At the level of society, globalisation continues, with more international mobility of talent, changing tax and IP environment, and value- and outcomes-based reimbursement. And last but not least, focusing research efforts on a world without disease, with emphasis on prevention, disease interception and cure for the most challenging unmet medical needs. These evolution in the pharma industry has a significant impact on our research models, and consequently also impacts the analytical support. First there is a need to have better results, but faster. Adaptions to hardware (from HPLC to UPLC, hyphenation, introduction of high-end mass spec,), software (data management, machine learning and artificial Intelligence) and new column development will be substantial enablers. Secondly miniaturization: from large standalone machines to benchtop technology and portable devices. Microelectromechanical systems (MEMS) technology will have an increasingly growing role in analytical applications including also the use of smartphones for monitoring. Finally, sustainable analytical lab design, minimization of the consumption of hazardous substances and maximized safety for analysts; the recycling, replacement, reduction of reagents and solvents and on-line treatment of waste will become very important in an environmental conscious society.

Industry pitch

Driving Proteomic NanoLC-MS to new crossroads, are we heading towards extra sensitive or increased throughput applications?

Robert van Ling, C. Mitterer, G. Jaouen, G. van Raemdonck, J. Op De Beeck
PharmaFluidics, Belgium

Since the late 1990s, NanoLC-MS has establised itself as a technique of choice for the analysis of highly complex proteomic samples. Especially in shotgun proteomics, the high separation resolution and peak capacities that can be achieved on a well packed nanocolumn have proven their worth, in protein identification and biomarker discovery research. In combination with high resolution mass spectrometry, large step have been made in knowledge and understanding of proteins, protein complexes and protein interactions.

With the emergence of Ultra High Pressure Liquid Chromatography (UHPLC) in NanoLC, smaller particles 2µm and longer columns have been utilized to further improve the analysis results. Drawback of these smaller particles is the increased back pressures they require, ranging towards 1200 bars. These high back pressure not only limit the length of the packed bed to typically 50cm, but also result in shorter lifetimes for the nanocolumns, requiring more column replacement time within the LCMS analysis.

A recent and highly attractive alternative for packed bed nanocolumns are the micro-Pillar Array Column technology columns, or µPAC™. These micromachined NanoLC chips have an perfectly ordered separation bed of freestanding µ-pillars, resulting in much lower back pressures, allowing for a wide flow rate flexibility. The high permeability and low on-column dispersion of the µ-pillar separation bed result in reduced peak dispersion, maintaining much more concentrated sample components during the separation, resulting in unprecedented separation performance. This allows column lengths of up to 200cm, with typical back pressures of only 120 bars.

Such 200cm long µPAC columns are ideally suited for deep diving proteomics, using gradient times of 4 to 6 hours, providing an increase peak capacities and maintaining excellent resolution. As we will demonstrate, outstanding results in both DDA and DIA analysis are readily achieved, independent of the NanoLC system used.

However, NanoLC-MS proteomics is, in the eyes of many, at a crossroad. Are we to persue more sensitive workflows for single cell analysis, or are we leaving the nanoliter per minute flow rates behind in favour of higher throughput in research fields like clinical proteomics. In this presentation, we will show the initial results for both approaches, utilizing the unique separation features of the µPAC column, especially in the 50cm length format.

Applications moving towards single cell analysis will be shown, demonstrating the sensitivity that can be achieved, as well as the reproducibility and robustness of the µPAC columns. For increased throughput or clinical proteomics workflows, the use of 1 µL/min and higher flow rates is demonstrated, utilizing gradients of 30 minutes and less.

Industry pitch

Experience the power of having a GC in your laptop: free modeling software to simulate GC separations

Jaap de Zeeuw, HansJorg Maier

Restek Corporation, United States of America

Our recently introduced Pro EZGC modeling software is a selectivity tool that relies on a pre-loaded library of thermodynamic retention indices. This makes it possible to predict retention times and optimize chromatographic methods without the need to analyze compound sets under many different conditions. The program allows the user to select the stationary phase and simultaneously see the separation result by changing: film thickness, temperature, column length, column internal diameter and carrier gas flow. Users can enter each compound or cut/paste large lists of compounds into the program. Also CASS numbers can be entered.

Since its introduction there have been thousands of searches across a broad range of compound classes. The program outputs: compound retention time, resolution and peak width along with the column conditions and dimensions. A model chromatogram is provided to illustrate retention, peak width and resolution. Users have the option to view compound mass spectral data with the added benefit of overlaying mass spectra for coeluting analytes. Specific searches can be saved and accessed at a later date. Examples of these features will be presented with a focus on challenging separations.

Industry pitch

Meeting Customer's Needs: YMC New and Innovative Solutions for BioLC
Daniel Eßer
YMC Europe GmbH, Germany

Over the last few years there has been a shift towards large biomolecules, namely proteins, antibodies or antibody-drug-conjugates. In addition, research and development activities in pharma/biopharma have further intensified towards peptides and oligonucleotides. The pharmaceutical world, in particular but not exclusively, has been constantly seeking for innovative separation solutions for this type of molecules.

New UHPLC/HPLC phases have to meet numerous challenging criteria including high temperature/pH stability, high resolution which lead to MS-compatibility or inertness. Properties such as highly robust phases and particularly excellent lot-to-lot reproducibility for QC purposes have become major priorities.

YMC's main focus for satisfying these needs for BioLC users has been the production and supply of reliable products together with the introduction of new, innovative products. This talk will provide a brief overview of the recent BioLC innovations from YMC.

Industry pitch

Characterization of New MS-Compatible Mixed-Mode RP/AX HPLC Columns

Thomas Henry Walter, Bonnie A. Alden, Melvin Blaze, Cheryl Boissel, Mathew DeLano, Jessica Field, Nicole L. Lawrence, Donna Osterman, Amit V. Patel

Waters Corp, United States of America

The separation of polar acids using reversed-phase (RP) HPLC poses significant challenges for currently available columns. Many acidic analytes of interest are poorly retained on conventional RP columns. Solutions to this challenge include the use of ion-pairing reagents or mixed-mode RP/anion exchange (AX) columns. However, most existing approaches are not compatible with mass spectrometry (MS) detection. In addition, binding of acidic analytes to metal surfaces in columns is a common problem. To overcome these challenges, we developed a new family of columns employing several novel technologies. The stationary phase is based on a new high phase-ratio hybrid organic/inorganic particle, bonded with C18 groups and a controlled low concentration of anion-exchange groups. The use of a bridged-ethyl hybrid composition allows the columns to be used with a broad range of mobile phase pH values. The material does not exhibit retention losses when used with 100% aqueous mobile phases. The anion-exchange groups have a pKa of approximately 7.5. Consequently, the columns exhibit anion-exchange retention at mobile phase pH values up to 8. The columns are compatible with mass spectrometry detection, exhibiting minimal ion suppression from column bleed. New column hardware was used to provide improved recovery of analytes containing phosphate and carboxylate groups that bind to metal surfaces in conventional columns. The chromatographic properties of the new columns have been characterized and compared to existing RP columns designed for separating polar analytes. Applications of the columns for separating several important classes of acidic analytes will be shown.

Industry pitch

Gaining insights into the complex chemistry of cannabis aroma

Laura McGregor[1], Matthew Edwards[1], Dave Bowman[1], Claire Keller[2]

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In recent years, there has been rapid, worldwide growth of the cannabis industry due to changes in legislation for both medicinal and recreational use. This growth is driving increased demand for robust testing of cannabis products.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS), enables the widest possible range of terpenes to be confidently identified across a range of sample matrices.

Odour thresholds can differ by many orders of magnitude, so high sensitivity is also essential to capture trace-level, yet pungent, compounds. In this study, we will demonstrate the use of trap-based secondary focusing to extend the performance of conventional sampling techniques (e.g. headspace and SPME) while retaining fully automated methods.

The acquisition of high-quality data is just the first step - efficient processing workflows are then required to allow meaningful conclusions to be drawn. Here, we use a novel comparative analysis tool to allow databases of terpene profiles to be created, for automated comparison of subsequent samples.

KL-09

Utilising the power and selectivity of supercritical fluid chromatography and mass spectrometry to detect and quantify polymeric materials across a range diverse applications

G. John Langley

University of Southampton, United Kingdom

Analytical methods are required to measure polymers that are used in diverse application areas, e.g. pharmaceutical, petrochemical and fuel additive chemistries as well as oligomeric series within polymer production. Each of these specialist areas present their own challenges to the analyst. In some cases the polymerics are present at low concentration in a complex matrix, in others the reactivity of the polymer chemistry constrains the analytics, demanding the removal all polar chemistries from the chromatography.

Supercritical fluid chromatography has been shown to be the preferred separation technique for a number of these application areas and the tunability and selectivity of modern atmospheric pressure ionisation techniques affords the optimal platform to address the challenging samples presented for analysis.

This presentation will discuss and show how the selectivity of supercritical fluid chromatography separation and mass spectrometry ionisation can be utilised to unravel the complexity of different polymeric materials, either as 'pure' materials or in formulations/complex matrices.

OC-09

Advanced mass spectrometry in biomarker discovery for emerging contaminants

Adrian Covaci

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Humans are daily exposed to a large number of (new) chemicals from the environment, food, and consumer products. While for many known chemicals, there is substantial knowledge regarding the appropriate human biomarkers of exposure for biomonitoring purposes, there are still many knowledge gaps for emerging contaminants.

Several approaches based on high-resolution mass spectrometry have been recently proposed for the identification of biomarkers of exposure relevant for the human exposure to emerging contaminants. Suspect screening of biological matrices (urine or blood) rely heavily on the availability of (home-made) databases with accurate mass information and, desirable, with (HR)MS/MS spectra. Since contaminants are metabolized to some degree in the human body, the identification goes beyond the parent compounds and extends to their metabolites. Furthermore, the presence of endogenous compounds in higher concentrations can in some cases lead to false positives.

To include a range of metabolites in the suspect screening of emerging contaminants, the biotransformation of the involved chemicals needs to be investigated. This is usually done using appropriate software and modelling approaches or through in vitro metabolism procedures with liver microsomes, S9 fractions, or hepatocytes, followed by detection and identification of the metabolites by LC-HRMS. The identification of relevant metabolites of emerging contaminants and their addition to the suspect screening improves very much the success in the process of biomarker discovery for emerging contaminants in human samples.

Indeed, one can setup biomonitoring programs only if the appropriate and relevant biomarkers of exposure (parent contaminants or its metabolites) have been adequately identified and characterized. An overview of the progress to date in this new research field will be presented, together with examples from the literature and from our own work.

Keywords: emerging contaminants, human exposure, biotransformation, high-resolution mass spectrometry

OC-10

Development of IP-LC-MSMS methodology to monitor Tau phosphorylation around T217 in human CSF as biomarker read-out in clinical study samples of Alzheimer diseased patients

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In Alzheimer's disease brain, tau is found aggregated in a hyperphosphorylated state that forms paired helical filaments and neurofibrillary tangles, a histopathological hallmark of the disease1. The combined measurements of Aβ1-42, total tau and phospho-tau at T181 in CSF with immuno-assays, have so far been commonly used as diagnostic and prognostic biomarkers for Alzheimer's disease. However, considering currently developed therapeutic strategies and ongoing clinical trials, we need additional biomarkers for a more precise and even earlier prediction of disease onset and a further increase in the accuracy of the diagnosis. Barthélemy et al. (2017) recently demonstrated by using LC-MSMS methodology that T217 phosphorylation (pT217) is considerably increased in AD patients. Moreover, T217 hyperphosphorylation occurred systematically in amyloid positive participants even at preclinical stage2.

In our lab, an immunoprecipitation (IP) LC-MSMS (6500, Sciex) assay was developed to determine low pM quantities of P-tau in human cerebrospinal fluid (CSF) and brain tissue homogenates (BH) of transgenic mice. CSF or BH is first immunoprecipitated with a pT212/pT217 tau specific antibody (Ab), followed by on-bead digestion with trypsin. Trypsinization of synthetic phosphorylated peptides containing the Ab epitope sequence showed that miscleavages can occur and are dependent on the site of phosphorylation. Miscleavaged peptides should therefore be considered in the search for P-tau tryptic peptides in BH and CSF samples. Acidic mobile phases typically used during RP-LC peptide analysis resulted in broad chromatographic peaks for the peptides of interest due to interactions of the phospho-moieties with free silica and stainless steel, and cis-trans isomerisation of a Pro-Pro moiety present in the sequence of the target peptides. Chromatography at elevated column temperature and basic pH mobile phase resulted in sharper peaks and improved selectivity and sensitivity. As ultimate sensitivity and selectivity are demanded for the detection of low pM P-tau levels in CSF, 1D LC-MSMS was compared with multi-dimensional LC-MSMS to remove matrix effects, resulting in increased sensitivity because of lower noise levels and increased peak areas. This setup was compared with an offline TiO2/ZrO2 clean-up selective for phospho-peptides. The optimized methodology allowed to quantify low pM P-tau levels in human CSF clinical samples. The IP-LC-MSMS setup was compared with an orthogonal single molecule array (SIMOA) assay: correlation was shown for pTau levels measured with both assays in human CSF clinical samples.

References:

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OC-11

Exploring the Application of Liquid Chromatography-Ion Mobility-Mass Spectrometry for Targeted and Untargeted Metabolomics

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Metabolomics is a rapidly growing area of application for mass spectrometry (MS) which aims to identify molecular changes in cells, tissues and bio-fluids related to disease and also the effect of pharmacological intervention on metabolic pathways. Liquid chromatography (LC) coupled to high resolution mass spectrometry is routinely used to increase confidence in identification based on retention time. Advances in ion mobility spectroscopy (IMS) allow metabolites to be separated based on their drift time and increase confidence in identification using collision cross section (CCS) measurements.

Typically in LC-MS metabolomics, metabolites are identified by accurate mass, isotope fine structure and tandem mass spectra however, this is not always sufficient to separate and annotate individual metabolites. Here we show that incorporation of ion mobility mass spectrometry into our routine metabolomics workflow is possible and provides a deeper coverage of the metabolome and greater confidence in metabolite annotations. Analysis was undertaken using previously established LC methods coupled to a Vion IMS-QTof and the benefits of CCS measurements using travelling wave IMS are demonstrated. Separation based on CCS allowed the fragment ions of co-eluting metabolites in cell extracts to be attributed to their precursor ions using data independent fragmentation. This improved coverage compared with data-directed methods and also resulted in reduced noise and increased confidence in annotation when compared with other data-independent methods. Comparison of CCS measurement with authenticated standards provided additional confidence in targeted workflows and the ratio of CCS to m/z reduced the incidence of false positives in metabolite identification.

We demonstrate the incorporation and advantages of routine IMS as part of an LC-MS metabolomics workflow including coverage of metabolite fragmentation and the measurement of CCS. This increased confidence in metabolite identification and was used to help the elucidation of unknown metabolites when employing untargeted metabolomics and reduce the probability of false identification. The benefit of CCS separation and measurement was also demonstrated for a derivatised LC-IM-MS workflow.

TU-04

High throughput comprehensive lipid/protein composition of lipoproteins in normal and dyslipidemic patients

John R. Barr, Zsuzsanna Kuklenyik

Centers for Disease Control and Prevention, United States of America

Lipoproteins are dynamic lipid-protein assemblies that function as transporters of various biomolecules circulating in blood. Lipoproteins consists of a core of cholesteryl esters (CE) and triglycerides (TG) surrounded by an amphipathic monolayer of phospholipids (PL) with the polar head groups positioned toward the aqueous plasma matrix. Traditional metrics of lipoprotein levels in serum or plasma are total cholesterol and total triglyceride concentrations. Studying the lipid and apolipoprotein composition of lipoproteins is important for better understanding energy homeostasis and the transport of various biomolecules in different disease states. Coupling a comprehensive analysis of lipid and proteins in size-fractionated lipoproteins allows for the analysis of lipoprotein composition and number as a function of particle size.

Serum (50 µL) was fractionated by asymmetric flow field-flow fractionation (AF4) and collected in 40 fractions with <2 nm size increments. Each fraction was then split into four aliquots for polar lipids, nonpolar lipids, proteins, and dynamic light scattering. Polar lipids were analyzed by extraction followed by HILIC LC and targeted MS/MS detection of major species of phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SM), and lysophosphatidylcholine (LPC). Nonpolar lipids (free cholesterol, cholesterol esters, and triglycerides) were prepared with a precipitation/evaporation/extraction sample preparation protocol and then analyzed by APCI with in-source nitrogen collision-induced dissociation (CID) followed by isotope dilution MS/MS. Protein analysis was done with on-line tryptic digestion followed by isotope dilution LC-MS/MS.

The workflow incorporating all three methods together was applied to a pilot study of 120 donors that were either normolipidemic, hypercholesterolemic, hypertriglyceridemic, or hyperlipidemic. The resulting comprehensive data set allowed the evaluation of correlations between absolute level and analyte ratio predictors specific to metabolic pathways and cardiovascular disease risk correlation. For example, in dyslipidemic subjects, we found higher apoC-III/apoE ratios on both LDL and HDL particles. Across individual samples, PE/PL and PI/PL ratios in the LDL fractions positively correlated with the affinity of LDL to several exchangeable apolipoproteins. Lower FC relative to SM on HDL particles emerged as potential indicator of FC transfer by HDL in dyslipidemics. The percent contribution of individual fatty acid species to corresponding PL classes showed higher abundance of polyunsaturated 36:5, 38:5 and 38:6 PCs in LDL fractions from normolipidemic samples. In samples with normal Total-TG levels, PE/PL molar ratios in the size fractions were significantly lower.

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matrix. Traditional metrics of lipoprotein levels in serum or plasma are total cholesterol and total triglyceride concentrations. Studying the lipid and apolipoprotein composition of lipoproteins is important for better understanding energy homeostasis and the transport of various biomolecules in different disease states. Coupling a comprehensive analysis of lipid and proteins in size-fractionated lipoproteins allows for the analysis of lipoprotein composition and number as a function of particle size.

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YES-7

HILIC-MS/MS Analysis of Histamine and its Main Metabolites in Human Urine Samples in the Search of Novel Biomarkers for Irritable Bowel Syndrome

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The irritable bowel syndrome (IBS) is a disorder of the lower gastrointestinal tract. Even though its underlying pathogenic mechanism is still undetermined, IBS is regarded as a cluster of diseases to which different pathogenic factors might contribute. Being present in 60% of the IBS population, visceral hypersensitivity (VHS), defined as an abnormal response to visceral stimuli, is widely recognized to play a key role in the development of its main symptom pain. Recently, researchers have established a causal relationship between VHS and the sensitization of TRPV1, an important nociceptor, through the activation of Hrh-1 (Histamine receptor 1) [1]. This effect could be blocked by the addition of ebastine, an Hrh-1 antagonist. A small-scale in vivo trial also demonstrated that pain could be alleviated in a subset of patients after administration of ebastine. As no difference was found in urinary histamine levels between ebastine responders and healthy volunteers, it is believed that one of its metabolites might be responsible for the activation of Hrh-1 and the subsequent sensitization of TRPV-1 [1]. Finding this compound will not only pave the way to innovative pharmacological interventions, but can also aid in the identification of patients who might benefit from them. Given the heterogeneity of the IBS population, it could also serve as an efficient tool for patient stratification in clinical trials.

Therefore, an LC-MS/MS method was developed enabling the separation and quantification of histamine and its main metabolites (imidazole acetaldehyde, imidazole acetic acid, methyl imidazole acetic acid, methyl histamine, acetyl histamine) in urine samples. Due to the high polarity of the metabolites, two BEH HILIC (2.1 x 100 mm, 1.7 µm) columns coupled in series, were used to obtain a satisfactory separation. Baseline separation was achieved in a timescale of 10 minutes, by using a linear gradient followed by an isocratic hold. In order to be compatible with the high organic content of the mobile phase, 10 µL of urine was diluted with ACN to a final volume consisting of 95% ACN. For methyl imidazole acetic acid, an additional dilution step was incorporated due to its high natural levels. The samples were stored at -20*C and centrifuged prior to injection.

Because of the natural occurrence of the compounds, matrix matched calibrators for quantification were unavailable. Due to the occurrence of matrix effects and the unavailability of labeled internal standards, the method of standard addition was employed as a viable alternative to solvent calibration. The validity of the method was shown in terms of accuracy and precision.

The method was adopted for the quantification of histamine and its metabolites in urine samples from healthy volunteers and IBS patients both before and after administration of ebastine. Levels were adjusted for creatinine concentrations in order to account for urinary dilution.

YES-8

Approaches from Relative to Absolute Quantification in Untargeted Lipidomics by Surrogate Calibration

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The hyphenation of liquid-chromatography (LC) and high-resolution tandem-mass spectrometry is the predominant analytical system that is currently utilized in untargeted lipidomics. Besides the general investigation of lipid profiles in various matrices, the principal goal of such studies is the generation or verification of hypotheses for novel biomarkers in health and disease. Although instrumentation, acquisition techniques and databases are steadily advancing for untargeted analysis in "omics" fields, the majority of methods still rely on the classic approach to elaborate relative fold changes of detected compounds between experimental groups. These relative results, however, have only limited potential for inter-assay or inter-batch comparability. In order to enhance the applicability of the gathered data, absolute quantification of as many features as achievable should be also pursued in untargeted analysis.

In general, absolute quantification requires preliminary calibration of the target analytes, preferably in the anticipated matrix and including internal standards to control for matrix effects and other variabilities in the instrument response. For biological samples and endogenous compounds like lipids, true blank matrices for the preparation of quantitative calibrant and quality control samples are usually not available. In consequence, alternative strategies, e.g. like surrogate calibration1 via stable isotope labeled analyte analogues, had to be established to achieve accurate calibration.

Eventually, two untargeted lipidomics methods were developed, in which several labeled lipids were included as lipid class-specific surrogate calibrants for large-scale lipid quantification. The first method was based on reversed-phase LC and yielded a separation of lipid species. However, mainly due to differences in matrix effects and the composition of the mobile phase during elution, nonuniform ionization efficiencies of the separated lipid species had to be compensated by response factors to the corresponding surrogate calibrant. In a second method, using hydrophilic interaction chromatography, lipid class separation was achieved and differences in ionization efficiencies were reduced due to co-elution of target analytes and surrogate calibrants. The quantitative performance of both methods was indicated by validation experiments that complied with international guidelines.

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YES-9

Colorectal Cancer :Biomarkers and Effect Size

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Colorectal cancer affects more than one million new persons each year worldwide, and kills more than 700.000.Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one of the approaches that can be used to solve these issues.

In the present study, we analyzed serum samples from patients affected by colorectal cancer (CRC, n = 18) and by colorectal cancer in remission (R-CRC, n = 17), and samples from healthy patients matched for biases (HC, n = 19 and R-HC, n = 17). The aim was to find candidate biomarkers able to diagnose the active state of the disease as well as to compare the concentration levels of the molecules of interest with the remission state to better understand the biolocal processes beneath the observed clinical and metabolic symptoms.

To do so, an optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS method we developed was used, that also included an in-house QC system and data processing based on multiple statistical techniques.Because the experimental design prevented a direct comparison between the active and remission samples, which were not directly matched for biases, we used a measure called effect size that has the advantage over significance testing to focus on effect (here signal/concentration variation) magnitude.

This presentation will therefore discuss effect size interest and application in metabolomics. Along with the results obtained in terms of the highlighted candidate biomarkers? Candidates that were identified using full mass spectrum, linear retention indices and accurate mass provided by state-of-the-art high-resolution (HR) time-of-flight mass spectrometry, and which discrimination potential was assessed using supervised and unsupervised models, discriminant analysis and ROC curves.

YES-10

Mimicking the blood-brain barrier by a biomimetic platform based on comprehensive two- dimensional liquid chromatography for drug diffusion studies.

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Scenario: The therapy of central nervous system (CNS) disorders has recently emerged as a public health priority. The incidence of most CNS disorders (e.g., Alzheimer's disease, stroke and Parkinson's disease) increases exponentially after the age of 65. The worldwide population aged over 65 years is projected to increase from 6.9% to 15.9% of the total population by 2050, constituting an extra billion elderly individuals. This trend is the result of the combination of the progressive increase in life expectancy and the baby-boom occurring in many countries during the two decades after World War II.

State of the art: Pharmaceutical research targeting CNS diseases is still fairly undeveloped and in its current state seems intrinsically unable to face this challenge. In fact, it takes longer to get a CNS drug on the market (12–16 years) compared to a non-CNS drug (10–12 years), while there is also a much higher attrition rate for CNS drug candidates than for non-CNS drug candidate. This can be attributed to a variety of factors, including the complexity of the brain, the liability of CNS drugs to cause CNS side effects, and the requirement of CNS drugs to cross the blood-brain barrier (BBB) in order to reach their target. The BBB is a highly selective, semipermeable membrane that separates the circulating blood from the brain and extracellular fluids in the CNS. The presence of tight junctions hinders the paracellular passage of smaller therapeutics, i.e. the migration of analytes between the discontinuities from cell to cell, but allows transcellular passage, that occurs by passive diffusion or active transport. The latter is the only way for polar compounds to cross this barrier. While several models are currently available to measure drug uptake in the brain, none of them seem to be adopted in the pharmaceutical industry due to their various disadvantages.

Aim: The present work aims at proposing novel in vitro platforms for BBB uptake assessment based on two- dimensional (2D) liquid chromatography (LC) that allow embedding biostructures, such as membrane lipids that emulate cell walls or proteins that transport drugs, in the separation mechanism. The chromatography is implemented in fully biomimetic conditions (pH, ionic strength and lipid/protein environment closely related to that of the in vivo BBB) and validated vs BBB uptake passage achieved in vivo on murine models collected from the literature.

Results and Conclusion: This unprecedented approach led to take advantage of the dramatically increased peak capacities achievable in 2D-LC and empowers the screening methodologies employed in the early stages of pharmaceutically drug development.

The developed method proved to be relevant in the screening of therapeutics for their uptake to cross the BBB, bringing substantial assets in reducing animal testing performed for pharmacokinetic and toxicological assessments.

PL-03

What are the challenges faced by the separation scientists in environmental analysis today?

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Separation science remains critically important not only for reliable monitoring of selected target analytes, but also in many other areas within the analytical pipeline including sampling, sample preparation, non-targeted analysis and in data interpretation for characterisation or classification of complex samples. This presentation will discuss a selection of recent advances with a focus on environmental applications where the numbers of compounds that require regular environmental monitoring in complex samples is growing or are in constant flux. Liquid (LC) and gas chromatography (LC) in single or multiple dimensions dominate this space and are used often in combination with mass spectrometry (MS). Recent developments for high throughput, direct injection LC-MS of ng L-1 concentrations of micropollutants such as pharmaceutical, illicit drug and pesticide residues in wastewater and river water are discussed and their success has been heavily dependent on stationary phase and column format selection. Furthermore, the re-emergence of supercritical fluid chromatography (SFC) coupled to MS has widened the analyte chemical space, especially to allow occurrence characterisation and risk assessment of polar metabolites and chiral species to gain understanding of population-level consumption and exposure patterns. Arguably, one of the most important technological advancements in recent years for simultaneous, flexible measurement of thousands of components in complex samples is high resolution accurate mass spectrometry (HRAMS). However, with this power came a flood of data to interpret, which is not always straightforward and in the absence of reference materials, chromatographic data is often overlooked. The opportunities for integration of machine learning into the analytical workflow to retrospectively predict chromatographic retention time is discussed for more rapid pollutant identification. Furthermore, following their original development for separation science applications, similar machine learning tools used with in situ passive sampling and micropollutant uptake in biota are discussed which allow time-integrated suspect screening and quantitation at high sensitivity and rapid shortlisting of new suspects for risk assessment based on predicted toxic effect pressures. For non-targeted analysis, some recent advances, opportunities and challenges also exist for separation science, especially in omics-type analysis for biota and humans following exposure to chemical pollutants.

KL-10

Dealing with Earth's most complex mixtures

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Peatlands cover only 3% of the Earth's surface but hold more carbon than all the vegatation on this plant combined. However peatlands can only store carbon if they are kept waterlogged and mainly anoxic. Unfortunately, the vast majority of peatlands have been damaged by human activities such as afforestation or grazing. All of these activities have led to peatland drainage that has removed the anoxic waterlogged conditions required to keep them functioning. Restoration of peatlands, mainly via rewetting, is a necessary part of climate change mitigation strageties. However whether these methods are working requires a whole system understanding on a molecular and microbial level

The solid component of peat consists principally of natural organic matter, which is commonly termed the most complex mixture on Earth, consisting of thousands of, mainly $C_xH_yO_z$ compounds. To date, no chromatographic tool has been able to separate the individal molecules contained with these mixtures to enable their identification.

This presentation will summarise where we are in 'seperating' these complex mixture to address the key functional questions regarding peat health.

KL-11

The importance of analytical chemistry and molecular knowledge in developing consumer-preferred food products

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Knowing which molecules are present and understanding how these affect quality and safety of a food product is key to developing consumer-preferred foods. It is for this reason that chromatography and mass spectrometry, and especially hyphenated and comprehensive couplings thereof, are widely used in food industry. With these methods we can detect thousands of compounds at levels of parts-per-trillion. Although impressive, it is often still insufficient. Certain sulphur species for example give a very clear smell at levels below this. And the flavor of a good tea can still contain many unresolved peaks, even if the most advanced comprehensive GC×GC-MS methods are used for analysis.

In this presentation we will present new chromatography and MS methods we recently developed to be able to answer the emerging questions from our food developers. Comprehensive GC×GC-high resolution MS was optimized for maximum resolution in the characterization of tea flavors. Very fast GC-MS with run times of a few seconds only was developed to monitor the release of garlic flavor in methods for controlled- and extended release of flavors from soups. Fast GC-MS was also applied to monitor lipid oxidation in dry powdered meal starters. This system allowed performing up to 30 analyses per hour. High temperature gas chromatography methods were developed for studying the interaction of foods with the (plastic) bottle wall. Methods to reduce this interaction were developed, resulting in a reduction of food waste as products were easier to squeeze out of the bottle. Particularly important is also the correlation of chromatography-MS data with sensory characteristics. Several high resolution methods, both in LC-MS and GC-MS, were developed to provide high-information density data sets that can be used in predictive models. Advanced chemometrics methods were applied for clustering of samples as well as for detecting new marker compounds. A particular application of the newly developed high resolution methods was also the detection of differences occurring in foods when the currently used food production methods were replaced by more sustainable methods.

The combination of chromatography and MS methods, off-line, online, hyphenated or comprehensive, is crucial for the development of foods that taste good, do good and can be sustainably produced. Still, further improvements in analytical chemistry, hyphenated methods and data-processing techniques are needed. Examples of situations where our analytical methods fall short will also be given.

KL-12

Determination of enzyme activity using time resolved (LC-) Ion Mobility- Mass Spectrometry

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The importance of quantitative time resolved data for mechanistic understanding of enzymatic processes has been recognized in many fields of study, including biochemistry, molecular biology, and food sciences. One example is the use of enzymes to modify lipids, that are naturally present in food, to change their organoleptic properties and nutritional values.

To investigate the kinetics of enzyme catalyzed reactions, progression curves must be obtained from a large variety of conditions and substrates. To obtain such curves, most of the current workflows make use of artificial substrates, spectrophotometric methods and discontinuous assays. However, there is a strong need to obtain enzyme progression curves in real application matrix and preferably in a fast and automated way.

Mass spectrometry enabling the direct/online analysis of application relevant substrates can be a good alternative to spectrophotometric methods. However, one of the major challenges to apply mass spectrometry for this purpose, is the incompatibility of MS with many of the matrix components. In this presentation, new analytical approaches will be discussed which are based on LC, and/or flow-injection (including Acoustic sample ejection) Ion Mobility - Mass Spectrometry.

TU-05

Tutorial lecture: Method development and optimization in analytical SFC

Caroline West

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With the introduction of modern instruments, supercritical fluid chromatography (SFC) has rapidly evolved in the past decade and attracted attention from chromatographers working in many different application fields. Although much has been learnt on this technique since its introduction in 1962, developing a method for a new sample is still a little tricky for the newcomers to the technique. In this tutorial lecture, I will explain the basic workflow to develop an analytical method with SFC, for achiral or chiral analytes. The selection of stationary phase and mobile phase compositions are critical and require a good understanding of the sample features to avoid wasting time on long screening steps. The workflow should also consider detection issues, particularly when SFC is hyphenated to mass spectrometric detection (MS). Finally, optimization can be done in an efficient manner with a design of experiments to define the best elution gradient, temperature and pressure conditions.

KL-13

A Closer Study of Ion-Pair Chromatographic Separations of New Therapeutic Oligonucleotides

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Polynucleotides constitute a growing field of various important therapeutic modalities[1] and offer most interesting challenges for the separation scientists. Typical antisense oligonucleotides are single-stranded DNAs and around 6–9 kDa in size and with more than 15 negative charges at pH 7–8. In addition, the complicated manufacturing processes and the storage routines generates numerous structure-like impurities and degradation products. Thus, the separation problems are far more challenging for therapeutic oligonucleotides as compared to yesterday's bestsellers, traditional synthetic drug molecules.

The best generic methods so far, for analysis of impurity profiles and also for preparative separation/fractionating of the smaller amounts needed in early drug evaluation in this high-tech life science area is ion-par reversed phase chromatography (IP-RPC). For larger preparative scales ion exchange chromatography is also used, or combined with ion-pair-RPLC. However, IP-RPLC is used with receipts in empirical ground.

We will therefore present a closer investigation of these systems. The strong impact of the type and concentration of the ion-pairing reagent and the selection of the column chemistry on diastereomer separations of therapeutic oligonucleotides was systematically investigated[2,3]. From these studies we can present how the operational conditions should be varied for either enhanced or suppress diastereomer selectivity. We will also present our most recent fundamental experimental and modelling research for oligonucleotide separation in ion-par liquid chromatography, considering, among others, electrostatic and hydrophobic interactions. The models used are based on firm physical chemistry for improved mechanistic understanding, allowing more reliable predictions of optimal experiments.

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TU-06

Think Bayesian: old and new solutions for massive chromatographic data-analysis

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Data analysis methods applied to chromatographic data, including base-line correction, peak detection, alignment and peak tracking, calibration and/or classification are a routine part of most modern analytical workflows. With the emergence of hyphenation (especially mass spectrometry) and two-dimensional methods (e.g. GCxGC and LCxLC) new challenges for the data analysis are also emerging. Analysing these enormous and complex quantities of data becomes a tremendous challenge, especially because of the need to do it automatically.

Traditionally, chromatographic data has been processed using the so-called frequentist approach. With this approach, we get just a final answer about hypothesis we are testing, but we have no information about its probability of being true. For example, if we want to know if compound A is present in a chromatogram, frequentist approach will conclude "the compound A is present" or "the compound A is not present", without informing the chromatographer what is the probability of both statements being true. The same holds for peak tracking ("where has my peak migrated in these other conditions?"), base-line treatment ("what is the correct base-line correction"), peak assignment ("are all these peaks belonging to the same compound?"), etc.

Contrary to the frequentist approach, Bayesian statistics offers a very interesting alternative. Instead of concluding "the compound A is present" or "the compound A is absent", it delivers an answer with its associated probability, e.g. "the probability that compound A is present is x%". This way of thinking opens a new world of tremendous possibilities, especially in the area of automated massive data treatment. Instead of being the algorithm the one that "takes decisions" based on pre-established parameters, it is the chromatographer that decides on a possible explanation of the data, once the probabilities for every configuration are given via Bayesian analysis. In this way, the chromatographer has no longer to "trust" the results of the data analysis, but (s)he has to decide on the different configurations that explain the data, based on the probabilities of each one.

We have applied this way of thinking to a broad range of examples in which the automation of the data treatment was a must. Several examples are going to be discussed in depth. Peak detection, peak tracking, peak alignment, peak assignment or peak deconvolution are examples in which Bayesian framework provides elegant solutions to very difficult problems. In all cases, the algorithm applied involve a paradigm shift in the way the chromatographer is working on data analysis. Basically, we are proposing to work with probabilities of hypotheses (and update them as long as more information/data is taken into account), opposed to deliver the final answer to the chromatographer.

YES-11

Establishing an Unique Open-Source Benchmark Dataset for the Comprehensive Evaluation of GC×GC Software

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Two-dimensional gas chromatography is amongst the most powerful separation technologies currently available. Since its advent in 1990, it has become an established method which is readily available. However, one of its most challenging aspects, especially in hyphenation with mass spectrometry, is the high amount of chemical information it provides for each measurement. The GC×GC community agrees that answering this challenge for data evaluation is the highest demand for action [1–3]. In response, the number of software packages allowing for in-depth data analysis of GC×GC data has risen over the last couple of years. These packages provide sophisticated tools and algorithms allowing for more streamlined data evaluation. However, the tools/algorithms and their functionality differ drastically within the available software packages.

This study focuses on two main objectives: first, establishing an open-source dataset for benchmarking, and second, streamlined evaluation guidelines for comprehensive comparison for GC×GC software. Thereby, the benchmark data includes, a set of standard compound measurements and a set of chocolate aroma profiles. On this foundation, eight readily available GC×GC software packages were investigated for fundamental and advanced functionality.

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YES-12

Unlocking automated method development: new peak-tracking algorithm for very fast data analysis of (LC×)LC-MS and (GC×)GC-MS data

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Comprehensive two-dimensional chromatography is increasingly indispensable for the analysis of complex samples, because it (i) profits from a potentially much higher peak capacity than 1D separations, (ii) exposes two chemical properties of the sample if the two separation modes are sufficiently different (i.e. "orthogonal"), (iii) allows highly complex samples to be separated and (iv) hyphenates separation modes with normally incompatible detectors, potentially providing additional sensitivity and selectivity.

In the case of LC×LC, these favourable traits do not prevent scientists and manufacturers from struggling to bring the technique to analytical labs in industry. While GC×GC is recognized as a successful, robust, established technique for the analysis of complex volatile mixtures, the potential of LC×LC is often shrouded by a widespread perception that the technique is not sufficiently mature and that its application is impeded by a seemingly insurmountable mountain range of challenges. The complexity of method development in LC×LC has sparked the development of computational optimization tools. Due to such improvements, more chromatographers can access the separation power offered by LC×LC and, consequently, the number of published applications continues to accelerate.

With the advent of more-sophisticated mass spectrometers and more-efficient separations, the amounts of data produced by these powerful separation systems is daunting and developing the ability of rapid analysis and using such data becomes vital. Both in the case of GC×GC-MS and LC×LC-MS it is imperative that data-related issues are overcome.

Our groups aim to support the proliferation of multi-dimensional chromatography in the routine analytical lab and we have heavily invested in its development. In this presentation, we will address the challenges associated with data analysis and method development. We will demonstrate our newly developed peak-tracking algorithm for LC×LC-MS and GC×GC-MS data. The usefulness of the algorithm will be shown within the context of both rapid automated data analysis and method development. Most notably, we will demonstrate the application of the algorithm on real datasets, including an LC×LC separation of antibody digests.

OC-12

The use of experimental design and automation for pharmaceutical solid oral dosage form sample preparation

Paul Ferguson

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Pharmaceutical manufacturers must demonstrate their medicines are manufactured consistently, are safe, efficacious, and of high purity. These quality attributes are measured using a variety of analytical instrumental approaches, where optimised methods are often developed over many days, weeks or even months. However, much less attention is placed on how the sample is prepared and ensuring quantitative transfer of all relevant sample components into the measurement system.

The complexities of preparing analytical samples from solid oral dosage forms (tablets and capsules) will be discussed briefly, alongside our use of experimental design to increase understanding and improve reproducibility. The presentation will close with discussion of future possibilities for automation and considerations for their use.

OC-13

QuEChERS: a versatile tool for monitoring strategies in life science, environmental and manufacturing industries

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We rely on accurate measurements in all aspects of our daily life to guarantee the efficacy and safety of a range of materials (e.g. therapeutics, food and nutrition, domestic cleaning products and fuels), for environmental and public health. Understanding the 'true' content of complex mixtures remains an unmet challenge for a range of applications and industries. This challenge is highly dependent on fit-for-purpose technologies and processes for which sample preparation remains a rate limiting step in this pipeline. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technology uses several separation approaches to target the removal of abundant interferences and offers a highly flexible preparation method for qualitative screening.

To test the breadth of the QuEChERS protocol we have developed and applied the technology for a range of challenging complex mixtures covering environmental, manufacturing and life science sectors. We have evaluated the technology for a range of chemical targets and undertaken some preliminary development work for the automation of the protocols. Initial results have proved positive for a range of biological samples, domestic and industrial wastes with reliable analyte extraction for a semi-automated process showing comparable results to the traditional manual protocol.

OC-14

Comparison of OMCL methods for N-Nitrosamine impurities in Sartans- The ongoing challenges and wider issues
Mark William Harrison
AstraZeneca

In July 2018 the Nitrosamine contamination issue in Sartans broke. Initially restricted to N-ntrosodimethylamine (NDMA) in Valsartan, the scope quickly expanded to include N-nitrosodiethylamine (NDEA) contamination in Losartan, Irbesartan, and Olmesartan, and has impacted a million plus doses globally. Regulators mandated testing for NDMA initially, followed by NDEA for all tetrazole containing Sartans, against extremely challenging timelines. A key risk factor being identified as the nitrosation chemistry involved in the tetrazole ring formation, and subsequently solvent contamination has been identified as a cause. In response to the evolving regulatory demand a rapid process of trace method development has been carried out by the official medicines control laboratories (OMCL) and allied groups. There are currently 19 published official methods in total for the analysis of n-Nitrosamines in Sartans. Initially headspace-GC/MS methods were developed for NDMA and NDEA and these have been refined into validated analysis capable of detecting not more than 5ppb of either analyte. Regulatory demand for 4 - (methylamino) Butanoic acid (NMBA) testing drove development of an LC-MS/MS method, which also added required specificity at the ultra trace levels. Regulators have further mandated N-nitrosodisopropylamine (NDIPA) and N-nitrosoethylisopropylamine (NDEIPA) to be monitored. The lowest LOD seen in any official method currently stands at 1ppb for NDMA, NDEA, NDEIPA and n-Nitrosodibutylamine (NDBA) in an FDA GC-MS/MS method. This LOD has not been proven for all Sartan matrices and the method cannot analyse for trace levels of NMBA directly. The importance of sample preparation has been further raised by the recent request by EMA and Health Canada to risk assess all chemically synthesised human medicinal products. Marketing authority holders must test where any risk is identified.

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KL-15

Infrared ion spectroscopy (IR-IS): a promising tool for metabolite identification!

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Infrared ion spectroscopy (IR-IS) represents the combination of infrared (IR) spectroscopy and MS and provides the opportunity to record IR spectra of mass-isolated ions directly inside a mass spectrometer. IR spectra can, therefore, be generated with the outstanding sensitivity and selectivity of MS, resulting in molecular structure information far beyond what MS data alone can supply.

Here we focus on the application of IR-IS in drug metabolite identification. There is often a need for structure identification of drug metabolites in order to understand potential consequences for pharmacology and/or safety. Mass spectrometry coupled with liquid chromatography is the analytical method of choice for metabolite identification. The localization of the exact site of metabolic biotransformations by mass spectrometric analysis alone is, however, often challenging or impossible since regio-isomeric metabolites are frequently not easily discriminated from product ion data, e.g., for positional isomers with a site of metabolism on an aromatic ring. In these cases, NMR is still the technique of choice but requires relatively large amounts of compound in high purity. IR-IS can sometimes be an alternative using a fraction of the sample needed and with limited or no requirements on purity as recently demonstrated in a proof-of-concept paper[1]. Moreover, IR spectra can be predicted by quantum-chemical calculations making reference free identification possible.

Here, we present our latest results in the novel application of IRIS in the identification of drug and other metabolites.

References:
[1] J. Martens, V. Koppen, G. Berden, F. Cuyckens, J. Oomens. Combined liquid chromatography – infrared ion spectroscopy for identification of regioisomeric drug metabolites. Anal. Chem. 89: 4359 - 4362 (2017).

KL-16

Molecular Weight Distribution Characterization of Reactive Higher Ethyleneamines using Size-Exclusion Chromatography

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Ethyleneamines have been commercialized for decades in the chemical industry for a diverse range of applications. They are often produced through a polymerization process. As a Consequence, final products are often a distribution of chain lengths that each correspond to a certain molecular weight (MW). The MW of a final product is therefore described as a molecular weight distribution (MWD) with MW averages calculated from the molecular weights of all the polymer chains in the sample. The MW averages are valuable metrics since they can often be related to the physical properties of the synthesized material and, accordingly, also to the ultimate product performance. A common technique to determine the MW averages is size-exclusion chromatography (SEC) in combination with refractive index (RI) detection which is frequently applied due to its robustness and ease of use. However, when dealing with ethyleneamines, the presence of amine functional groups provides them opportunity to adsorb onto surfaces which can make them a very challenging sample matrix to analyze using separation techniques.

In the present study, a new aqueous SEC-RI approach, which enables MWD characterization of higher ethyleneamines, is going to be introduced. The sample preparation will be based on the dilute-and-shoot methodology. A surface-modified SEC column with positively charged groups attached to the stationary phase is going to be explored. Optimization of the mobile phase composition (salt concentration, pH) will be considered in order to suppress interaction between the ethyleneamines and the packing material. Effect on the measured MW averages and MW distribution due to various experimental parameters (e.g., system variability, mobile phase preparation, sample concentration) will be studied.

OC-15

Is chromatography still at the heart of the future lab?

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Everyone talks about efficiency. In the context of the analytical lab, usually the chromatographic process is considered to be the key parameter for increasing the overall efficiency. Moreover, very complex hyphenation is used to extract a maximum of information of a sample. But what are the technologies that will have a major impact on the design of the tomorrow's lab environment.

In this presentation, the state of the art of hyphenation is discussed, including two-dimensional liquid chromatography coupled to ion mobility-mass spectrometry, as well as online solid phase extraction coupled to nuclear magnetic resonance spectroscopy. The trend of automation and digitalization facilitates new workflows and new coupling technologies in order to save more resources and to obtain results faster. This includes collaborative robotics as well as a lab environment that can be changed according to the needs of projects.

OC-16

Automated preparation and analysis of haloacetic acids in water by GC/MS

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Haloacetic acids (HAAs) are toxic organic components that are formed during the disinfection of water intended for human consumption. They originate from the reaction of popular disinfectants such as chlorine and chloramine with natural organic matter and account for more than 25% of the total halogenated disinfection byproducts (DBPs) measured in chlorinated water. HAAs are toxic components and their high water solubility renders routine monitoring obligatory. Currently, nine representative components are included in routine monitoring programs imposed by the European Environmental Agency (EEA). Legislative reporting limit is currently set at < 80 µg/L for the sum of their concentrations.

Due to their high aqueous affinity, HAA analysis requires a labor-intensive extraction and derivatization step prior to GC/MS analysis. Here, a method is presented for routine analysis of the nine representative HAAs by means of GC-MS/MS after on-line sample preparation using a dedicated XYZ autosampler that is installed on top of the GC instrument. Briefly, 10 mL of sample is transferred to a 20 mL headspace vial, sulfuric acid and sodium sulphate are added to the vial. Afterwards, the sample is transferred to the robotic sampler, which performs the extraction, derivatization and finally injects 1 µL of the extract onto the GC-MS/MS. The method exhibits excellent reproducibility %RSD below 5% for all HAAs and detection limits < 0.2 µg/L.

TU-07

Advances in two-dimensional liquid chromatography

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Two-dimensional liquid chromatography (2D-LC) is rapidly emerging as a powerful technique for the separation of complex mixtures. While the technique is most frequently applied within academia, the introduction of robust commercial instrumentation has significantly facilitated its use in industry.

What is 2D-LC? Why is it powerful and when should it be used? What is needed for application of the technique? How can it be applied effectively? How do I use and interpret the resulting data? In this tutorial we will address each of these questions and learn how to rapidly develop a method.

You will learn about key principles including peak capacity, orthogonality and selectivity to understand the origin and power of 2D-LC. You will see how these principles and the concept of sample dimensionality can pragmatically be applied to select orthogonal retention mechanisms as first- and second-dimension methods.

Much attention will also be devoted to the modulation interface, which allows us to transfer first-dimension effluent to our second dimension. You will learn how a sensible selection of retention mechanisms can be advantageous, and how to solve or avoid potential drawbacks.

Finally, some attention will be given to optimization of our developed method as well as the analysis of our data. How to use the output? All concepts in this tutorial will be illustrated with representative LC×LC chromatograms.

The tutorial is intended for analytical chemists who are confronted with the characterization of complex, non-volatile mixtures. For such mixtures LC×LC offers some very attractive features. It may provide excellent separations in a relatively short time and it may yield structured, readily interpretable chromatograms.

YES-13

Enhancing sensitivity in 2D-LC by hyphenating temperature-responsive phases with reversed phase liquid chromatography

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Achievable sensitivity of online 2D-LC systems is still hampered in comparison to conventional (U)HPLC and is traded for a gain in peak capacity. This limitation is due to the modulation problem. To achieve highest orthogonality, incompatible modes are combined with each other, such as HILICxRPLC or NPLCxRPLC. Thus, non-miscible solvents are transferred between the two dimensions, requiring the use of narrow column diameters in the first dimension, allowing for low flow rates and thus limited transfer volumes. Consequently, also sample-loading capacities are decreased. To allow for fast gradients without exceeding backpressure, the second dimension columns then have to be very broad, but combination of a 1 mm column in the first dimension, paired with a 4.6 mm column in the second dimension leads to a ≥20-fold loss in sensitivity for concentration-sensitive detectors due to the inevitable dilution of the sample. Alternatively, in RPLCxRPLC, depicting suboptimal orthogonality, at best 2 mm IDs are combined with a 3 mm ID column, also leading to lowered detection sensitivity. The underlying cause of this problem is a too high eluotropic strength of the first dimension solvent, and following the risk of incomplete refocusing of the analytes at the second dimension column head. Possible solutions to this problem usually focus on the dilution of the first dimension solvent prior to transfer to the second dimension[1,2], potentially complicating 2D-LC. To address this methodical complexity, here, temperature-responsive liquid chromatography (TRLC) is used in the first dimension and paired with RPLC in the second dimension. TRLC is based on a smart polymer, poly(N-isopropylacrylamide), which depicts increasing hydrophobicity for rising temperatures. This type of stationary phase allows for retention and separation in a purely aqueous mobile phase, offering the chance to overcome the dilution problem by the use of identical column IDs in both dimensions. Because full solute refocusing at the second dimension column head is obtained in TRLCxRPLC, the dilution problem experienced with all concentration sensitive detectors in comprehensive and heart-cutting 2D-LC even offers the prospect to be inverted. These aspects are studied for various column ID combinations (e.g. 2.1x4.6mm, 2.1x3.0 mm, 2.1x2.1 mm and 2.1x1 mm) in heart-cutting and comprehensive 2D-LC-UV. This approach can then also be extended, whereby a broader 4.6 mm column ID is used in the first dimension, which should allow for enhanced sensitivity in LCxLC-UV compared to conventional LC-UV. The potential of this strategy is investigated, expecting improvement in impurity profiling in drug products or analysis of low-abundant molecules in food or natural products.

YES-14

Developing active-modulation interfaces for hyphenation of light-induced-degradation reactors with LC separations

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Organic compounds can undergo photochemical conversion through exposure to (UV) light. While this sometimes is exploited in specific applications, such as in water purification, it is often undesirable. One example is the fading of colorants in cultural-heritage objects, which reduces their esthetical value. Another case in point are changes in food products due to degradation of healthy ingredients (e.g. vitamins).

Studying this photochemical conversion is challenging and potentially time consuming. Often it is difficult to establish a strong link between the degradation products and the starting materials, which results in poor degradation-prediction models.

To solve these issues, a separation system is being developed based on comprehensive two-dimensional liquid chromatography featuring reaction modulation. In this TooCOLD method (Toolbox for studying the Chemistry Of Light-induced Degradation) the separated compounds from the first dimension are transferred to an exposure cell. In this reaction modulator the effluent and all its constituents are degraded under the influence of light. The degradation products are then injected into a second LC system for further analysis.

By connecting a degradation cell to another liquid-chromatography separation, new challenges present themselves. For example, the relatively large cell volume required for efficient light-induced degradation, as well as the connections to and from the cell, result in significant dispersion of the degraded fragments. These extra-column band broadening and incompatibility issues between the degradation matrix and the second-dimension stationary phase may be resolved through active modulation. The composition of the first-dimension effluent is then altered before injection into the second-dimension separation. The two most used forms of active modulation are solid-phase-assisted modulation (SPAM) and active solvent modulation (ASM). Because SPAM can both reduce the extra-column band broadening and remove the degradation matrix, it is the preferred method. SPAM is not limited to a maximum volume, which increases the applicability of the TooCOLD method. One of the major disadvantages of SPAM is the potential loss of less-retained compounds, since SPAM relies on the retention of analytes on a small solid-phase cartridge.

In this presentation the applicability of SPAM in a TooCOLD set-up is investigated. The unpredictable composition of the degradant mixture for every new sample demands a new approach to rapidly assess the feasibility of the modulation technique. This approach relies on retention modelling under extrapolated conditions and its applicability for the present purpose will be demonstrated. To cover a wide variety of chemical properties, analyte standards were selected from various classes and modelling accuracy will be investigated for each of these. The acquisition of suitable data for such studies will be discussed in detail.

YES-15

Application of evolutionary algorithms to optimise one- and two-dimensional gradient chromatographic separations

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Method development is an essential procedure to achieve the full potential of a chromatographic separation. However, since it is intrinsically a multi-parameter optimisation problem, this procedure can be quite complex and time-consuming. This is especially true for two-dimensional chromatography, where twice the number of parameters have to be optimised. When applying classical optimisation methods, this leads to a drastic increase in complexity and time consumption.

With the advent of artificial intelligence, novel possibilities to search among numerous combinations have arisen. These include so-called evolutionary algorithms, which mimic biological evolution to solve multi-parameter optimisation problems. They are less sensitive to local optima, as well as to an increase in the number of parameters.

In the present contribution, three evolutionary algorithms were applied to the optimisation of gradient parameters of chromatographic separations: a genetic algorithm, a non-adaptive evolution strategy and a covariance matrix adaptation evolution strategy. In a first phase, they were adapted to method development to reduce their time consumption. In a second phase, their time consumption was compared to that of a grid search method. Both first and second phase were based on in silico meta-experiments, simulating the chromatographic separation of numerous computer-generated samples, to guarantee statistical significance of the results.

The first phase led to insights in the design of evolutionary algorithms. Somewhat surprisingly, the 'one individual per generation'-design appeared to be the most efficient. The second phase showed that the evolutionary algorithms have a considerable advantage over the grid search method, which increases with the complexity of method development. Notably, this advantage is most pronounced in the case of two-dimensional chromatography, demonstrating the potential of evolutionary algorithms for method development in hyphenated techniques.

Since evolutionary algorithms are versatile, future research could improve their performance by combining them with other optimisation methods or extend their applicability towards other hyphenated techniques.

KL-17

HTA and the clinical world
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'Routine' clinical analyses by their very nature demand rapid turnaround times, either to inform a clinical diagnosis for patients, or to inform a change in treatment or pharmaceutical dosage. Whilst many such analyses are undertaken on fully-automated, random-access, immunoassay-based analytical 'track' systems, which offer very rapid turnaround (often less than one hour from sample receipt), specialist tests which use chromatographic methods are often slower and more labour-intensive. There are clear benefits from increasing throughput in chromatographic methods in the clinical world – this lecture will review approached to high-throughput analysis, including automated sample preparation, ultra-rapid chromatographic separations and novel approaches to multiplexed analysis utilising the specificity of mass spectrometric detection.

KL-18

The Robot in your Lab; Friend or Foe?
Kathy Ridgway
Anatune Ltd.

The use of autosamplers for instrumental analysis is now common place and manual injection is largely a thing of the past. However, there are now many options for robotic systems that are much more than simple autosamplers. Manual tasks previously performed by lab technicians can now be fully controlled by software and data output linked to external systems for on-line analysis solutions. Intelligent software enables tasks to be overlapped and the majority of procedures traditionally involved in sample preparation protocols can now be fully automated. These include preparation of calibration solutions, liquid extraction, enrichment (solid phase extraction), evaporation, centrifugation and derivatisation. This talk will outline some of the solutions available and discuss how new technologies have influenced many laboratory work flows. The advantages of automating sample preparation will be highlighted with case studies illustrating why robotics are becoming more common place in modern high throughput laboraties.

OC-17

Will Bioanalysis surrender to the robotic army?
Arundhuti Sen
GSK

The performance of most analytical instrumentation platforms has improved rapidly and continuously over the past decade, resulting in more robust and reproducible bioanalytical assays. To further improve assay robustness and reproducibility; however, we need to minimise the errors that arise from the steps prior to the analytical measurement (e.g. sample handling, extraction or processing). This is particularly important for complex, multi-step assays involving derivatisation or biological reagents. The development of such complex assays often requires the iterative optimisation of many parameters, meaning that traditional manual, one-factor-at-a-time optimisation strategies add substantially to assay development timelines. The efficiency and efficacy of manual method development workflows are dependent largely on the skill and previous experience of the responsible analyst, and the sizeable data generated by such manual workflows can be difficult to interrogate later. Automating method development in bioanalysis has the potential to significantly reduce the time and effort associated with assay optimisation, the quality of the data generated during this process, and the quality of the resultant assays.

In this contribution, we will be discussing GSK's automation initiative, and the transition from a sporadic use of laboratory automation to an environment where automated approaches to data generation and analysis are standard. We will focus on the implementation of a modular automation strategy within a regulated bioanalytical laboratory, to support method development and validation for both small and large molecules. We will also highlight the power of bespoke data capture systems to increase the adoption and utility of automated workflows. Both automated (end-to-end) and semi-automated workflows incorporating a variety of informatic (e.g. GCAL system for API handling) and liquid-handling technologies (including the D300, Tecan Evo, Bravo AssayMap and ECHO Acoustic Dispense systems) will be described, and examples of the impact these workflows have had on the development of LC-MS and immunoassay-based methods will be shared.

OC-18

Capillary Electrophoresis : Speed and Selectivity for High Throughput Analysis
Gordon Ross
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From the inception of Capillary Electrophoresis (CE) as an instrumental method of electrophoretic analysis the technique has evolved into an analytical technique complementary to LC based separations and a disruptive and replacement technology in the field of size based separations. CE is characterised by its provision of high resolution separations which can be achieved in a relatively short time period. This speed of separation, while preserving its efficiency, makes a clear contribution to high throughput analysis. Additionally, the basis of CE separations, specifically Capillary Zone Electrophoresis, provides a selectivity in a single run which might require multiple LC methods in order to emulate. For more complex sample matrices with a wide range of analyte characteristics, this reduction in run number can be practically useful. CE can be operated in a number of modes and its use in gel based separations, based on compound size, allows not only increased speed but, with its microscale format, also allows multiplexing of analyses.

KL-19

Advantages and limitations of HILIC in the second dimension of comprehensive two-dimensional liquid chromatographic separations: A kinetic evaluation

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The combination of HILIC and RP-LC separations in comprehensive two-dimensional LC (LC×LC) is attractive due to the high degree of orthogonality of these separation modes. Despite the challenges associated with the hyphenation of HILIC and RP-LC due to the divergent elution strengths of their mobile phases, on-line HILIC×RP-LC in particular has found extensive application, especially in the separation of phenolics. There are however several potential benefits to swapping these separation modes for phenolic analyses. Application of RP-LC in the first dimension (1D) offers improved chromatographic efficiency and the opportunity to inject relatively large volumes of highly concentrated aqueous extracts, thereby improving sample loading compared to HILIC in the 1D. On the other hand, HILIC in the second dimension (2D) provides the benefit of allowing high flow rates (due to the low viscosity of HILIC mobile phases) and improved compatibility with electrospray ionisation (ESI)-MS detection.

In this contribution, we present a detailed kinetic evaluation of the performance of on-line HILIC×RP-LC and RP-LC×HILIC systems for phenolic analysis. Active solvent modulation is used in both cases, where the 1D effluent is diluted with a weak 2D solvent to minimise injection band broadening. An automated Pareto-optimisation protocol is used in combination with experimentally determined chromatographic parameters and solubility ratios for selected model phenolics to predict performance in terms of practical peak capacity, dilution, sensitivity for both concentration- and mass sensitive detectors, two-dimensional resolution and analysis time. The predicted findings are experimentally confirmed for a range of complex natural product phenolic fractions in combination with diode array-ion mobility spectrometry-high resolution MS detection, and are discussed critically in terms of the sample type and analysis goals.

TU-08

Approaches Towards Method Development in Two-Dimensional HPLC

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Two-dimensional liquid chromatography (2D-LC) is becoming a routine technology as more and more instrument solutions are becoming commercially available from instrument vendors.

The main operation modes in 2D-LC are full-comprehensive for very complex samples as well as heart cutting and multiple heart cutting (selective comprehensive) for samples of low to medium complexity. Although 2D-LC is inherently a high-resolution technique, it is a challenging task to develop and optimize separation methods depending on the desired separation goal.

The method development strategy for full-comprehensive operation aims mostly at achieving a desired peak capacity in the shortest time possible. In this context, the gradient kinetic plot model (KPM) can be employed to find the best column dimensions and operating conditions for the 2nd dimension (particle size, column length and ID, flow rate, temperature etc.). The model takes limiting factors such as maximum operating pressure or flow rate into account. An extended version of the KPM also includes the impact of external band-broadening that limits the use of very short, narrow columns.

If the sample is only of limited complexity or if only certain parts of the 1D chromatogram are of interest, selective comprehensive 2D-LC can be used to achieve the desired separation in a shorter time by analyzing only the areas of interest. In this case method development will aim more at achieving specific selectivities (through selection of stationary phases, 2D eluent, gradient range and slope) than aiming exclusively at optimizing peak capacity. Here a combination of kinetic optimization and retention modeling is useful.

Other important factors to consider are compatibility of separation modes in the two dimensions and possibilities to overcome negative impact of solvent mismatch when injection large volume in the second dimension.

The authors will discuss possible strategies of for developing methods in full-comprehensive and selective comprehensive 2D-LC modes.

OC-19

Development of Microfluidic Chip Technology for Spatial Three-Dimensional Liquid Chromatography

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Multidimensional liquid chromatographic separations strategies are advancing towards higher separation performances, driven by the analysis of contemporary life-science samples used in biomarker discovery studies. These samples are typically characterized by a large sample complexity (often containing ~1,000,000 compounds) and broad dynamic range, requiring separation technology which provides the resolution required for the identification and quantification of all compounds present in a complex sample mixture.

A microfluidic device for spatial three-dimensional LC holds the promise to provide ultra-high peak capacities. In spatial 3D-LC chromatography components are separated inside the microchannels of the device with each peak being characterized by its X, Y, and Z coordinates in the separation body. Furthermore, due to parallel developments of all fractions in the second- and third-dimension separations, the analysis time is greatly reduced compared to conventional column-coupled multi-dimensional LC approaches. The different design aspects to create spatial multi-dimensional chip technology will be discussed, including flow distributor design and channel layout. During the different developments in X-, Y-, and Z-direction, the analytes and therefore also the flow should not convolute in other dimensions. Aspects of flow control mechanisms, which includes the use of physical barriers to achieve flow control, and implementation of polymer-monolithic stationary phases, will be discussed. We extended an earlier introduced concept, in which active flow confinement is achieved by applying a rotating interface. To allow for automated process operation, mechanisms applying advanced robotics have been successfully integrated on-chip. Furthermore, approaches to integrate functionalized monolithic stationary phases inside the microfluidic device have been realized, which are essential to realize a spatial 3D-LC separation.

OC-20

From batch to continuous processing: purification of a bioactive peptide by means of Multicolumn Countercurrent Solvent Gradient Purification (MCSGP)

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The interest around the use of peptides for therapeutic scopes is continuously increasing. Thanks to their high specificity and inherent affinity for target molecules, peptides represent an effective solution to unresolved medical issues avoiding typical toxic side effects of exogenous chemical drugs. From an industrial point of view, they are produced mainly by means of solid phase synthesis. However, during the synthesis, many undesired impurities (truncated or modified peptides, by-products, scavengers, etc.) are produced. Reversed-phase single-column preparative liquid chromatography is the preferred choice to obtain the target peptide at the desired degree of purity for pharmaceutical and therapeutic scopes. However, the presence of product-related impurities, chemically similar to the target, can generate several peak overlapping regions. In these cases, the purification is most likely governed by a yield-purity trade-off. This means that in order to obtain a pool with acceptable purity, the collection window need to be narrowed at the cost of yield (and vice-versa).

In this work, we have investigated the possibility to overcome this trade-off limitation by applying twin-column Multicolumn Countercurrent Solvent Gradient Purification (MCSGP), an innovative method for purification based on the internal recycling of the overlapping regions between two columns connected in series. The presence of valves allows to alternatively operate the two columns interconnected or in batch mode (disconnected). The principle of operation is very simple: briefly, in the first column (upstream) the gradient is performed; the portion of the peak which respects the purity requirement is collected from the upstream column (in this moment the columns are disconnected); on the other hand the overlapping regions between product and weakly/strong adsorbed impurities (front and tail of the main peak) are recycled in the second downstream column which is also filled with fresh feed (in this phase the columns are interconnected). Then the two columns virtually change the position and another half-cycle starts by performing now the gradient in the second column. One full cycle is completed when the columns return in their original position (first column in upstream and second column in downstream). Steady-state conditions are usually reached after 4 or 5 cycles. These operations allow to increase recovery without losing purity.

The design of the MCSGP methods is based on a single column batch chromatogram (named "design batch chromatogram") where a portion of the main peak satisfies the purity requirements. This work will illustrate the optimization of experimental batch conditions for the purification in MCSGP of a raw glucagon mixture industrially synthesized. The results obtained with single columns batch purification will be compared to those obtained with MCSGP in terms of purity, yield and other important parameters such as solvent consumption.

TU-09

Exotic fragrances of Namibia: Application of current fragrance analysis trends

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The essential oils and aromatic extracts of a number of Commiphora species indigenous to southern Africa is of commercial interest to the region. These plants have a variety of traditional uses, including being used by the Ovahimba women of Namibia as the main ingredients for their perfume and as soap. Although these products have drawn some international attention in recent years, their detailed chemical composition were not known. The chemical characterisation of the oil and extracts is important, since this knowledge may be used by perfume, cosmetics and detergent manufacturers to guide the formulation of their products and also to assess the safety of these oils and extracts when used as ingredients. In our studies the detailed chemical characterisation of the volatile constituents of the essential oil and aromatic extracts of Commiphora resin and gum was performed using GC-MS and GC-FID. The latest requirements for the identification of flavour and fragrance compounds using retention indices were applied.1 In addition, enantioselective GC analyses were performed in order to determine the enantiomeric excess of the major chiral constituents. The current quantitative analysis practices were also followed, including the use of predicted relative response factors when using FID.2 Complex mixtures of compounds were detected in the oils and extracts, and different types of compounds, including α-pinene (enantiomeric ratio 85S:15R), decane and maltol, were found to be the major constituents in the products of the different Commiphora species. Using the current fragrance analysis trends, it was possible for us to execute these detailed chemical characterisations successfully with a very limited number of reference standards.

YES-16

Development of an untargeted and targeted multi-class method for cannabis products

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The recent trend towards the commercialization of legal cannabis in several countries has generated novel opportunities to understand the potential benefits for medical purposes. Together with the growing need for safer cannabis products, the quality control inspections and methods for their characterization increased exponentially. Generally, quality control analyses include

multi-chemical class testing for potency, terpenes, pesticides, residual solvents, heavy metals, mycotoxins and microorganisms.

In this presentation, the importance of the profiling and the determination of terpenes, cannabinoids, and pesticides will be discussed. In addition, a unified method for their qualitative and quantitative analysis in a single analytical run will be provided. The method involves a sorption-based extraction followed by comprehensive two-dimensional gas chromatography coupled to (low- and high-resolution) mass spectrometry, i.e. GC×GC-MS. The extraction method was optimized to have optimal recovery for the chemical classes of interest. A factorial design of experiments was used to determine the most advantageous combination of the extraction conditions (solvent type, salt addition, extraction time and temperature).

The overall method was validated on a variety of recreational cannabis flowers and cannabis oil samples. It will be demonstrated that the method allowed to efficiently highlight the difference between the various cannabis strains based on the multi-chemical class information provided.

YES-17

European lacquer in Context. Strategies to find THM-GC/MS resin biomarkers and application on historical lacquered objects.

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After four years, the "European Lacquer in Context" project (ELinC) has come to an end. The project gained chemical, art historical and technological insights on the production, the trade and the chemistry of lacquered art objects from Western Europe. European lacquers are mainly complex, layered combinations of various natural materials, including resins, oils and solvents, refined with inorganic materials such as pigments, fillers and metals. With their diverse constitution and important impact of aging on their molecular composition, analysis of lacquer can be challenging. Central in the unravelling of the composition of European lacquers is Thermally assisted Hydrolysis and Methylation- Gas Chromatography/ Mass Spectroscopy (THM-GC/MS). Pyrograms of European lacquer can feature a large variety of known and unknown compounds. In order to detect small amounts of ingredients, to cope with aged ingredients or to differentiate between two similar ingredients, more chromatographic markers could be useful.

Two different strategies to detect additional natural resin markers and to assist in a better interpretation of these THM-GC/MS data will be discussed during this presentation.

In a first approach, a chemometric approach was investigated. A method normally used in metabolomics for generating biomarkers of interest for medicine and biotechnology was successfully tested to generate markers for copals [1]. In the second strategy, a structured marker database for AMDIS was created in a semi-automated way starting from a large amount of chromatograms of different ingredients before, during and after artificial aging. The temperature in the pyrolyzer is important for the result.

References:

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YES-18

Optimization of untargeted screening workflow for the characterization of lung fluid samples

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The ballistic rise of high-resolution analytical technologies has opened a large playground for all type of untargeted "omics" screening. Comprehensive two-dimensional gas chromatography (GC×GC) has become a method of choice for complex mixture characterization. The two chromatographic dimensions and the possibility to hyphen high-speed high-resolution time-of-flight mass spectrometers (HRTOFMS) locates GC×GC as a method of choice for untargeted metabolomics. In this quest of the big picture, it is important to carefully apprehend every step of the analytical workflow from the sampling to the statistical process. The complexity of the approach cannot impact the analytical robustness.

In this study, Bronchoalveolar lavage fluid (BALF) samples were analyzed by solid phase microextraction (SPME) coupled to GC×GC-TOFMS. These samples were analyzed as part of a discovery study for lung inflammation mechanisms characterization. First, a QC mixture was designed by pooling an aliquot of the different liquid samples spiked with internal standards. This QC solution was used for optimization and daily system monitoring. Central composite design is a method of choice to establish optimal analytical conditions. For SPME, the peak intensity was used as a quality metric versus the fiber type, incubation time and temperature as variable parameters. For the GC×GC-TOFMS, normal and reversed column combinations were tested. Based on these optimal conditions, the samples were injected and the optimization was performed for the pre-processing parameters. Different alignment, normalization, and data transformation approaches were compared using unsupervised clustering.

The resulting workflow offers robust and controlled conditions for the second phase of this project. The following step in collection of BALF sample from patient with different lung conditions. The preliminary results indicate the capacity of the technique for the identification of chronic lung inflammation.

KL-22

Membrane-Assisted Solvent- and Sorbent-Phase Microextraction of Difficult Liquid Matrices

Hian Kee Lee

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Since their advent between twenty and thirty years ago, sorbent- and solvent-based microextraction in all their various modes have demonstrated their practical applicability to many types of liquid (aqueous) matrices. Together with gas or liquid chromatography coupled to especially mass spectrometry, these extraction techniques have proven to be advantageous for, e.g., environmental analyses. One particular challenge is dealing with environmental analysis is the complexity of the sample matrix. A solution that addressed this issue with reference to the liquid-phase microextraction of aqueous samples was the use of polymeric membranes. In the two to three decades hence, membranes continue to represent one of the most appropriate approaches in microextraction to deal with complex liquid samples. This talk will cover the use of polymeric membranes in sorbent-phase and liquid-phase extraction procedures, including how they can not only be integrated with chromatographic analyses, but also with water sampling, to afford a fully automated workflow.

KL-22

High-resolution two- (and three-) dimensional liquid chromatography
Peter Schoenmakers
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Comprehensive two-dimensional liquid chromatography (LC×LC) is gaining maturity as a high-power, high-resolution separation method, thanks to the increasing availability of suitable commercial hardware and accompanying software. LC×LC offers additional selectivity and a much greater separation power (peak capacity) in a much shorter time for the analysis of very complex mixtures of individual analytes [1]. As a rule of thumb, one-dimensional LC may offer peak capacities up to about 1,000 at approximately one peak per minute, while LC×LC offers peak capacities up to about 10,000 at about one peak per second.

One domain in which LC×LC has proven indispensable is that of synthetic polymers. Polymeric samples can be extremely complex, but the sample dimensionality [2] is relatively low, so as to give rise to structured separations. High-resolution LC×LC-MS provides highly detailed characterization relatively low-molecular-weight polymers. Different types of synthetic polymers call for the exploitation of different types of LC retention mechanisms.

Potentially, spatial two- and, especially, three-dimensional LC offer even greater separation power than the established column-based LC×LC. Spatial 3D-LC promises peak capacities of the order of one million in a relatively short time and at modest pressures. However, it requires a paradigm shift in terms of separation devices. Prototypes are being developed using 3D-printing techniques, based on theoretical insights and computational simulations. Progress in this direction will be discussed.

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KL-23

Porous polymer monolithic structures in hyphenated chromatographic techniques

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The modern monolithic columns emerged about 30 years ago. Their well-known advantages include ease of the preparation, robustness, high permeability to flow, mass transport via convection, and a vast variety of chemistries. The early polymer-based monoliths were used almost entirely for the rapid liquid chromatography separations of proteins and other large molecules. A number of new chemistries and functionalization methods were meanwhile developed to produce monolithic columns for the separations in various chromatographic modes including gas chromatography, electrochromatography, and microfluidics. In addition to typical chromatographic applications, new uses were recently described thus confirming versatility of the monoliths. For example, reversible functionalization via attachment of gold nanoparticles to thiols provides materials for highly sensitive surface enhanced Raman spectroscopy (SERS). Thin monolithic layers are gaining more attention as well since they enable efficient separations of proteins using very simple means followed by an easy detection using mass spectrometry or SERS.

KL-24

Shedding light on novel zwitterionic-teicoplanin chiral stationary phases for liquid chromatography: from fundamentals to innovative applications

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Zwitterionic teicoplanin CSPs represent an innovative version of traditional, well-known teicoplanin-based ones where, thanks to a proprietary bonding protocol, both a protonable amino group and the acidic moiety of teicoplanin are simultaneously present on the chiral selector

It has been demonstrated that zwitterionic teicoplanin CSPs are able to efficiently separate different classes of compounds, including N-protected amino acids, aryloxy acids and anti-inflammatory drugs under various types of elution conditions, such as RP, normal phase (NP), hydrophilic interaction (HILIC), polar organic mode (POM), WAX and weak cation exchanger (WCX) and supercritical fluid (SFC).

In this presentation, the effect of different experimental variables, including mobile phase composition, particle geometry, pore size, etc. will be valuated from a thermodynamic and kinetic viewpoint.

Focus will be given to relevant applications of practical relevance, e.g. in pharmaceutical analysis, about the simultaneous separation of chiral active pharmaceutical ingredients (APIs) from their counterions in the same chromatographic run. This comes from the properties of the zwitterionic version of the teicoplanin CSP, able to retain both cations and anions (while the traditional version of the CSP excludes anions due to Donnan repulsion).

YES-19

Detecting STAMPs of Microfluidic Separations by SERS and MALDI-MS

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Science is progressing towards knowing the composition and understanding the properties of very complex mixtures in ever increasing detail and at ever increasing speed. For this purpose extremely high separation powers are required. The STAMP (Separation Technology for A Million Peaks) project, funded by the European Research Council (ERC), is aimed at obtaining a peak capacity of one million by using spatial three-dimensional (3D) liquid chromatography (LC). One of the challenges within the project is the development of (imaging) detection principles, initially for spatial two-dimensional (2D) LC and, at a later stage, for spatial 3D-LC.

In the absence of an interface between the spatial separation devices and existing detection techniques, an offline-method is proposed by STAMPing the effluent from the device on a modified substrate. In the present research a STAMPbot was designed and assembled, capable of moving in the x, y and z directions to facilitate the STAMPing process. As STAMPing substrate, glass surfaces roughened to different degrees were investigated, in combination with sputtered gold layers of various thicknesses [1]. These "rough" surfaces allow for Surface Enhanced Raman Spectroscopy (SERS) as a STAMP detection technique. Because SERS is non-destructive, the STAMPs can subsequently be measured by Matrix-Assisted Laser Desorption Ionization (MALDI)-MS or even Surface-Assisted LDI (SALDI)-MS [2]. SERS allows for fast analysis with very low detection limits, whereas MALDI-MS can provide (confirmative) identification, all with little or no sample preparation.

References:

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YES-20

Unlocking the composition of gasoline gum content by application of GC-MS and UHPSFC-MS

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New UHPSFC-MS methods have been developed to separate and detect gasoline gum components that were undetectable by GC-MS. The gum is the residue that remains after the evaporation of the gasoline and contains semi- and non-volatile components. The gasoline gum content is under investigation to identify suspect molecules with low abundance within the fuel that can be related to deposit formation, which can lead to decrease of engine performance, increased emissions or failure of engines parts.

Concentration protocols need to be applied produce gums, which enable speciation and concentration of different deposit precursors, enabling their subsequent analysis by mass spectrometry. The gum samples were prepared following two different approaches that serve different purposes; the industrial standard method IP131/ASTM D381-12 (EI/ASTM gum) and a nitrogen gas evaporation method (Soton gum). The EI/ASTM gum contains any oxidation products formed in the sample prior or during the relatively mild conditions of the test procedure and serves a complimentary role to the analysis of fuels that correlates to the quality of the gasoline. The Soton gum removes the volatiles and concentrate semi- and non-volatile components with low abundance within the fuel.

The analysis of EI/ASTM and Soton gum by GC-MS had revealed hydrocarbons and homologous that were also observed in the fuel analysis. However, when the same samples were analysed by UHPSFC positive ion ESI MS, polymeric material (e.g. carrier oils, detergents) and homologous series with molecular weights above 400 g/mol, different nitrogen content and degree of unsaturation across series, were detected. In addition, the analysis of EI/ASTM and Soton gum by UHPSFC positive ion APPI MS has revealed a difference of species being present in the two types of gum. The Soton gum contained homologous series with aromatic and olefinic content that were not observed in the EI/ASTM gum. These material are described by the General Motors fuel patent and can be related to deposit forming chemistries.

The combination of both GC-MS and UHPSFC-MS analysis of gasoline gum content is providing a holistic picture of the composition of the gum allowing deeper understanding for the cause of deposit formation in gasoline direct injection engines.

YES-21

UHPSFC-MS to unravel complex PEG derivatives used in pharmaceutical formulation

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Novel UHPSFC positive ion ESI MS methods have been developed to characterise poly(ethylene) glycol (PEG) derivatives (Brij®58, mPEG acid 2000, PEG diacid 2000 and Tween®20). PEGs are added to pharmaceutical formulations to help with the delivery of the active principal ingredient (API) to the biological target. Furthermore, more variability can be introduced by the functionalisation of one or two of the hydroxyl end groups in PEG, allowing to tune in the manner in which the API is encapsulated, i.e., as a micelle or a bilayer. The control of PEG formulations is mandatory in terms of quality control and the regulatory bodies involved. The oligomer dispersion within the polymer is characterised as the polydispersity value. Additionally, possible impurities from synthetic by-products and/or compounds originated from UV, temperature or packaging exposure compromise the efficacy of the final formulation. Hence, the industry is demanding newer and faster analytical methods to fully characterise PEG-based pharmaceutical formulations.

For this, novel UHPSFC positive ion ESI MS methods have been developed to characterise PEG derivatives in pharmaceutical formulations. Baseline separation was obtained for several linear PEGs differing only by the functionality of the end group (PEG and mPEG of different molecular weight, Brij®58, mPEG acid 2000 and PEG diacid 2000). Additionally, some characterisation is presented for Tween®20, a multi chain PEG-based polymer. An SFC separation strategy is proposed based on the correct choice of the stationary phases and the mobile phase additives. Per example, the use of ammonium salts results to be the obvious choice due to the ion pair formed between the oligomeric PEG chains and the silica-based stationary phases. Additionally, ammonium salts enhances positive ionisation ESI towards the formation of a podand structure between PEG and the ammonium ions in the media, with predominance of multiply charged ammoniated species for larger polymers.

Finally, the use of ion maps facilitate data visualisation and interpretation, a 2D-representation of the chromatographic retention time in the x-axis and the m/z value in the y-axis. Ion maps facilitate the observation of the ionisation charged state of the oligomeric series and, for some mixtures, some impurities/degradation products can be readily observed away from the polymeric mixture that are not revealed using the BPICC.

YES-22

Development and implementation of multi-dimensional LC-MS setup for a faster and more effective characterization of bio-therapeutic products

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The identification of critical quality attributes (CQAs) is an important step in the development of bio-therapeutics products1, such as monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and bispecific antibodies (BsAbs). Characterization of post-translational modifications (PTMs) such as oxidation, deamidation, glycosylation or glycation is required before regulatory approval since these attributes may impact patient safety and product efficacy2. Although cation exchange chromatography (CEX) is typically used to separate mAb charge variants in control system testing, the characterization of each CEX fraction performed at the peptide level by LC-MS/MS, requires a time-consuming and tedious off-line process.

To circumvent these limitations, an on-line 4D-LC/MS (CEX x Reduction-RPLC x Digestion x RPLC/MS) workflow was developed for the quantification of common PTMs. To compare the performance of our workflow with a conventional off-line procedure, a proof of concept study on a mAb and a bispecific antibody was performed. Overall, the use of the automated online 4D-HPLC/MS workflow shows comparable performance to that of the conventional off-line procedure, but with a much faster turnaround time (1 day compared to several days/weeks).

Besides the use of CEX in the first dimension, a Protein-A column was also tested as first dimension for the characterization of mAbs from real cell cultures. Two analytical multi-dimensional LC workflows were developed for the monitoring of PTMs within the same system. In one workflow a 4D-LC/MS (Protein-A x Reduction-RPLC x Digestion x RPLC/MS) method enabled the characterization of oxidation/deamidation modifications at the peptide level. In another workflow the glycosylation patterns of mAbs were characterized by a 3D-LC/MS (Protein-A x Reduction-RPLC x HILIC/MS) method at the reduced level.

In summary, the present work illustrates how the use of multi-dimensional LC-MS workflows can be effectively implemented for the characterization of mAb variants by combining the analysis at the reduced and peptide levels within the same system.

References:

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KL-25

The devils in the detail of our data processing
David Kilgour
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As we try to understand the world, at least from a chemical or biochemical perspective, we need to find ways of being able to reliably detect and measure relatively small changes, in very complex and naturally variable samples. In many cases, the signals we are trying to find are not simple, but are the correlation of combinations of multiple smaller signals, each of which can only be distinguished from the natural variability and noise by statistical means. So, in order to find these signals, it is likely that we will need to rely, ever more heavily, on bioinformatic or artificial intelligence methods, working on huge populations of highly dimensional datasets (e.g. PI-LC-IMS-MS/MS). These data are commonly much larger than could ever be comprehensively reviewed by hand – so we are dependent on the algorithms we employ. But, it is very easy to confound these approaches with accidentally biased or distorted data.

There are many aspects of the solutions to these problems. In this presentation, I will be looking at a key part – how can we make the instruments and the data they produce more useful and accessible? How can we develop more intelligent instruments that are capable of much more efficient and automated self-monitoring and optimization, or even just capable of reporting their own confidence in their output? And, if we have that capability, our current data-formats were not designed to encapsulate this extra information, in order to make it easily accessible to downstream processing algorithms.

We're not going to solve these problems now they are well known. But, this meeting offers a great opportunity to continue this conversation, so the aim of this presentation is, hopefully, to promote these discussions.

KL-26

Data Analytics: the journey from complexity to clarity
Camilla Liscio

Anatune, United Kingdom

In an analytical world which is dealing with constantly increasing sample complexity, separation capabilities have never been more cherished, especially as the triptych of money, time and quality has become a constant driver in data delivery. On the quest from complexity to clarity, availability of additional separation approaches is not only an option, but a necessity. Post-acquisition data processing in combination with chemometrics are certainly a less orthodox separation approach but surely just as effective to separate analyte information from background noise. This talk will discuss some of the latest additions to the data analytics capabilities portfolio and it will put them in perspective showcasing some applications developed in our Applications Development Laboratory.

OC-22

Extracting value from untargeted e-cigarette aerosol analysis: a metabolomics approach

Justin Frosina, <u>Michał Brokl</u>

E-cigarettes populate a diverse product category that continues to evolve rapidly, presenting technical challenges for product assessment. Evaluating the chemical profile of the e-aerosol is an important element in characterising consumer exposure and evaluating toxicological risk. A typical aerosol chemical profile contains tens to many hundreds of detectable features depending on the complexity of the added flavours. Comprehensive assessment of such large numbers of features on a regular basis cannot be achieved practicably. However, if the ingoing chemical ingredients are carefully managed, assessment can focus on the differences between an e-liquid and its resultant aerosol to target detection of chemical changes that occur during the aerosolisation process. By combining a thermal desorption-gas chromatography-time-of-flight mass spectrometry methodology and a metabolomics-based approach applying Genedata software, the changes in chemical profiles can be assessed thereby streamlining data analysis and interpretation. Utilising student's T-test, Wilcoxon rank sum and absent-present statistical tests in combination with fold-change, aerosol features that are significantly increased or newly detected relative to the e-liquid can be readily detected and categorized. This approach typically reduces the number of features of relevance from several hundreds to less than 20. Additionally, referencing the data against in-house libraries and results from previous analyses can further simplify data interpretation and increase the confidence of chemical identity assignment.

OC-23

Non-targeted analysis of organic contaminants in complex environmental matrices- Applications of metabolomics data workflow

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Solid waste (tars) and wastewaters arising from thermal conversion of coal are predominantly constituted of complex mixtures of organic hydrocarbons, such as alkanes, aromatic compounds and phenols. Chemical characterisations of these types of samples have often been limited to a suite of targeted analysis. Historically, this has been due partly to known or suspected toxicity of certain compounds but also to the readiness of available analytical methods. Near exhaustive non-targeted analysis of complex hydrocarbon samples, however, has the potential to increase significantly our understanding of the formation and fate of these types of samples. To extract reliable information from exhaustive approaches, robust methods must be developed for sample extractions, analysis and data treatment. Here we will show how we are applying data workflow that have been used in the field of metabolomics for biomarker discovery to better characterised 1) a hydrocarbon forming process and 2) biological transformations of complex hydrocarbon mixtures.

We will present, first, our investigation of the formation of semi-volatile organic compounds (SVOCs) during underground coal gasification (UCG)1. A method for comprehensive extraction of SVOCs in UCG wastewaters was developed and samples were analysed by GC-MS. An in silico workflow was set up that included chromatograms alignment, peak searching, deconvolution and integration and returned a data table with the integrals for each feature for each sample. Chemometric analyses were carried out using the Metaboanalyst R package to identify compounds that could be used as markers to characterise the gasification process efficiency.

Then, we will introduce our investigation of the microbial ecology and chemical signatures of soils from two different sites contaminated with coal gasification tars2. Exhaustive extractions of SVOCs in the soil samples was carried out by pressurised liquid extraction and the extracts were analysed by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS). GCxGC chromatograms were processed using the Leco proprietary software ChromaTOF © and aligned using the R2DGC R package. DNA extraction methods were developed, and DNA extracts were amplified using 16S rRNA primers and sequenced. The sequencing data was processed through previously developed bioinformatics pipelines. The N-integration algorithm DIABLO was used to classify and discriminate features that correlate between microbiome and GCxGC feature to provide both chemical and biological markers of biodegradation processes. We will discuss the current limitations of the approach.

KL-27

Recent Progress on the Simulation of Second Dimension Separations for 2D-LC, with Application to Biomolecule Separations

Dwight Stoll[1], Gabriel Leme[1], Tyler Brau[1], Thomas Lauer[1], Gregory Staples[2], Sarah Rutan[3]

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Two-dimensional liquid chromatography (2D-LC) separations are continuing to grow in popularity in diverse application areas ranging from small pharmaceutical molecule analysis to lipidomics. Although the addition of a second dimension of separation holds tremendous potential to augment the resolving power of an existing one-dimensional LC separation, realizing this potential is usually dependent on making good decisions during method development. The performance of the second dimension separation is particularly sensitive to development decisions, since it is common to inject volumes of effluent from the first dimension separation into the second dimension column that approach the dead volume of the second column. Under these conditions, choosing the proper parameters can make the difference between a terrible, useless separation, and a great separation.

Over the past several years we have been working to develop a simulation approach that can be used to support method development for 2D-LC by taking into account the extreme conditions commonly encountered in the second dimension in particular (e.g., large injection volumes, significant mismatch between sample solvent and mobile phase, and active modulation techniques). In this presentation we will provide an update of recent progress in this research area. This includes: 1) improvement in the simulation speed and development of a web interface to make the tool more broadly accessible and useful to a wider user group; and 2) improvements in flexibility that enable simulation over a range of injection volumes from 5 to 360 µL, and simulation of Active Solvent Modulation (ASM). We will present results of studies aimed at validating these features by comparison of results from experiments and simulations. Finally, we will demonstrate the use of the current version of the tool to support development of 2D-LC separations of biomolecules, including fat soluble vitamins, peptides, and glycopeptides.

KL-28

Exploiting Comprehensive Two-Dimensional Liquid Chromatography (LC×LC) for the Determination of Bioactive Compounds in Natural Products

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Natural products are rich source of bioactive molecules with pharmacological activity e.g flavonoids, alkaloids, saponins, and so on. Although many studies have been carried out on the chemical characterization of these samples, there is still plenty of interest in the discovery of novel compounds. Conventional one-dimensional liquid chromatography (1D-LC) does usually provide sufficient resolving power for their determination. However, in some cases their complexity overwhelms the separation capability afforded by 1D-LC requiring more powerful analytical technologies for their characterization. Comprehensive two-dimensional LC (LC×LC) involving the coupling of two or more orthogonal or quasi-orthogonal separation systems is an interesting alternative being in many cases also selective and sensitive enough to detect minor components.

For the analysis of natural products, in the last two decades, several LC×LC methodologies e.g. NP×RP, RP×RP and HILIC×RP coupled to photodiode array and mass spectrometry detection have been successfully investigated for tackling such a task. In this contribution, selected applications are discussed with a particular emphasis on the employment of a micro LC pump in the first dimension of the LC×LC systems, allowing high repeatability and stable retention times.

OC-24

Automated flexibility to implement two-dimensional and multiple LC-MS methods in a single instrument setup

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Multiple chromatographic methods for the analysis of a single sample are an increasing demand to solve complex analytical problems and fulfill more efficiently and in a timely manner the requirements.

In particular, samples of increased complexity with high numbers of analytes and structurally closely related substances present an analytical challenge in terms of resolution power, efficiency and selectivity. In order to overcome these problems without extensive method development, the same sample or fraction of the sample needs to be processed with more than one analytical separation. Although it is in general possible to analyze the same sample with two or more independent chromatographic methods, this may require for example the use of several instruments, more sample amount, or offline collection of fractions. This kind of approaches are very difficult to automatize, require more human interaction and as consequence they are more time consuming and prone to errors. Thus, several more sophisticated techniques like parallel LC, serial column coupling, column switching or two-dimensional LC (2D-LC) gain increasing importance.

Those approaches are finding in the last years many different application fields and are not considered anymore "pure research" methods. Some applications examples are related to comprehensive profiling and screening in biological and food areas, or to differentiate contaminants and interfering substances from the compound of interest in the pharmaceutical industry. Herewith, we present a heart-cut 2D-LC method for the separation of two regioisomers, desmedipham and phenmedipham, two herbicides that are difficult to analyze by 1D-LC. In the case presented, a heart-cut 2D-LC with analyte trapping during fraction transfer enabled straightforward peak identity and purity confirmation by HR-MS detection, despite MS incompatible mobile phases in the first dimension separation. The use of a double injection unit made possible to switch from a 2D-LC setup to two independent 1D-LC systems without the need of replumbing. This increases system flexibility and usability, without the need of user interaction. Another example is related to the simultaneous analysis of metabolites and lipids of C. elegans extracts from one single vial. Due to the high sample complexity, specific sample preparations are required to produce a metabolite-enriched sample and a lipid-enriched one. Those are, thereafter, generally analyzed by different chromatographic methods to cover the complete range of polarity. Herewith, we developed a single analysis based on a LC with a double injection unit and a second independent flow path. Furthermore, in-vial dual extraction was used. Metabolites from the lower phase were analyzed using a tandem-HILIC-RP setup, while lipids from the upper phase were injected into the second flow path. Mass spectrometric analysis was performed using a HR-MS in positive ionization mode.

OC-25

Approaches towards multidimensional LC within the biopharmaceutical industry

Isabelle François

This contribution discusses the various approaches to multidimensional LC which can be used within the biopharmaceutical industry. Heartcut as well as comprehensive 2DLC both represent significant advantages to an analytical scientist. The applied technique should be carefully selected based on the need of the analysis: complete elucidation of highly complex samples or identifying only relevant information on specific parts of the sample. Both approaches will be discussed together with benefits and pitfalls.

TU-10

Basic principles of analytical method validation

Erwin Adams

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Analytical measurements are performed in nearly all scientific disciplines. However, the science of measurement is a language that has to be learned. In order to obtain reliable, consistent and accurate results, analytical methods need to be validated so that they meet the requirements for their intended purpose. Moreover, validation data should not only be reported, but also statistically interpreted.

The objective of this tutorial is to provide an overview of the basic concepts and tools for implementing method validation.

First an introduction will be given discussing the different aspects of method validation as well as the general framework. This will be followed by a short overview of the regulatory guidelines that have been issued. Since there are many misconceptions, participants will be familiarized with the typical terminology related to method validation. Different terms will be explained and discussed like for example specificity, selectivity, repeatability, reproducibility, accuracy, linearity, sensitivity and robustness. Special attention will be paid to the interpretation of the calibration data. Indeed, although mostly a linear relationship between the measured signal and the concentration or amount of analyte is assumed, this is not always the case. Besides the well known and frequently used determination coefficient, alternative options to evaluate the calibration curve will be offered (lack-of-fit test, residual plot, etc.). All these aspects will be illustrated with some examples from the pharmaceutical area.

YES-23

Characterization of the Functionality-type × Molecular Weight Distribution of Complex Polyesters Using NPLC×SEC

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Polyesters are important polymers used in various applications and industries. The most known use of polyesters is for the production of food packaging, mainly PET bottles. Furthermore, polyester fibers are used as reinforcements with high-energy absorbing properties in safety belts, fabrics for conveyor belts and coated fabrics.

Polyesters are formed by the esterification of a diol and dicarboxylic acid. Hence, the formed polymers can vary in molecular weight and end-group functionality including di-OH, di-COOH and OH-COOH. Furthermore, branching of the polyester can be introduced by incorporating triols in the polymer backbone, resulting in distributions in topology (linear vs. branched polyesters).

Understanding the relation between chemical characteristics and the above described chemical distributions of synthetic polymers is one of the challenges faced by analytical chemistry in industry. This is a complex task as polymers are not synthesized as single molecules but are populations of chemically similar compounds that distribute over several properties. In order to deconvolute the multidimensional distribution of polyesters, we have developed an online comprehensive two-dimensional liquid chromatography method (LC×LC) coupling a polymer functionality-based separation (NPLC) with a method describing the molecular weight distributions of polyesters (SEC). Furthermore, NPLC×SEC will be hyphenated to chemical informative detector(s) to obtain information on the topology of the polyesters.

YES-24

Analysis of base oil mixtures with automated machine learning

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Aims/Introduction: Automotive lubricant development is increasingly focussed on better fuel economy and lower carbon emissions. This influences the base oil choices that product formulators have to make and has changed the base oil landscape, with new processes, new base oil types and complex mixtures observed in many products. This complexity is passed on to the analytical scientist when detailed understanding of lubricant composition is required. Digital technologies and the potential advantages they bring can help extract insight from complex data and ultimately give market competitors an edge if leveraged correctly. The topic of this presentation will be how digital technologies have helped us look at problems in the base oil market focusing on mixtures of base oils.

Experimental and Results: High-resolution field ionisation (FI) mass spectrometry data was collected for a series of mixtures of base oils from different American petroleum institute groups. FI effectively ionises non-polar analytes without significant fragmentation and provides a hydrocarbon profile of these samples. The sample-set was pre-processed within Python, where the molecular formula for each component was assigned within a ppm limit. The data were analysed further within a Python workspace using libraries such as Pandas, Scikit-learn and Plotly for downstream analysis and visualisation.

The base oil data was collated into a dataset, where it could be easily filtered, sorted and manipulated. From this, trends were identified by applying different statistical and numerical techniques. These were most effectively visualised using both static and interactive plots, which highlighted chemical changes and correlations that could be exploited by machine learning.

A variety of machine learning models were applied to the data-set using proper validation procedures. Ultimately, the best models were obtained through the application of automated machine learning. A genetic algorithm was employed to determine the best pre-processing, feature engineering and supervised models to use for this data-set, giving the strongest predicting models

Conclusions: These tools leverage the complexity of data obtained through mass spectrometry to allow the extraction of chemical information and to identify trends in the data that can be exploited by machine learning. In summary, mass spectrometry combined with machine learning has enabled BP to predict the ratios of base oil mixtures, giving them an edge in this competitive market.

YES-25

Intelligent invertebrate toxicology (iNVERTOX): Linking metabolomics to behavioural changes in a freshwater invertebrate.

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The (pseudo)persistence of emerging contaminants and other organic micropollutants in the environment represents a risk for wildlife that are exposed to them. 'Omic technologies, such as metabolomics, are providing a powerful tool within environmental toxicology to better understand the effects of these exposure scenarios. However, application of metabolomics in environmental toxicology is currently very limited and the subsequent interpretation of metabolite data for the understanding of toxicological responses is challenging, not least due to the scale and complexity of the data generated. Furthermore, behavioural data are currently generating debate for the use in risk assessment as a sub-lethal indicator of effect. Thus, we characterised metabolomic changes in a freshwater invertebrate, G. pulex, upon exposure to several psychoactive drugs. Behaviour was monitored across a 7-day exposure period to environmentally relevant concentrations of selected drugs. Animals were sampled across the exposure phase to determine both internalised drug concentrations and altered endogenous metabolites to establish links between cause and effect relationships. An optimised pre-processing method using XCMS in R was used for peak picking across non-target MS data for the metabolomics workflow. The results indicated that both endogenous metabolites and activity of the animals were altered depending on psychoactive drug treatment. Additionally, endogenous metabolites were shown to be affected by laboratory acclimatisation, moulting cycle and biological sex of the animals. Behaviour was affected during the first 24-hours of the experiment suggesting that handling of animals can cause significant effects for subsequent behavioural measurements. Internal concentrations of drugs reached steady-state very quickly and experimentally estimated bioconcentration data suggested that these organisms are capable of rapid turnover of these drugs. Overall, the characterisation of metabolic variance for invertebrates along with the use of metabolomics shows a very powerful approach for understanding adverse effects that may be associated with environmental contaminants.

YES-26

Monolithic molecularly imprinted polymer and nano-liquid chromatography for on-line miniaturized trace analysis in biological fluids

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Evolution of the instrumentation in terms of separation and detection allowed a real improvement of analysis sensitivity. However, trace analysis in biological fluids requires in many cases a step of purification before the chromatographic separation. Solid phase extraction (SPE) is a powerful method to achieve this purpose. However, the majority of the available sorbents mainly promotes the development of hydrophobic interactions during the extraction procedure that leads to the co-extraction of a huge amount of interfering compounds. To overcome this lack of selectivity, sorbents based on molecular recognition mechanism are necessary for selective extraction of a target molecule and its structural analogs. Molecularly imprinted polymers (MIPs), which synthesis leads to the formation of specific cavities mimicking the recognition site of the antibodies, constitute powerful tools to improve the selectivity of the extraction procedure. Their miniaturization is particularly attractive from a general point of view, and even more when templates or monomers are expensive or difficult to synthesize. The aim of this work was therefore to develop a miniaturized analytical procedure with the on-line coupling of a monolithic MIP, in situ synthesized into a 100 µm internal diameter fused-silica capillary, to nano-liquid chromatography (nanoLC).

A first study was conducted on a monolithic MIP using cocaine as template after the screening of different synthesis conditions. The homogeneous morphology of the MIP was investigated by scanning electron microscopy and its permeability was measured. The selectivity of the MIP was evaluated by confronting it with a non-imprinted polymer, synthesized under the same conditions but in the absence of template, allowing the determination of imprinting factors for cocaine (3.2 ± 0.5) and its main metabolite, benzoylecgonine (2.2 ± 0.3), on polymers resulting from independent syntheses, showing the high selectivity and repeatability of the synthesis. After optimizing the extraction protocol, the monolithic MIP was successfully on-line coupled with nanoLC-UV for the direct extraction and analysis of cocaine present in spiked human plasma and saliva samples. The developed procedure provided a low detection limit and reduced the impact of the interferences from these complex biological samples. Moreover, the repeatability of the extraction recovery, between 85.4-98.7%, was high with RSD values lower than 5.8% for independently synthesized MIPs. Then a transfer of know-how was carried out and the same experimental protocol was implemented for the study of an ephedrine imprinted monolith for the simultaneous extraction of ephedrine and dopamine.

PL-04

Synthesis and functionalisation of nanostructured porous polymer materials for analytical applications

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The majority of materials for chromatographic applications are silica-based, due largely to the excellent mechanical properties and well established protocols for synthesis and chemical modification. However, there remains a number of chromatographic modes and applications where polymer-based materials offer superior performance. This presentation will focus primarily on polymer-based monolithic materials for which the relatively simple preparation, robustness, high permeability to flow, mass transfer via convection and flexible chemistry has since seen these materials used in a range of applications such as chromatography and as supports for synthesis, catalysis and immobilized enzymes. A key factor in improving the performance of polymer monoliths, in particular for analytical applications is the degree of bed heterogeneity. This presentation will introduce a number of approaches we have recently demonstrated to form ordered porous polymers with improved bed heterogeneity, suitable for analytical applications including chromatography, sample preparation and microsampling. Polymerization at sub-zero temperatures (cryopolymerisation) with and without unidirectional freezing is shown to produce highly ordered monolithic structures with porous properties that can be easily tuned according to the freezing/polymerization conditions. The incorporation of nanoparticles (neutral or charged polymer, inorganic, metal) during the polymerization process also leads to significantly improved structural homogeneity, reflected in chromatographic separation performance. Examples of new interconnected polymerized high internal phase emulsions (polyHIPEs) and medium internal phase emulsions (polyMIPEs) will also be presented. A range of different emulsion stabilizers including nanoparticles, novel amphiphilic block co-polymers and surfactants will be demonstrated, resulting in control of both the morphology and surface chemistry. The use of controlled radical polymerization will also be demonstrated as a technique to produce new polymer materials for high performance separation of proteins and peptides.

Monolithic micro-sampling devices prepared within disposable pipette tips will be described, suitable for in-tip separation of blood cells from plasma and as immobilized enzyme reactors (IMER) for protein digestion. New, high surface area polymeric monolithic sorbents with controlled mesoporosity tailored for extraction of small and medium sized molecules will also be introduced including application to bioanalysis and selective extraction of perfluorinated compounds in environmental samples. Finally, novel silica coated fibre technology will be introduced as a less invasive method suitable for in vivo analysis of drugs and metabolites.

PL-05

Reflections on half a century of research in capillary gas chromatography

Pat Joseph Sandra

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When a "senior" scientist (" " in the social demographic meaning) is invited to present a plenary lecture at an international meeting, it is or a courtesy request or an invitation to give a review-type lecture related to the topics of the meeting. I do hope it's the latter. In my scientific career spanning from 1969 to 2019, capillary gas chromatography (CGC) and its pre- and post-column hyphenation were keywords. I started my Ph.D. studies in 1969 (year of the student revolt) and the subject of my research was very Belgian: "The contribution of hops to the flavour of beer". Hops are directly responsible for the bitterness of beer but there was an on-going discussion in how far the aroma of hops (dominated by high concentrations of apolar terpenes) was contributing, as such or modified, to the flavour beer. It was immediately clear that the complexity of the beer flavour required high resolution techniques explaining our move to CGC.

The fundamentals of CGC were well-described by its inventor Marcel Golay, but practical capillary GC for analysis of mixtures like essential oils stood in his infancy and important CGC tools had to be made in-house starting with glass capillary columns for inertness reasons. In 1979, Dandeneau and Zerenner introduced fused silica columns opening the technique to routine application. In the following years, dedicated stationary phases were synthesized and immobilized in the column. Today we have an array of columns available to solve our separation problems in a one-dimensional (1D) or two-dimensional (2D) set-up and we don't realize anymore how challenging, but also rewarding, that period was!

If the column is considered the heart of the CGC system, injection is its Achilles heel. Numerous injection systems have been developed over the years, all with pros and cons that we have to know for successful implementation in our analytical schemes. Unfortunately, in the past decades inlet characteristics and performances are often not provided in publications although this is key information to understand the figures of merit of a method! More recently inlet systems are incorporated as part of a robotic system for on-line sample preparation. In the era of hyphenation and automation, it is difficult to understand why nowadays sample processing is often still done manually (also in official methods!).

In post-column hyphenation, the early successful combination of CGC with selected detectors and especially with mass spectrometry has opened the way to endless combinations of different detection systems. A short overview of the most performant systems, both in R&D and in routine analyses, will be presented. The performance of the combination capillary GC-MS/FTIR, not yet very popular, will be highlighted.

DATA-01

Reinforcement Learning for the Optimization of Scouting Runs and Retention Modeling in Liquid Chromatography

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Optimizing chromatographic conditions to obtain a good separation for analytes in complex samples is one of the great challenges in chromatography. An adequate separation of such mixtures is crucial for everything from detecting and identify impurities and degradation products in drugs, to detecting contaminants in wastewater. The main aim of this project is to develop decision-algorithms (e.g. reinforcement learning algorithms) to automate the decisions that have to be made to obtain an adequate separation for a complex mixture. Therefore, in this study, Markov Decision Processes (MDPs) will be introduced to optimize scouting runs that have to be made prior to building retention models for further method development.

Specifically, the percentage of modifier will be studied as the chromatographic parameter to be altered to obtain adequate separations. Modeling the retention factor k as a function of the percentage of modifier provides the ability to predict k's of the compounds in the studied sample for all different percentages of modifier, for both isocratic and gradient runs. By optimizing the scouting runs with an MDP to obtain these models, the number of required experiments will be reduced significantly. The MDP will be trained for nearly one hundred representative small molecules under a large set of isocratic and gradient conditions. From this, the MDP will learn to decide the next step to take after starting with just one single isocratic scouting run, with a positive response/reward if the observations result in a good predictive model (measured as a mean-squared distance between predicted and experimental k). In future experiments, this MDP will be applied to unknown samples and will continue to learn based on the experimental outcome following the decisions made by the MDP.

DATA-02

KairosMS: A new tool for the processing of hyphenated ultrahigh resolution mass spectrometry data

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The coupling together of ultrahigh resolution mass spectrometry with separation methods can provide further insights into complex mixtures such as petroleum, dissolved organic matter (DOM) and bio oils; however, challenges exist in processing the resultant large datasets and the accurate identification of extracted ion chromatograms (EICs) within the data. Current workflows typically involve segmenting the total ion chromatogram (TIC) into a series of time slices and then for each: producing a mass spectrum, assigning elemental compositions, and then visualising the results. This process is laborious and can incur a loss of time resolution. KairosMS is presented as a tool that addresses many of the current issues.

KairosMS accepts data exported from instrument vendors' software, processes the data and then produces a peak list that can be used in third-party software to produce elemental assignments. Assignments are then imported back into KairosMS, which merges the processed data and assignments to create a data matrix which is then used for various interactive visualisations. Examples of plot types include, but are not limited to: heteroatom class distributions, van Krevelen plots, and double bond equivalents (DBE) vs. carbon number plots. Additionally TICs, mass spectra (as a function of time) and EICs can be visualised and compared, allowing the observation and matching of isomers and the tracking of species with respect to time.

To date, KairosMS has been tested with gas chromatography (GC), liquid chromatography (LC) and trapped ion mobility spectrometry (TIMS) data, from both Fourier transform ion cyclotron resonance (FTICR) and Orbitrap mass spectrometers. Sample types covered so far include: petroleum, bio-oils, DOM, peptides, and environmental samples. The use of open data formats means KairosMS is not limited to any particular hyphenated technique or instrument vendor.

To conclude the new workflow afforded by KairosMS significantly improves the speed of analysis of complex mixture data by hyphenated ultrahigh resolution mass spectrometry. Analysis that previously took multiple hours or days now can be performed within minutes. By removing the bottleneck that previously existed, researchers are now able perform greater in-depth analyses of complex mixture data. KairosMS is also usable with direct infusion mass spectrometry data, creating a single, expandable, scalable platform for complex mixture analysis and visualisation. Development is ongoing with the aim of covering a wider range of sample types, visualisations, and statistical analyses.

DATA-03

Mass spectrometry with operation at constant ultrahigh resolution (OCULAR): advances for the analysis of complex mixtures

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Complex mixtures such as crude oil and their fractions can contain hundreds of thousands of individual molecular compositions, and therefore ultrahigh resolution mass spectrometry is the only technique currently able to provide a detailed analysis of these samples. For instance, a mass spectrometer with a resolution of 400,000 at m/z 400 is typically used to ensure resolution of a mass difference of 0.00337 Da corresponding to species with C3 vs SH4. This allows the assignment of sulfur-containing compounds that can poison catalysts, act as atmospheric pollutants, and prove to be corrosive at elevated temperatures. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) affords the highest performance and is therefore ideal for the analysis of the most complex mixtures. Some limitations need to be overcome however, when complex samples are analysed. For instance, for a given m/z range, the resolving power is inversely proportional to the m/z. Unfortunately, it is at higher m/z that it is possible to have a greater number of compositions per nominal mass, and so this is the region where ultrahigh resolving power is most needed. FT-ICR instruments must also deal with a balance of the total number of ions trapped in the cell. A stable ion cloud can be formed with approximately 100 ion per m/z and a maximum of a few million ions can be detected in a single experiment. Thus, FT-ICR mass spectrometers can detect a maximum of tens of thousands of peaks within a single experiment, limiting the dynamic range.

In this work, we present a new method, known as OCULAR (operation at constant ultrahigh resolution), to overcome the traditional limitations of resolving power and dynamic range in FT-ICR instruments. The method consists of a modified stitching method which uses increasing time domain data length with increasing m/z, incorporates the development of software that determines the best position for overlapping the many segments, corrects the relative abundances of the ions in the segments, and then automatically stitches the segments together. OCULAR was used to analyse the heaviest fraction of crude oil analysed to date: the truly non-distillable fraction of a crude oil. With OCULAR, segments were acquired with near constant ultrahigh resolving power of an order of millions (typically >3,000,000 FWHM) across a broad m/z range (m/z 260–1500). This has enabled the resolution and assignment with ppb mass accuracy of 244,779 individual molecular compositions, some of which differ in mass defect by less than the mass of an electron. The compositions spanned dozens of heteroatomic compositions, contained up to 114 carbon atoms, and up to 51 double bond equivalents (DBE). The high number of species with heteroatomic compositions helped to explain the

extremely low volatility of the sample and highlight the enormous challenges that will need to be addressed to upgrade heavier crude oils.

FOOD-01

Profiling of phenolic compounds using UPLC–MS for determining the geographical origin of green coffee beans from Ethiopia

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A total of 100 samples of green coffee (Coffea arabica L.) beans from the major producing regions of Ethiopia were studied using ultra performance liquid chromatography-mass spectroscopy to determine if the phenolic content could be linked to their geographical origins for authentication purposes. Principal component analysis allowed the most discriminating compounds to be identified. Based on their concentrations, 3-O-caffeoylquinic and 4,5-O-dicaffeoylquinic acids were found to be characteristic markers for Northwest and East (Harar) region coffees, respectively. Sub-regional coffee types from West, except Jimma B, could be distinguished by their 3,5-O-dicaffeoylquinic to 4,5-O-dicaffeoylquinic acid concentration ratios, while Yirgachefe coffees from South could be distinguished by their 4,5-O-dicaffeoylquinic to 3,4-O-dicaffeoylquinic acid concentration ratios. Linear discriminant analysis provided a classification model with recognition and prediction abilities of 91% and 90%, respectively, at regional level, and 89% and 86%, respectively, at subregional level. This is important for the detection of fraud, including the selling of inferior Ethiopian coffees under the label of the more expensive Harar coffees.

FOOD-02

The importance of sufficient chromatographic separation for identity confirmation

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Naproxen belongs to the group of non-steroid anti-inflammatory drugs (NSAIDs) and is not registered for use as veterinary drug within the EU. As part of the Netherlands Residue Monitoring Program, the presence of residues of NSAIDs in samples milk and meat from various animal species is monitored. Suspect samples will be confirmed using the criteria as described in Commission Decision (CD) 2002/657/EC. Two criteria were established to confirm the identity of residues measured with LC-MS/MS. The first criterion describes a maximum deviation of the ion ratio of two fragment ions between a suspect sample and reference standard. The second criterion describes a maximum deviation of the retention time. Both criteria were applied to samples that were found suspect for residues naproxen. The ion ratio and the retention time met the criteria described in CD 2002/657/EC. However a shift in ion ratio was observed when the suspected samples were enriched with naproxen for quantification using the principle of standard addition. In addition an experiment using chiral chromatographic separations was applied using a LUX i-Amylose column instead of reversed phase C18 chromatography. Results showed that the suspect samples did not contain naproxen but an unknown compound with the same mass and fragment ions. This shows that besides meeting up with identifying criteria as described in CD 2002/657/EC, sufficient chromatographic separation is very important. Especially since nowadays, new LC methods become more general for multi-class applications and become faster and faster. The presented example shows that a critical view of identification criteria is required.

FOOD-03

Multivariate Calibration of Chromatographic Fingerprints to Predict Antioxidant Potential in Argan kernels: a Metabolomic Approach

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Argan oil, extracted directly from Argan kernels (Argania spinosa L.), is used for centuries in southwestern Morocco in traditional diets, cosmetology and dermatology.

An untargeted metabolomic approach combining UPLC-MS fingerprints with chemometric tools to model and predict the antioxidant potential, and to indicate the responsible components in Argan fruit kernels, is presented. Argan kernels polyphenols-containing extracts have a pronounced antioxidant activity. In the present study, the antioxidant capacity of 120 samples from five Moroccan Argan forests (Ait-Baha, Agadir, Essaouira, Tiznit and Taroudant) was evaluated using a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay. The polyphenolic extracts were also subjected to untargeted UPLC-MS analysis, and the metabolite profiles acquired. Metabolomics-related data analysis, such as multivariate curve resolution alternating least squares (MCR-ALS), has been investigated on various types of LC-MS profiles. These approaches were compared on the extraction of relevant metabolites and then were investigated in the modelling of the antioxidant potential. The relationship between the polyphenolic fingerprints and the in-vitro antioxidant activity was investigated by Partial least squares regression (PLS) and Orthogonal projections to latent structures (O-PLS) modelling. The models were compared, and their abilities discussed.

Both strategies, coupling MCR-ALS on LC-MS with multivariate calibration (PLS or O-PLS) to relate chromatographic fingerprints and pharmacological activity, provided good results. Advantages and drawbacks of the two metabolomics strategies are also debated. A tentative identification of the potential biomarkers (metabolites) responsible for the antioxidant potential was assessed as well.

The work demonstrates that the fingerprint analysis based on metabolomic data handling could be applied for the quality control evaluation of Moroccan Argan kernels and its extracted oil, and that the constructed model is appropriate for depicting the fingerprint–activity relationship.

FOOD-04

Analytical workflow for untargeted analysis: sampling, separation, detection, and data analysis methods to unravel aroma complexity

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Headspace gas chromatography has been frequently used for aroma profiling because of its ability to naturally exploit the volatility of aroma compounds, and also to provide information on the composition of the sample [1]. Its main practical advantages are its simplicity, no use of solvent, amenability to automation, and the cleanliness of the extract.

In the present contribution, the most effective sampling (dynamic sampling), separation (multidimensional gas chromatography) and detection (mass spectrometry) techniques are combined, showing their potential in unravelling aroma profiles in beverages.

In addition, a neat workflow for data analysis is discussed and used for the successful characterization and identification of different beer flavors, if the steps in the analytical process are properly controlled. From the technological viewpoint, this is the first time that a purge-and-trap (P&T), comprehensive 2D gas chromatography (GC×GC), and mass spectrometry (MS) are exploited in combination. A newly-thought flow modulation approach allowed for multidimensional 2D gas chromatography, with the full eluate transfer onto the second dimension and the MS detector with no need to divert the flow, making the overall method highly sensitive and selective [2-3].

Acknowledgments

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FOOD-05

Radiokitchen: Use of the 14C-radiodetector UPLC/HR-MS/MS approach to investigate the fate of pesticides during food processing

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Analysing the fate and metabolism of ingredients or environmental and technical contaminations in complex matrices, such as food, is a major challenge in food technology. Modern food processing aims to combine more and novel ingredients and to apply combinations of physical processing factors. By using radioactively labelled compounds their remains, e.g. metabolites and fragments are identified and monitored along processing chains. Pesticide residues are of major public concern, this study focuses on the fate of pesticides during food processing. Thereby it is possible to reveal potential hazardous compounds that have not yet been identified with common techniques and to optimize food processing strategies to yield safer food.

First results of this project indicate that current guidelines to elucidate the fate of pesticides (e.g. OECD 507) do by far not represent all chemical reactions in food processing. Additional degradation products were observed e.g. of the imidazole fungicide Prochloraz when heated in the presence of rapeseed oil. Heating radiolabelled [imidazolyl-2-14C]-Prochloraz at temperatures up to 240 °C in closed vessels for 45 minutes leads to an extensive degree of degradation of the active substance by more than 70 %. Using radio-UPLC methods coupled with high-resolution mass spectrometry, in total eleven degradation products were found. Several of the degradation products cannot be formed by simple bond breakage but were shown to be formed by chemical reactions of the active substance with matrix components (e.g. fatty acid moieties).

These observations demonstrate the limitations of the OECD guideline in which such complex matrix reactions cannot be discovered. These first results show that further investigations on the fate of chemicals in food processing have to be conducted and that realistic food processing steps need to be considered for pesticide regulation. Thereby it is possible to further elucidate and finally assess potential hazards caused by unknown process metabolites.

FOOD-06

Comparative study of various sorbents for determination of ochratoxin A and ochratoxin B in archive Tokaj wines using on-line SPE-HPLC

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Various sorbents for on-line extraction and determination of ochratoxin A (OTA) and ochratoxin B (OTB) in archive Tokaj wine have been compared. Tokaj wine is a special type of sweet wine from grapes infected with mould Botrytis cinerea produced in Hungarian Tokaj wine region and in Slovakia. Our method involved on-line extraction using precolumn coupled to liquid chromatography with fluorescence detection and enabled fast and sensitive control of mycotoxin contamination. Four types of fibrous sorbets including polyethylene microfibers, polypropylene microfibers, polycaprolactone microfibers/nanofibers composite, and polycaprolactone microfibers/polyvinylidene difluoride nanofibers composite, typical fused-core C18 sorbent, and commercial molecularly imprinted polymers were compared while varying extraction approaches. The polymer fibers filled in a cartridge were directly connected to HPLC system and the clean-up efficiency and the subsequent chromatography separation optimized. Typically, 50 μL wine was directly loaded and preconcentrated in extraction column. The separation was then carried out using analytical column Kinetex Phenyl-Hexyl (100 × 4.6 mm, particle size 2.6 μm) followed by fluorescence detection (Ex 335 nm, Em 463 nm). Solvents suitable for extraction and separation were methanol or acetonitrile and 0.5% aqueous acetic acid. The separations were carried out in the gradient elution mode at a flow rate of 1.0 mL/min. These conditions provided reliable validation results with a limit of detection of 0.03 – 0.06 μg/L and recoveries exceeding 90% were determined for both OTA and OTB in archive Tokaj wines. The maximum tolerable limit for OTA in wines authorized by the European Union is 2 μg/L. Among the tested nanofibers, polyethylene enabled the best results while other nanofibrous materials are unsuitable for the analysis of ochratoxins. Comparable results were obtained using molecularly imprinted polymers, fused-core C18, and polyethylene microfibers. However, the last sorbent excels in the affordability. A more detailed comparison of sorbents will be presented.

FOOD-07

Pushing the boundaries of hyphenation: Comprehensive aroma profiling of food and beverages

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Aroma profiles of food and beverages are composed of a broad range of chemical classes, including terpenes, phenolics, fatty acids, esters, lactones, aldehydes, as well as nitrogen- and sulfur-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

In this study, we will demonstrate the use of trap-based secondary focusing to extend the performance of conventional sampling techniques (e.g. headspace and SPME) while retaining fully automated methods. Using real world examples, we will demonstrate unique, high-performance workflows including SPME-trap with enrichment and high-capacity sorptive extraction which allow significant improvements in profiling applications

Nevertheless, the aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. The enhanced separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) is now frequently used to tackle this challenge.

Here, we apply a multi-hyphenated analytical system to obtain comprehensive aroma profiles. The use of parallel detection by three different techniques ensures that three complementary datasets are obtained from a single run:

Robust quantitation of high-loading species by flame ionisation detection (FID)

Highly-sensitive, confident identification of aroma-active species by time-of-flight mass spectrometry (TOF MS)

Highly specific detection of sulfur odour taints by sulfur chemiluminescence detection (SCD)

We will show that the result of using this multi-functional setup is confident aroma profiling and off-odour detection, with fully automated workflows and simple data processing.

FOOD-08

Volatile profile of white wine with an easy-to-use GC×GC diverting flow modulator: towards the routine evaluation of the winemaking process

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The volatile composition of Pinot Blanc wines from South Tyrol (Italy) was investigated using a mono-dimensional GC and a two-dimensional GC system equipped with an easy-to-use diverting flow modulator (only injection time and modulation period must be set). Pinot Blanc wines were produced using two different winemaking protocols and were characterized after nine months of storage in bottles. To extract volatile compounds from wine a headspace solid phase micro-extraction (HS-SPME) using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was applied in manual and automatic injection modes.

Different combinations of stationary phases (high, mid and low polarity) were tested in instruments coupled with a quadrupole MS or a TOF MS.

The results were compared in terms of reproducibility and signal-to-noise to establish guidelines for an improved characterization of the volatile composition of white wines intended for a routine analytical application. The profile of volatile compounds allowed the discrimination of the two different vinifications used for Pinot Blanc wines.

FOOD-09

Rapid Separation of trans/cis Fatty Acid Methyl Esters with Agilent DB-FastFAME GC column

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The GC analysis of fatty acids as their methyl esters derivatives (FAMEs) is an important tool in the characterization of fats in the determination of total fat and trans-fat content in foods. Traditionally, the detailed separation of complex FAMEs requires the use of a long (100 meters or more) capillary column coated with a high polarity cyanopropyl stationary phase to differentiate between the multiple FAME isomers. However, some of the carbon chain lengths usually overlap on the high polarity phase, causing problems in peak identification. Therefore, long analysis time (more than 70 minutes) is required to achieve good FAME separations.

The Agilent J&W DB-FastFAME GC column was specifically engineered for the fast separation of FAME mixtures, including some key cis-trans separations. This application shows that the 90m DB-FastFAME GC column can effectively separate 57-component FAME mixture including 37 representative FAMEs and some representative trans FAMEs within 40 minutes, and most of cis-trans isomers can be baselined separated. Analysis of 63-component FAME mixture on DB-FastFAME GC column also demonstrates that DB-FastFAME capillary GC columns can provide rapid analysis and the necessary selectivity to resolve cis-trans pairs in food samples to ensure the food conforms to label requirements.

FOOD-10

Simple, reliable determination of biogenic amines in wine. Direct analysis of underivatized biogenic amines by LC-ESI-MS

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Bioactive amines may be both essential and harmful to health. When originating from natural metabolic pathway, they are called natural polyamines and play a several biological roles. When they are formed by microbial and enzymatic amino acid decarboxylation, they are called biogenic amines (BA). Their concentration in food depends on presence of microorganisms capable of decarboxylate amino acids. Wines are known also to contain many BA and they can originate from grape or produced during alcoholic fermentation by yeast or malolactic fermentation by bacteria. The main BA found in wine are tyramine, histamine, putrescine and phenylethylamine. Tyramine and histamine are the most effective. They show severe adverse effects on the central nervous and vascular systems. These pharmacological effects depend on individual sensitivity and on simultaneous presence of co-factors (ethanol, drugs, other amines). This study deals with the comparison between two different methods, LC-UV and LC-ESI-MS, for the determination of eleven BA (tryptamine, methylamine, ethylamine, agmatine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine) in wine samples. The wine samples, 12 white wines and 10 red wines, produced in different regions of Italy, are analysed. With the LC-ESI-MS method the most prevalent amines resulted to be histamine in white wines (4.15 mg l-1) and tyramine in red wines (6.75 mg l-1). Putrescine concentrations were much lower than the values normally encountered in wines, with levels slightly higher in red wines (up to 2.47 mg l-1) than in white wines (up to 1.00 mg l-1). Both LC-UV and LC-ESI-MS methods demonstrated to be effective methods for the determination of the 11 BA in wine samples. The LC-UV method resulted to be more versatile and cheaper compared to the LC-ESI-MS method as it allowed, using a less expensive apparatus, the determination of all the 11 amines studied while with the MS method the volatile amines, such as methylamine and ethylamine, were not detectable. The most significant improvement of LC-ESI-MS method is the possibility of avoiding the tedious and time-consuming derivatization step, necessary in the LC-UV, thus decreasing the analysis time, being the MS technique compatible with the detection of raw amines. Besides, no sample clean-up or pre-concentration procedures are required. This fact is of relevance in respect to the green chemistry. Moreover, LC-ESI-MS method contributed to improve significantly the sensitivity with LODs values lower than those obtained with LC-UV and better recoveries. LC-UV detection can be successfully used for preliminary experiments as both volatile and not volatile amines can be easily detected, but the LC-ESI-MS method, which does not require any sample clean-up or pre-treatment, demonstrated to give definitely better results in terms of time of analysis, cost, sensitivity and accuracy.

FOOD-11

Chemical profiling of taste related compounds of various Eastern Scheldt seaweeds

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Seaweed is a promising renewable resource for a wide range of chemicals and a viable source of protein. The demand for vegetable proteins is increasing globally. Contrary to Asia, the consumer acceptance of seaweed as food application in Europe is relatively low, partly due to their distinctive taste i.e. umami. In this study, non-volatile taste-active components, e.g. 21 free amino acids and 12 free nucleotides, were analyzed by means of reversed phase and mixed mode HPLC respectively coupled to fluorescence and UV-Vis detection. Thereafter, their impact on the taste was evaluated by calculating the equivalent umami concentration (EUC). The EUC was used to determine the synergistic effect of the flavor enhancing nucleotides and umami amino acids to assess the umami taste sensation of various seaweed species. Variation in the content of these compounds and subsequently in EUC was found between species. This knowledge contributes to a better understanding of which seaweed species would fit European palatability and shows how chemistry can help in providing sustainable solutions for socially relevant issues.

Keywords: taste enhancing molecules, umami, HPLC-fluorescence, derivatization

FUN-01

**Morphology optimization and assessment of the performance limits of
nanostructured polymer monolithic columns for the analysis of intact proteins**

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Rigid polymer-monolithic stationary phases have emerged as attractive alternative for packed-bed columns, mainly due to ease of synthesis, the availability of wide range of surface chemistries, and also the flexibility of tuning the structure defining kinetic performance limits. By precisely-tuning the macropore and microglobule size on multiple length scales, the chromatographic performance of current state-of-the-art packed columns can be surpassed. To achieve high resolving power within a short analysis time, monolithic entities in the submicron range need to be synthesized, with macropores ranging between 100 – 500 nm (for fast analysis) or 500 nm – 1 μm (for high-efficiency separations).

The existing possibilities and limitations regarding the effect of structural inhomogeneity on chromatographic dispersion of high-permeability monolithic columns will be a central point of discussion of this contribution. Aiming at high-resolution separation of biomolecules, high-porosity poly(styrene-co-divinylbenzene) monolithic materials featuring nano-sized globule entities were developed. The thermodynamic and kinetic properties of the reaction were systematically tuned by varying the porogen ratio, crosslinker density, initiator content, and temperature. Attempts in decreasing the globule and macropore size below a certain threshold led to a point where structural inhomogeneity (A-term) became significant. Optimized polymer monolithic entities yielding separation impedance values <1000 were achieved. High-resolution separations of intact proteins were achieved, considering the impact of the gradient volume on the overall performance of these columns. While minimizing the extra-column contribution to band broadening, application for high-throughput analysis is also demonstrated, showing 5 runs of ballistic separation of 6 proteins in a minute (total cycle time of 12 seconds).

FUN-02

Development of an improved protocol for the measurement of molecular diffusion coefficients of biopharmaceuticals

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A precise knowledge of the molecular diffusion coefficient (Dm) of an analyte of interest is crucial to assess the individual contributions to mass transfer. The Taylor-Aris open capillary method is an absolute method for the accurate measurement of Dm–coefficients. This method is based on measuring the peak broadening of an analyte when flowing through a long capillary under conditions wherein longitudinal diffusion can be ignored [1]. Important prerequisites for the Taylor-Aris method are that the analyte concentration is fully radially equilibrated[2] and the contribution of extra-column effects to the total variance is negligible. Considering a typical HPLC set-up, this necessitates the use of capillaries with an ID of 500 μm and lengths of 10–20 m. Such long capillaries can only be handled in a practical way when rolled up into a coil, therefore limits must be set to the maximum velocity that can be applied to avoid recirculation of the fluid in the coil. This results in Dm–coefficients that are typically obtained in analysis times of 30–40 min for small molecules[3]. For large biomolecules, this is 10 times longer, since the flow rate will have to be reduced further to avoid recirculation in the coil. There is hence a clear need to develop the Taylor-Aris method further, in order to speed up the determination of molecular diffusion coefficients of large molecules, such as biopharmaceuticals. For this purpose, an interactive tool is presented that allows to assess the influence of different tubing set-ups and different instrumental set-ups on the accuracy of the resulting Dm value. The interactive tool indicates if for an estimated Dm value and a specific set-up, all above conditions are fulfilled. It also gives suggestions on how the design and operating parameters should be modified if the above conditions are not met. The main focus of the interactive tool is on biopharmaceuticals such as insulin, bradykinin, lysozyme and monoclonal antibodies. To validate the interactive tool, the Dm values for this group of molecules are measured on different tubing IDs and lengths, using different injection volumes and flow cells, for a range of flow rates and different mobile phase compositions and subsequently checked with the predictions made by the tool. All the above investigations are done with the goal to find the conditions that allow to measure Dm values of biopharmaceuticals in the most accurate way and in the shortest possible time.

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FUN-03

Determination of the Diffusion Coefficients in SFC for a Wide Variety of Samples and Conditions

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Over the past decade, the introduction of reliable instrumentation has led to a resurgence of the interest in supercritical fluid chromatography (SFC). Whereas theoretically several solvents could be used as a supercritical fluid, the non-toxicity and non-flammability, and the rather mild conditions to reach a supercritical state, have made sCO_2 the mobile phase of choice for SFC. In order to apply the technique to a

wide range of samples over a large enough range of molecular weight and polarity, the sCO_2 is mixed with a co-solvent, often MeOH, in volume fraction from 3-5% up to 30-40% in gradient elution. More recently, separations taking up the entire window from pure CO_2 up to pure MeOH have been performed for the simultaneous analysis of hydrophilic and lipophilic substances [1]. Whereas a vast amount of studies have been performed to investigate the properties of pure supercritical CO_2, only scarce data is available of the typically employed mixtures of CO_2-MeOH in SFC. For one of the most important parameters in any separation process, i.e. the diffusion coefficient Dmol, only few compounds, often not relevant for current applications in SFC, have been investigated. Dmol is not only the underlying parameter that drives longitudinal diffusion (B-term) and improves mass-transfer (C-term) in packed bed SFC, but is only an important factor in countering peak dispersion due to packing inhomogeneities (A-term). However, before these contributions can be investigated, it is essential that accurate values for Dmol can be obtained. Whereas several methods exist to measure diffusion coefficients, the use of supercritical fluids put an extra constraint, i.e. high pressure operating, on the experimental system. Luckily, one of the most use methods, i.e. the Taylor-Aris dispersion method, can readily be implemented on modern SFC instrumentation. In this methodology, the dispersion in a well defined piece of open tubular capillary is measured and related to the theoretical expression derived by Taylor-Aris for a straight capillary to derive the diffusion coefficient. In practice, the required tubing length to obtain sufficiently accurately measurable dispersion and to avoid transient effects, is however too large (several meter) for practical implementation. When using coiled tubing, care has to be taken to avoid secondary flow effects that can affect radial dispersion and thus the apparent diffusion coefficient. Finally, the entire set-up needs to be temperature controlled and a method needs to be developed to ensure measurement of the diffusion coefficient in the desired mobile phase and not in the initial sample plug. The present contribution will present novel designed experimental procedure to measure diffusion coefficient in SFC. Dmol-values and the effects of solvent composition, temperature and pressure will be presented for a large range of neutral pharmaceutical molecules.

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FUN-04

An experimental procedure for the in-depth evaluation of band broadening phenomena in capillary and nano-size columns

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With the development of Nano LC-ESI-MS for bioanalytical applications, nano LC has gained much attention in the last few years [1]. Despite the increasing interest, only a few studies have focused on the evaluation of the kinetic performance of nano LC. In this study, an in-depth evaluation of band broadening phenomena was carried out in capillary and nano-size columns. Plate heights were measured for a number of nucleobases and nucleosides with phase retention factors ranging between $k = 2$ and 10 at different velocities to construct plate height curves. An accurate assessment of the effective and intraparticle diffusion coefficients was made via peak parking experiments. The external porosity was measured through inverse size exclusion chromatography. It was demonstrated that the procedures used to study band broadening phenomena in conventional columns, are also perfectly applicable to capillary and nano-size columns. These procedures were subsequently applied to HILIC monolithic columns prepared by different monomers and different crosslinkers to investigate whether a more in-depth understanding of the mass transfer phenomena underlying their observed band broadening could help direct the production process of these columns in the future.

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FUN-05

Design and evaluation of flow distributors for radially elongated hexagonal pillar arrays column using computational fluid dynamics modelling

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Pillar array columns (PACs) have been introduced to suggest a more uniform structure as a new support in liquid chromatography on a chip to improve the separation efficiency through decreasing the Eddy diffusion.This support consists of two flow distributors (FD) along with the regular arrays of pillars with identical shape and size. In these columns, flow distributor spreads out the flow from the narrow injecting channel to a much wider beginning channel of PACs. Experimental and theoretical studies proved that the radially elongated hexagonal pillars with the high radial over axial aspect ratio (more than 15) are the best shape in terms of the design and resulting band profiles owing to the high tortuosity [1].

The present study aims at making a broad theoretical survey to design flow distributors that can be used in conjunction with the high aspect ratio radially elongated hexagonal pillar arrays column. Five different flow distributors have been compared as a function of their ability to distribute small sample volumes over the entire width of radially elongated hexagonal pillar array columns. The investigated designs are: bifurcating (BF), radially interconnecting (RI) with different pillars' shape, and mixed mode (MM) distributors. The aspect ratio of each pillar and axial distance between the pillars are in each case equal to 20 and 3 mm respectively. All distributors had the same inlet and the same number of outlets. The quality of the flow distribution and performance of each flow distributor were evaluated numerically using computational fluid dynamics (CFD). The fluid and the traced species used in the simulations were liquid water. All the simulations were performed with COMSOL 5.3. Mesh independency has been checked through decreasing the mesh size half the previous size. The effect of clogging on the band broadening has been investigated. CFD results showed that, in the absence of channel blockage, all distributors produce symmetrical peak with a little difference in band broadening. However, in the presence of channel blockage, RI flow distributor with rectangular pillars produces the broaden unsymmetrical peak in comparison with RI flow distributor with hexagonal pillars. Also, MM distributors which are the combination of BF and RI distributors, both in the absence and presence of blockage, produced symmetrical peaks.

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FUN-06

Polymerization of the Through-pores in HPLC Columns for Enhanced SEM Based Assessment of Packing Order

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Recent developments in chip-based column technology, whereby plate heights corresponding to the domain size can be obtained,[1] suggest that it might also be possible to improve the lower minimal plate height limit in packed column HPLC, corresponding to twice the particle size, down to lower values if improved packing procedures can be developed.

In order to allow improved understanding of the order in packed HPLC columns in this work a methodology for immobilizing silica particles is therefore developed based on the polymerization of a monomer and a cross-linker in the interstitial pores of HPLC columns. Subsequent cutting by mechanical cross-sectioning then allows scanning electron microscopy (SEM) based imaging of cross sections of the packed bed over the entire length of the column.

In this way the packing efficiency of in-house packed and commercial HPLC columns comprising the same packing material can be compared. The methodology is developed for native silica used in e.g. hydrophilic interaction liquid chromatography and the information obtained is cross-referenced with external porosity measurements obtained via inversed size exclusion approach.[2,3]

Results indicate that the home-made columns typically depict a lower total porosity compared to the commercial columns but a higher external porosity, indicating a lower density of the packed bed. This is reflected into losses in efficiency and into more disordered SEM data. The tool can allow fine optimization of packing procedures.

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FUN-07

Creating Monolithic Stationary Phases in Targeted Regions of 3D-printed Titanium Devices

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Researchers from many fields aim to fully characterize complex mixtures in a very short time. Multi-dimensional chromatography has proven to be a successful and fruitful option, but there is still room for improvement. In the STAMP project we aim to obtain a peak capacity of one million in analytical liquid-phase separations by using spatial three-dimensional liquid chromatography. The separations in the three dimensions will occur perpendicularly to each other. The second- and the third-dimension separations are carried out in parallel, so that the analysis time is greatly reduced. Spatial 3D-LC requires microfluidic devices that are structurally complex, with many channels and fittings.

3D-printing is an attractive manufacturing method, since it is fast and cheap. However, the possibilities are limited by the resolution of the 3D-printer and by the type of material that can be used. The solvent compatibility of the printed devices proves to be a major obstacle. Therefore, our research is focused on a 3D-printable material with outstanding mechanical, chemical, and thermal resistance, i.e. titanium.

The 3D-printed devices require in situ synthesis of different stationary phases, selectively created in different regions of the separation body. In this poster we show how Peltier elements can be used to locally create styrene-co-divinylbenzene-based monoliths within glass capillaries encased in a titanium device. By creating hot and cold zones in devices, we can perform confined thermal polymerization of monolithic stationary phases. Such phases have also been synthetized directly in a 3D-printed titanium device that contains heating and cooling jackets, with good attachment of the monolith to the inner surface. In this poster we describe several parameters to characterize the different monolithic stationary phases, such as porosity, permeability, repeatability of retention time, and spatial resolution.

The proposed method is a promising way to implement stationary phases within complex structures, such as those necessary for spatial three-dimensional separations.

FUN-08

Unraveling the molecular interactions driving retention and selectivity of a sphingomyelin-based stationary phase by QSPR interpreted through block relevance analysis

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State of the art: Sphingomyelin (SPH) is a type of sphingolipid found in animal cell membranes in a range from 2 to 15% mol/mol in most tissues. However, SPH features higher concentrations in red blood cells, the ocular lenses and nerve tissues, especially in the membranous myelin sheath that surrounds some nerve cell axons. As a consequence of its characteristics, SPH stationary phase represents an ideal tool to mimic the interactions taking place between active pharmaceutical ingredients and neurons.

Method: The SPH stationary phase (0.821 mg), synthesized by the Separation Science Group in 2012[1], was suspended in methanol (7.0 mL) and the resulting slurry packed (600 bar) in an HPLC column (10 cm x 2.1 mm). The column was operated at 300 mL min^{-1} at 25 °C using a mobile phase consisting of 60/25/15 Dulbecco's phosphate buffer saline pH 7.4/methanol/acetonitrile. The elution was achieved isocratically and monitored by UV detection at 220 nm. The databased assayed consisted of 36 neutral compounds, 26 basic molecules and 26 acids.

Results: QSPR study allowed accurate prediction and mechanism elucidation of the retentive behavior of pharmaceutically relevant compounds on the SPH stationary phase. Moreover, block relevance analysis assisted in rationalizing the intermolecular driving forces involved in the chromatographic retention.

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FUN-09

Evaluation of system performance in ultra-high-pressure operation mode

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The current state-of-the-art UHPLC technology comprises instrumentation allowing to operate columns packed with 1.5 um core-shell particles at operating pressures up to 1500 bar. To effectively utilize the potential of these columns, yielding peak volumes ranging between 0.5 and 10 uL, extra-column dispersion contributions need to be minimized.

In the current study, the instrument configuration of a UHPLC system with 1500 bar pressure capabilities was systematically altered and its effects on separation efficiency and retention were assessed in both the isocratic and gradient modes. Key factors affecting extra-column dispersion were investigated and the optimal system configuration with respect to extra-column dispersion and flow resistance is discussed for isocratic and gradient LC mode. Furthermore, pressure-induced retention effects have been evaluated, which affect method development and HPLC to UHPLC method-transfer strategies.

GC(xGC)-01

Development and application of PLOT GC columns on the Agilent Intuvo 9000 GC

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This work describes the development of HP-PLOT Al2O3 KCl PT, Al2O3 S PT and HP-PLOT Q PT GC columns for the Agilent 9000 Intuvo GC. Because of the column shape with a small diameter we have to demonstrate that the particle layer is stable.

An example analysis of a standard hydrocarbon gas mixture with the Agilent Intuvo 9000 GC and a Al2O3 PLOT GC Intuvo GC column is shown [1]. The system featured a gas sampling valve, a postcolumn D1/D2 detector splitter chip, and detection by flame ionization and thermal conductivity. Results were comparable, in terms of retention time and peak response, for both detectors in the flow path.

The Intuvo 9000 GC combined with Agilent J&W HP-PLOT columns, provides a smaller, more productive option to separate and quantify gas samples with confidence.

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GC(xGC)-02

Chemical characterization and in vitro antioxidant activity of essential oil from Commiphora kraeuseliana resin

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The Burseracaea family is best known for its aromatic resin producing plants. Resins consist of highly concentrated, volatile oils known as essential oils, as well as non-volatile solids which tend to make the resin thick and sticky. Essential oils are typically extracted via distillation or cold pressing. The genus Commiphora is the largest of the Burseracaea family, and the only genus present in both South Africa and Namibia. Resins from plants of the genus Commiphora are commonly used as antiseptics, to treat various skin infections, in cosmetics, as flavouring substances and as pharmaceuticals, due to their antimicrobial, anti-inflammatory and antioxidant properties. Commiphora essential oils typically consist of various secondary metabolites, such as terpenoids, steroids and flavonoids. The Opuwo Processing Facility is interested in producing and commercialising essential oils from the Commiphora genus, including Commiphora kraeuseliana essential oil. Commiphora kraeuseliana is a dioecious shrub, which is indigenous to the north-western region of Namibia, and typically grows on rocky hill slopes.

In this study, essential oil was extracted from the C. kraeuseliana resin using hydrodistillation. The oil could be produced at an average yield of 2.5%w/w. The chemical characterization of the volatile constituents of the resulting oils was performed using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionisation detection (GC-FID). The contituents were quantified using methyl octanoate as the internal standard and their concentrations (%w/w) were calculated using predicted relative response factors. Sixteen compounds were identified in the oil. The major compound was identified as nonane (92.5%w/w). Enantioselective GC analyses were performed, using a β-DEXTM 110 column, in order to determine the enantiomeric excess of the chiral constituents. A DPPH radical scavenging assay revealed that the antioxidant potential of the oil is rather poor (IC50 of 9.3 mg/ml). These results may be used by cosmetics or pharmaceutical industries to guide the formulation of their products and also to assess the safety of this oil when used as an ingredient.

GC(xGC)-03

Turning up the heat on WAX GC columns without getting burned

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100 % polyethylene glycol (PEG) columns, also known as WAX columns, are used for a wide variety of applications, such as industrial chemicals and petrochemicals. Traditional WAX columns have a maximum temperature limit of 250 °C isothermal and 260 °C programmed due to issues with decreased thermal stability, reducing the potential applications range. The Agilent J&W DB-HeavyWAX has an extended temperature limit, up to 280 °C isothermal and 290 °C programmed and increased thermal stability, increasing injection-to-injection retention time reproducibility and column lifetime. The increased upper temperature limit allows for faster analysis while minimizing possibility of carryover sample to sample.

GC(xGC)-04

Innovations in GC×GC software for characterisation of petrochemicals
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Comprehensive two-dimensional gas chromatography (GC×GC) has been proven to be an ideal platform for the analysis of complex mixtures, but the data processing aspect earned a reputation as being difficult and time-consuming.

GC×GC data exploration, filtering and mining need not be complex, and can be implemented in an environment that is based on classical peak detection algorithms and chromatographic rules.

For example, scripts are valuable time-saving tools even in simple analyses, but they offer particular benefits for highly complex samples investigated using GC–MS or GC×GC–MS, to identify specific compounds and/or chemical classes. The incorporation of soft ionisation and/or accurate mass information will be shown to greatly enhance the use of filtering scripts for precise group-type analysis of petrochemicals.

In this poster, we will demonstrate simple yet effective GC×GC data processing for a range of file types, proving that this technique can be a productive contributor to any high-throughput laboratory, by enabling sophisticated peak merging, flexible data navigation and streamlined data-mining workflows.

GC(xGC)-05

The PHySICAL Project: Research Protocol Applied on a Japanese Buddha Statue

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Asian lacquers being thermosetting polymeric coating materials with exceptional qualities in terms of high gloss, durability, deep black color and sophisticated decoration techniques have since prehistory been highly valued.

Lacquer is known to be impermeable and unaffected by water or any solvent in a polymerized state. However, upon light irradiation the lacquer surface polarity changes radically. This renders top surface lacquer layers to be highly sensitive towards polar solvents and water, which complicates cleaning practices, being an essential and complex part in the conservation treatment of cultural heritage objects.

The project "Profound study of Hydrous and Solvent Interactions in Cleaning Asian Lacquer" (PHySICAL) is an interdisciplinary research project dedicated to the cleaning of Asian lacquer objects.

In this contribution selection criteria applied for implementing objects in the study and how sampling and analysis is performed, is illustrated. The research protocol is further explained through the chemical study of a Japanese buddha statue in which a rare South East Asian lacquer formulation was identified with thermal hydrolysis and methylation - pyrolysis gas chromatography - mass spectrometry (THM-Py-GCMS). Organic solvent Immersion studies were thereafter analyzed with conventional GC/MS in splitless mode (as an aim to define the efficiency and risk of using solvents during cleaning). This was performed using single solvents on artificially light aged mock-up samples, mimicking the lacquer composition of the statue. This allows to search for an ideal solvent (mixture) formulation to define best practices to clean the statue.

GC(xGC)-06

Revealing the reactivity of isomers of bio-oils by gas chromatography coupled to Fourier transform ion cyclotron resonance mass spectrometry

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Given the high complexity of bio-oils, chemical characterization is inherently challenging. The difficulty in understanding how the upgrading methodologies affects the composition of a bio-oil arises from the many possible multiple functionalities that may be present in the arrangement of thousands of compositions, combined with poorly understood reaction routes under the reaction conditions. Previous analysis of bio-oils by direct infusion Fourier transform ion cyclotron mass spectrometry (FTICR MS), have reported that bio-oil compositions include highly oxygenated compositions (Oxclasses, where x = 2 – 10) and NOx(x = 1 – 6) classes, among others. Despite the high performance of FTICR MS, mass spectrometers cannot discriminate between structural isomers present in complex mixtures on the basis of m/z. This limitation can be overcome by coupling a chromatographic method to the FTICR MS instrument, such that structural isomers can be separated prior to mass spectrometry. In this study, a pyrolysis bio-oil obtained from softwood and its esterified product were analysed by gas chromatography hyphenated to FTICR MS (GC – 7 T FTICR MS) equipped with quadrupole phase detection. The data analysis was performed using in-house software (KairosMS) that allows the user to process the data and to compare hyphenated data sets. The peak detection performed by KairosMS allowed the determination of 10,544 and 18,512 structural isomers in the softwood bio-oil and its esterified bio-oil, respectively. A direct comparison of individual molecular isomers across each EIC in the data can help to understand the reaction behaviour of specific isomers with a carbon number up to 32 during the acid-catalysed esterification of the bio-oil. For instance, some isomers were not detected in the esterified sample, and therefore, these species contain carboxylic acids, ketones, or aldehydes functional groups that were effectively esterified (highly reactive isomers). Additionally, some isomers were only detected after esterification which indicates isomers containing esters and acetals functional groups that were produced after upgrading (esterified products). Finally, the peaks detected in both samples contain alcohols and ether functional groups or correspond to low reactivity isomers (low/non-reactive isomers).

Approximately 29.6% of the isomeric compositions correspond to species that were effectively transformed under the esterified conditions and 25.1% remain after esterification due to the low- or non-reactivity of these chemicals under these conditions. Thus, a direct comparison of the isomeric distribution of bio-oils under different upgrading conditions can reveal remarkably detailed information of the reactivity of multiple chemicals that comprise the bio-oil. For instance, the effectiveness of different pyrolysis conditions, chemoselectivity of different reaction methodologies, and multistep synthetic strategies can all be better understood.

GC(xGC)-07

Polymer Sequence investigations with pyrolysis-GC

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Polymer characterization is essential to understanding material properties by creating a link between polymers and their chemical structure. A polymer is not one defined molecule, but rather a distribution of molecules that may differ in molecular size, chemical composition, functional groups, end groups, branching, molecular architecture and sequence length. Contemporary materials comprise complex polymers and often require the application of multiple separation and/or detection methods for their characterization [1].

The sequence-length distribution of a copolymer, also sometimes referred to as the block-length distribution or the blockiness, is an important parameter to its physical properties. Monitoring the blockiness is challenging as sub-units of the polymer (e.g. dyad, triads) must be characterized. Currently, the golden standard to determine the sequence of a polymer is NMR spectroscopy [2,3]. However, several disadvantages of this method for quantifying the sequence distribution include (i) the need for expensive equipment, (ii) long measurement times and (iii) large amounts of required sample. One alternative method to determine polymer sequence distributions is pyrolysis-GC as demonstrated by Wang et al.[4]. While this method must be calibrated with standards with a known sequence distribution, it requires less sample and allows for faster measurements than NMR.

These advantages render pyrolysis-GC especially useful when hyphenation to other methods such as liquid chromatography is desired. In the present work we perform offline SEC-pyrolysis-GC to monitor both the composition and sequence of styrene-methyl methacrylate copolymers across the molecular-weight distribution. The method allows for the distinction between random copolymers, block copolymers and blends. When coupled to SEC, the method can distinguish differences in microstructure and composition across the molecular-weight profile.

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GC(xGC)-08

Thermal desorber – gas chromatography with offline and inline liquid calibration for the determination of residual solvents in drug loaded albumin

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The conventional approach for residual solvent (RS) analysis is headspace-gas chromatography (HS-GC). This starts from a homogenous sample solution and is based on the equilibrium of the analyte between the sample and the gas phase. Unfortunately, an aqueous solution of albumin forms irreversible hydrophobic aggregates when it is heated above 50 °C. Consequently, the use of HS-GC for RS analysis in albumin becomes problematic due to the formation of an additional solid phase in the HS vial.

In this work, a method using a thermal desorber (TD) combined with GC was developed for the determination of RS in drug loaded albumin. Samples were immobilized between two double layers of quartz filter (QF) in a polytetrafluoroethylene (PTFE) insert which was placed in an empty desorption tube prior to TD-GC analysis.

The liquid standard mix consisted of ethanol (EtOH), acetone (Ace), dichloromethane (DCM) and chloroform (Chlor) dissolved in toluene. Calibration occurred in two different ways: offline liquid calibration (OLC) and inline liquid calibration (ILC). OLC was applied by introducing 2 µL of the standard mix under counter flow of an inert gas into the TD tube containing a mixed bed of mesoporous silica immobilized between two double layers of QF.The completeness of release from the reference tube in OLC was verified using the ILC approach based on a heated GC injector installed on the TD. The results were found to be similar as the analyte release for OLC relative to ILC was found to be close to 100 %. So, in further experiments, OLC was used as this can be performed without modification of the equipment.

In a next step, the completeness of release of the RS from the albumin was checked. Hereto, a method based on the complete enzymatic digestion of albumin combined with conventional HS-GC was developed.

The validation results revealed that both the TD-GC and HS-GC methods showed good recovery (> 98 %). R2-values (> 0.998) indicated good linearity over a wide range with low quantification limits. RSD-values, as measure of the repeatability, were lower than 3 %.

Finally, the two methods were applied for the determination of EtOH and DCM in different albumin samples loaded with experimental drugs. Statistical comparison indicated that there was no significant difference (p > 0.05) between the methods. However, the HS-GC method following enzymatic degradation is much more expensive and time consuming.

GC(xGC)-09

Total volatilization headspace gas chromatography for the determination of volatile methyl siloxanes in personal care products

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Siloxanes are oligomers of organo-silicone polymers or silicones. The fully methylated siloxanes are called methyl siloxanes and are volatile. Mostly thanks to their surface tension and harmless nature, they have become very popular additives to enhance sensorial properties of personal care and cosmetic products. However, two of them (octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane) have been recently identified as substances of very high concern and a limitation in their use in wash-off products in Europe has been issued.

Gas chromatography (GC) is the most reported method of analysis for such molecules, which are brought to the column by direct injection (DI) or thermal desorption (TD) depending on the application. Those methods focused on environmental monitoring and, although they achieved low limits of detection, system contamination by condensation of different siloxanes that may cause carry over, is often reported. Moreover, if not-fully methylated siloxanes are present in the sample, this may cause side reactions such as backbiting. In headspace GC (HS-GC), the analytes are volatilized in a sealed vial and part of the gas phase is transferred to the GC. As analytes are already in gas phase before reaching the GC system, there is less chance of inlet contamination and carry over. Moreover, the inlet acts only as a liner between the heated transfer line and the column. Thus, no silanized wool is required at the inlet port, greatly reducing the risk of condensation. Although, HS-GC presents higher limits of detection than DI-GC, it has great potential as a robust method for routine control considering the legal limit of 0.1 % w/w in the final formulation.

Using high boiling point solvents is customary in HS-GC experiments to prevent saturation of the HS. However, despite being called volatile methyl siloxanes, some of these analytes present a rather high boiling point (> 200 °C), limiting the solvent choice for conventional HS-GC. Nevertheless, using small amounts in the vial can prevent the saturation of the HS even when working with high volatility solvents. This technique, known as total volatilization (TVT), allows using the same solvents as used for DI-GC methods such as n-pentane. Hence, we have developed a method for the analysis of methyl siloxanes by TVT HS-GC as an alternative to DI-GC for routine analysis. The method was validated for external calibration (linear regression with R2 > 0.996, accuracy > 99 % and repeatability < 2.5 %RSD) and solvent blank analysis proved that this method avoids the aforementioned complications. Next, it was applied to four different samples that represent typical matrices for these analytes: a medical adhesive remover, a hair oil, a skin care cream and a shampooing. For the last two, a Box-Behnken design was applied to optimize a single step solvent extraction procedure, achieving recoveries between 86 % and 108 % in all cases.

GC(xGC)-10

Considerations on adsorbent materials for in vitro and ex vivo VOCs (bio-)sampling

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Thermal desorption (TD) tubes are often used to trap and extract VOCs in many applications, from biomonitoring to food aroma characterization [1-3]. Recently, there is a general growing interest in VOCs from biological fluids (breath, serum etc), specially as possible biomarkers of specific disease states.

Because of the wide variety of adsorbent materials, the tube can be filled with, it may be challenging to select the optimal tube for biological samples. Indeed, these trapping materials can be used alone or in combination, and depending on the characteristics (chemical and physical), the selectivity can be tuned, as well as the sensitivity and repeatability.

In this study, TD adsorbent materials sampling performance were compared in biological samples, both in in vitro and ex vivo situations. Specifically, 7 different adsorbents (Tenax TA, Tenax GR, Carbopack B, 5TD, 1016, X and Sulphicarb) were used, packed singularly and in combination, on Fetal Bovine Serum (FBS) and human breath. A mix of 19 standards were employed to monitor and evaluate the sensitivity and repeatability.

Regarding the in vitro sampling, spiked FBS was used to mimic the biological matrix, and a dynamic headspace extraction was performed. For the in vivo part, breath was collected in Tedlar bags in which standards were successively flash-vaporized. In both cases, after extraction, the tubes were thermally desorbed on a comprehensive two-dimensional gas chromatography system coupled to a time-of-flight mass spectrometer (GC×GC-TOF MS).

For both sample matrices and in the targeted analysis on selected VOCs, the tubes packed with Tenax TA alone resulted the most sensitive with the highest repeatability, in the range of 2-22 RSD % for in vivo and 2-32 RSD % for in vitro sampling. In untargeted analysis on serum and breath, Tenax TA confirmed to be the most suitable material for sampling in terms of analyte coverage, recovery, and repeatability.

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GC(xGC)-11

Optimization of a multidimensional gas chromatographic separation. Classical steps toward a better characterization of the separation space

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The chromatographic separation in multidimensional gas chromatography is significantly affected by the interactions between the involved experimental parameters[1]. In order to achieve the best separation possible, these parameters need to be thoroughly optimized. Chromatographic procedures are optimized by varying one parameter at a time, thereby disregarding interactions between the two separation dimensions and resulting in time-consuming procedures[2]. Moreover, the metrics of chromatographic optimization are sometimes not clearly defined or solely based on the user's expertise and/or intuition.

In the present study, chromatographic factors were measured from a designed set of GC×GC-ToF-MS analysis of standards. These factors were used to derive specific outcomes that allowed for a detailed characterization of the two-dimensional (2D) separation space. Furthermore, the result of a design of experiments approach allowed for a statistical investigation of commonly optimized factors, namely flow rate, modulation time, and temperature ramp based on the derived outcomes.

Initial findings suggest that differences in chemical groups tend to impact the 2D separation. It also highlights a correlation between the influencing factors and the chemical composition of the studied compounds. Additionally, special attention was paid to the structural similarities between the studied compounds, as well as to wraparound effects and their consequences on the derived outcomes.

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GC(xGC)-12

Can increased Instrumental Sensitivity Replace Extensive Sample Preparation?

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The optimization of gas chromatographic separations is often a necessary step to push the limits of detection and quantitation to their utmost limits. Parameters controlling injection and separation conditions need to be adjusted and optimized thoroughly for rugged and reproducible conditions. Thereby, careful attention needs to be paid to the sample preparation stage, since it often enhances the instrumental sensitivity. Moreover, sample preparation is highly prone to error, as well as time-consuming[1]. For this reason, techniques that offer a reduction of the overall sample preparation procedure are of increasing interest.

Two-dimensional comprehensive gas chromatography (GC×GC) offers a wide scope of advantages allowing for some slightly simplified sample preparation. For example, analytes of interest can somewhat be separated from remaining traces of the interfering matrix due to the increased separation capacity, while the cold zone compression allows for increased sensitivity and improved peak shapes. Especially the hyphenation of GC×GC and highly sensitive mass analyzers can potentially reduce steps needed in existing sample preparation procedures.

In this study, the authors evaluate the performance of a GC×GC-ToF/MS for a detailed analysis of the volatile organic profile in Belgian chocolate. To evaluate the impact of the MS's sensitivity, a previously established sample preparation procedure was compared to a minimalistic sample preparation approach [2]. Although the majority of compounds could be determined in both approaches, initial findings indicate differences in between the two investigated approaches. Thereby, the differences are not only limited to the total number of compounds detected but also the detected compound classes differ within the compared approaches. Depending on the analytical challenge, the applied approach has to be chosen.

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GC(xGC)-13

Tackling challenges for the adoption of two-dimensional gas chromatography in Forensic Sciences

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In the last decade, comprehensive two-dimensional (2D) gas chromatography (GC×GC) has been successfully established as an essential tool for the analysis of complex mixtures in the field of environmental science, in the petrochemical industry, and for the exploration of biological samples. Despite its numerous advantages in comparison to one-dimensional (1D) GC, such as increased peak capacity, higher sensitivity and the possibility of group-type analysis, its application in forensic sciences remains rather limited. A major reason is that the adoption of a novel analytical technique to the needs of the modern forensic science laboratory is usually lengthy and challenging due to the strict requirements that are enforced in forensic laboratories. The time investment for method validation through laboratory accreditation, as well as potentially increased acquisition and maintenance costs, are both contributing factors to reluctance in the field. Regarding the implementation of GC×GC-based techniques, doubts may arise whether information could be lost using this new technique, because only few studies have been presented documenting method translation from 1D GC to 2D GC in forensic sciences to ensure that there is no loss of metadata. However, in the case of GC×GC, more information about sample composition is acquired in comparison to 1D GC. Therefore, additional software tools are needed to assist with the process of extracting meaning from multivariate data for objective decision-making. Furthermore, the complexity of the technique, a limited degree of automation in the workflow, and the need for additional training for laboratory technicians and scientists, are significant factors that impede full adoption in forensic laboratories.

Conventionally, novel GC×GC instruments were provided as a packaged solution. However, increasing options are becoming available to retrofit existing GC-MS systems, such as those available in forensic laboratories currently using traditional quadrupole MS (qMS). This may serve to make GC×GC more accessible to industries that are reluctant to purchase and adopt a completely new technique over their pre-existing instrumentation and workflows. Also, with the increasing improvements in quadrupole mass spectrometer scan rates, these tools now have increasing options for coupling to GC×GC. In this study, a GC×GC with qMS and simultaneous flame ionization detector (FID) with a reverse fill/flush (RFF) flow modulator was employed to exploit higher flows and slightly wider peaks in a dual detection approach. The objective of this study was to demonstrate quality assurance procedures when translating a GC-qMS method to a GC×GC-qMS/FID instrument using reverse fill/flush modulation retrofitting for the analysis of volatile organic compounds (VOCs). Figures of merit and calibration curves using GC-qMS and GC×GC-qMS/FID are presented, documenting the transfer and adaptation of the original method without a loss in data quality.

GC(xGC)-13

Development of a new polymeric ionic liquid stationary phase for use in High-temperature GC

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Gas Chromatography (GC) is of major importance to routine analysis. However, a significant number of polar compounds cannot be measured by GC due to lower volatility. Therefore, improving upon the thermal stability of polar stationary phases would lead to an increased amount of compounds measurable by GC. In this regard, the discovery of ionic liquids, which boast high thermal stabilities is one of major importance. These ionic liquids have been reported to show dual nature characteristics [1], meaning that they can separate both polar and apolar molecules as if they were polar or apolar stationary phases respectively. The thermal stability of these stationary phases is further increased by means of polymerization to yield polymeric ionic liquids (PILs) [2]. Therefore, a new PIL stationary phase, poly[ViC5Im+][NTf2-], achieved through free radical polymerization of the [ViC5Im+][Br-] monomer, which consists of a 1-vinyl imidazolium with an isopropyl chain on the imidazole moiety.

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HYP-01

Double barrel ESI source and novel tandem nanoLC-MS setup enables 24/7 proteome profiling with close to 100% MS utilization

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NanoLC coupled with high-resolution accurate-mass (HRAM) mass-spectrometry (MS) is the gold standard in discovery proteomics that requires deep profiling of complex proteomes. The unmatched sensitivity of nanoLC-MS, however, is often linked with relatively low MS utilization (the ratio of peptide elution window vs. total run time). The time that is not utilized for the acquisition of useful MS data is needed for sample injection and loading, column washing and equilibration, and samples traveling through the analytical column and fluidics to reach the MS interface. All these limitations can be eliminated by combining tandem nanoLC configuration where samples are separated on 2 columns as well as many loading, equilibration, washing steps are parallelized with the double-barrel source that allows to interface two columns with HRAM MS without post-column flow splitting to maintain the high chromatographic resolution of nanoLC.

This novel tanem nanoLC separation platform comprises a tandem UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) and a Double Barrel column oven (Sonation GmbH) installed onto the Nanospray Flex Ion Source. The tandem nanoLC platform was coupled with Q Exactive HF-X MS. The nanoLC system was configured with direct or pre-concentration onto trap cartridge fluidics using 10-port 2-position switching valves installed in the column compartment. The peptide separation was completed on two analytical columns packed into the emitter (C18, 75 μm I.D. x 40 cm). The optimized nanoLCMS methods incorporated "look ahead" injections and intelligent automated switching between columns to provide a user experience similar to standard nanoLC-MS sequence setup and execution. The HeLa protein digest peptides were separated at 250 nL/min and HRAM MS was used for DDA. The data were processed with Proteome Discoverer 2.4 with 1% FDR at protein and peptide levels.

We completed extensive testing of double-barrel ESI source and novel tandem nanoLCMS setups with gradients from 45 to 120 min and MS utilization above 95%. The record performing results were obtained while operating system 24/7. For example, a 90-min method resulted in the identification of more than >73,000 peptide and >6,700 protein groups in each replicate This corresponded to the identification of > 800 peptides during each minute of 24/7 system operation. More than 97% proteins and 90% peptides overlapped between individual runs that shows excellent result reproducibility.

Overall, the developed tandem nanoLC-MS platform with double-barrel source is an excellent choice to increase MS utilization in proteomics to almost 100% while maintaining the advantages of nanoLC-MS sensitivity for deep proteome profiling.

HYP-02

Hyphenated Method (LC-MS/MS-Fluorometric) for Determination of Nucleoside Triphosphates and Analogs in Peripheral Blood Mononuclear Cells (PBMCs)

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We report a validated doubly hyphenated technique (LC-MS/MS-fluorometry) for determination of nucleoside triphosphates and analogs (TPs) in peripheral blood mononuclear cells (PBMCs). The analytical process involves sequential lysis of a PBMC sample, extraction and selective determination of TPs in the total extract volume by LC-MS/MS, and subsequent determination of sample cell counts by use of a DNA-specific fluorogenic intercalant to flourometrically quantify DNA in the residual pellet of the extracted sample. The amount of DNA is calibrated by standards prepared from commercially available purified reference human genomic DNA. Since a known, constant amount of DNA is present in every human PBMC (and within ±1.5% between males and females), the amount of DNA in a PBMC sample can be used to determine the number of PBMCs in the sample. The overall amount of TPs in a PBMC sample can then be presented as mass of TPs per million cells, which can be further converted to an intracellular molar concentration of TPs by use of the known mean volume of a PBMC and the TP molecular weight. Although the initial PBMC sample lysis for TP extraction and the corresponding TP quantification method need to be developed and validated for the specific TP(s) of interest, the subsequent determination of PBMC number in the residual pellet sample applies to any PBMC sample, regardless of drug analyte; therefore validation and application of the cell count method is independent of drug analysis and can be performed once.

As an example, we present a hyphenated method validated for simultaneous determination of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) in a PBMC sample by LC-MS/MS and determination of the PBMC count by fluorometric detection. Both TFV-DP and FTC-TP are TP metabolites of dosed prodrugs (tenofovir alafenamide fumarate and emtricitabine, respectively) that are administered in combination as treatment for HIV. Validation results for both the analyte and PBMC components of the LC-MS/MS-fluorometric hyphenated method are summarized and meet relevant expectations of regulatory guidances for bioanalytical methods.

HYP-03

Characterization of Polymers by Hyphenating Pyrolysis with GPC-MS

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PyroVial® is a filament-based pyrolysis unit and micro-scale reactor commercialized by GERSTEL. This unit is controlled by an MPS autosampler allowing automation of pre- and post-pyrolysis treatment, such as addition of a reagent or a solvent. This analytical pyrolysis interface gives the opportunity to subsequently analyze the pyrolysis products by other techniques to study polymer microstructure, like liquid chromatography (LC) or size-exclusion chromatography (GPC/SEC) in contrast to the traditional approach using pyrolysis-GC. Although similar information is offered by alternative techniques like nuclear magnetic resonance (NMR) and LC, the Pyrovial® pyrolysis overcomes problems related with low or no polymer solubility.

In this poster an overview of the analytical options offered by the PyroVial® will be presented. Further, analysis of different commercially available polymers by pyrolysis-GPC and detection by mass spectrometry (MS) and refractive index (RI) will be illustrated.

HYP-04

A non-targeted analysis approach for screening of volatile and semi-volatile compounds by off-line sampling coupled to ATD-GC-MS (automated thermal desorption gas chromatography mass spectrometry)

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Philip Morris, Switzerland

The effect of exposure to material pollutants on human health has been a topic of investigation for several decades in the field of toxicological assessment. The associated concern is mostly linked to a broad range of volatile and semi-volatile compounds released from materials. As a consequence, the emission rates of materials are assessed to monitor the release of potentially hazardous substances in the context of toxicity clearance of materials. Multiple approaches are described to date have limitations in sample preparation (e.g., aliquoting by cutting pieces of material, thus biasing the measurements), compound identification, or throughput. In addition, comparison based on untargeted approaches is time consuming (1–2 months) and might be subject to potential errors when multiple manual interpretation steps are applied. To address these challenges, we have developed an innovative hyphenated approach facilitating the assessment of chemical emissions from new semi-finished goods or materials with comparison to reference materials.

A non-targeted method was developed to detect, identify, and semi-quantify volatile and semi-volatile compounds released from new semi-finished goods or materials and to compare the obtained profiles with reference or certified materials. In the first step, the entire sample is placed without additional preparation in a pot of a microchamber thermal extractor (µCTE) and heated to 50–250℃ for a specified period of time. The temperatures are adjusted to either investigate the accelerated release of volatiles from the material or mimic realistic use conditions (e.g., 65℃ as described in automotive norms). During this period, the released volatiles are trapped on adsorptive material (Tenax TA®). The trapped volatiles are then desorbed and analyzed by ATD-GC-QTOF-MS. An in-house library search tool has been implemented which combines the benefit of high-resolution mass spectrometry and retention indices as well as predictive tools for structural identification, delivering the probability of presence of compounds with an enhanced level of confidence.

Emissions from entire samples were trapped off-line through the µCTE. Bulk and surface samples were measured by using a semi-automated workflow that is suitable for analysis of approximately two samples per week and enables measurement of compounds released within previously defined temperature and chemical ranges. Chemicals released in three-fold higher concentrations relative to the references or absent in the references are then subject to semi-quantification.

Off-line sampling of entire samples combined with the use of an enhanced non-targeted approach improves the confidence and speed of analysis of material emissions. This new paradigm we have proposed here will facilitate toxicological assessment for material clearance with a reasonable sample throughput, allowing comparison of new semi-finished goods with reference materials.

HYP-05

Coupling of Thermal Gravimetric Analysis with Fast Gas Chromatographic Separation - Mass Selective Detection and its Applications for Material Testing

An Adams, Wilco Hoogerwerf, Pascal Pijcke, Ron Bassie, Brian Dickie

Dow Benelux

The coupling of a chemical detection technique to thermogravimetric analysis (TGA) offers added value, as it allows for more advanced characterization of materials under study by identifying the gaseous compounds evolving from the sample upon heating. On-line real-time detection is commonly realized by hyphenation with Fourier transform infrared spectroscopy (FT-IR) or direct mass spectrometry (MS). However, identification of individual compounds can be very complicated and often impossible due to overlapping signals. Hyphenation with gas chromatography (GC) as a separation step enables enhanced differentiation and more detailed analysis of complicated mixtures.

This presentation will discuss the in-house development of a TGA coupled to a fast GC system with MS detection, as well as the use of this instrument for different applications in material characterization where the technique has proven its value. Application of TGA-GC-MS allows to connect the generation of specific volatiles with heating temperature and weight loss, and thus enables to differentiate evaporation from degradation processes.

HYP-06

Is my Decaffeinated Coffee Caffeine-Free? Engaging Chemistry Undergraduates with Mass Spectrometry and Chromatography

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A new laboratory based practical has been developed to introduce first year undergraduate chemistry students to modern mass spectrometry and high performance liquid chromatography. At this early stage of their degree programme, the students have had little exposure to analytical chemistry techniques. This practical element forms part of a classroom based "Introduction to Analytical Chemistry" course. The course aims to provide students with an understanding of the theory and use of analytical techniques such as separation methods, mass spectrometry, UV Vis, atomic spectroscopy and simple statistical analysis.

Groups of ~5 students are introduced to the concept of ambient ionisation techniques using a Direct Analysis in Real Time (DART) video. The importance and advantage of quick and easy screening tests for alcohol and drugs of abuse etc. is also discussed in this context. The students are then given a demonstration of Atmospheric Solids Analysis Probe (ASAP) MS in the mass spectrometry/chromatography facility. An "empty" cocaine bottle is used as an example to show the sensitivity and ease of use of ambient ionisation. Whilst data are being collected, the concept of ionisation is introduced.

The group then use ASAP to analyse a range of different drinks, e.g. coffee, tea, soft drinks and energy drinks to determine the presence of caffeine; each student undertakes one test on a different drink. At the end of the session the students are asked follow-up questions:

- Is caffeine present?
- If yes, how much caffeine is present?
- What else is present in the drink?
- What other information might be useful/required?

The second stage of the project is group works to explore the literature and decide how to answer these questions and the students a prompted by the staff to consider how the level of caffeine could be determined. To answer this quantification question they are provided with calibration data together with the analysis for each drink using LC-MS. The multiple data sets coming from one of the in-house UHPLC or UHPSFC-MS instruments. Each group is required to take peak areas for the data and determine the level of caffeine in their samples. The different levels of caffeine across all drinks are also compared.

Each student returns an individual technical report, and this is followed by a group oral presentation to an expert panel.

HYP-07

Particle number and stoichiometric characterization of lipid-protein nanoparticle assemblies in biofluids by size fractionation and comprehensive quantitative LC-MS/MS analysis

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Lipoproteins are nanometer-size lipid-protein assemblies that can be utilized as diagnostic and therapeutic targets. Because of the complexity of biofluids, particle number and composition assessment of lipoproteins requires preparative fractionation, size measurement and selective lipidomic and proteomic analysis of numerous size fractions. We developed a hyphenated workflow that combines asymmetric flow field-flow fractionation (AF4), dynamic light scattering (DLS) and three LC-MS/MS methods. As demonstrated in this presentation, the main advantage of our comprehensive workflow is the ability to determine not only absolute concentrations of individual lipid and protein constituents in size fractions, but also the ability to calculate particle number and average particle stoichiometry through molecular volume calculations.

Serum samples were fractionated by AF4 and collected in 40 fractions with 1-4 nm size increments covering a 6-100 nm particle diameter range. Each fraction was then split into four aliquots including aliquots for phospholipids, nonpolar lipids, proteins, and particle size measurement. Main phospholipid classes (PC, LPC, SM, PE, and PI) and nonpolar lipids (FC, CE, and TG) were analyzed by high throughput, precipitation/evaporation/extraction protocols followed by LC-MS/MS. Protein analysis was done with on-line tryptic digestion followed by isotope dilution LC-MS/MS. The absolute concentrations of lipoprotein constituents in the fractions were multiplied with corresponding theoretical partial specific volumes to obtain the sum of molecular-volume-concentrations (nmole*nm3/L), while the particle size data was utilized for the calculation of volume by particle (nm3). The ratio of the sum of molecular volumes and the particle volume yielded particle numbers (nmole/L). The calculation of mole/mole ratios of specific analytes and particle numbers at specific particle size ranges yielded particle stoichiometry measures.

Evidence for the accuracy of our comprehensive approach was obtained by comparison with 2D non-denaturing gel electrophoresis and protein cross-linking. The calculated stoichiometry and physicochemical properties of lipoproteins also closely agreed with literature reported values, including apolipoproteins/particle (i.e. apolipoproteins A-I and apoB), particle density, phospholipid monolayer thickness, and core diameter. Analysis of serum samples from individuals with various disease states (atherosclerosis, diabetes, hyperlipidemia, metabolic syndrome) revealed significant correlations between protein stoichiometry with phospholipid monolayer or core-lipid composition. Our approach for combination of size fractionation, size measurement, high throughput sample preparation, quantitative LC-MS/MS analysis, and volumetric assessment of particle numbers can be applied to other nanometer-size lipid-protein assemblies present in biofluids, such as extracellular vesicles, exosomes, lipid droplets, liposomes, etc..

MDLC-01

Complementary Dual LC as Alternative to Multi Heart-Cut LC for Samples of Medium Complexity resulting in improved precision, sensitivity and productivity

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The separation of highly complex samples (100-1000 components) is commonly addressed with comprehensive multi-dimensional LC techniques that require advanced instrumentation and software. Comprehensive 2D-LC implies long analysis times and difficult accurate compound quantification due to slicing of peaks from the first dimension. If sample complexity is reduced to a level below 100, multi heart-cut 2D-LC that transfers unresolved sample zones into a 2nd dimension with orthogonal selectivity is a viable alternative. While this reduces analysis times and simplifies quantification, it is still relatively slow and demanding with respect to the fluidic set-up and instrument control.

This study addresses the question to what extent the transfer of fractions into a second dimension can be replaced by injecting the whole sample into two orthogonal LC methods. At sufficiently orthogonality and moderate sample complexity the probability is high that non-resolved zones in the 1st method are well resolved in the 2nd method or vice versa. The combined information will then allow to quantify all components from well resolved peaks of the respective dimension. It will be statistically modelled for orthogonal methods with given peak capacity what the maximum number of components and sample dimensionality according to Giddings1 can be resolved with this approach.

The experimental evaluation is done on a Vanquish Duo that allows simultaneous injection of one sample into 2 independent flow paths that run the orthogonal methods. Hence, the total analysis time is equivalent to a corresponding 1-dimensional separation. The complementary Dual LC approach is experimentally compared to multi heart-cut analysis of a model mixture with a given number of compounds and dimensionality in regards to achieved precision, sensitivity, analysis time and productivity.

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MDLC-02

Development of optimization strategies for heart-cut two-dimensional liquid chromatography

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Two-dimensional liquid chromatography (2D-LC) is a suitable method for the separation of complex mixtures. The effective peak capacity is increased when two different separations are applied. To achieve the best separation, two different retention mechanism with different selectivities ("orthogonal") are selected for both dimensions [1]. However, the development of a 2D-LC method requires the simultaneous optimization of two separate dimensions. This results in a large number of options and time-consuming method development. Due to the complexity of 2D-LC methods, industry mainly uses heart-cut liquid chromatography (LC-LC), where exclusively fractions of interest are subjected to the second-dimension separation.

The drawback of LC-LC is that only a limited number of cuts can be analysed in the second dimension, which is restricted by the combination of the first-dimension flow rate and second-dimension analysis time [2]. The development of multiple heart-cut LC (mLC-LC) enables the system to store multiple fractions prior to the second-dimension analysis with an interface that contains multiple loops [3]. The decoupling of the first and second dimension also allows for a more independent optimization of the first and second dimension, which provides even more possibilities in the method optimization for LC-LC. In this poster, the general principles of LC-LC method development will be explained. The parameters of interest and an approach for LC-LC method optimization will be discussed.

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MDLC-03

Numerical and Experimental Investigation of Sample Loss and Dispersion Occurring in Sample Loops Used in 2D-LC Setups

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With the increased interest in multidimensional LC separations over the past years, several easily usable 2D-LC systems have been introduced on the market. Depending on the separation problem, all separation volumes from the first dimension are captured and sent to the second dimension (comprehensive) or only a (small) number of selected parts of the first dimension chromatogram (heart-cutting). It is however of high importance to avoid sample loss in such systems, as may occur when underestimating the volume of the sample loop required to avoid breakthrough. The latter is the result of the parabolic flow profile inside the open tubular loop, which results in a velocity that is twice as large in the center than the average velocity expected for a given flow rate. This breakthrough, but also the resulting dispersion of this flow profile is on the other hand countered by radial diffusion of the sample compounds. It is therefore dependent on residence time, which is in turn affected by the loop diameter and the first dimension flow rate. In a second step, the sample is eluted from the sample loop, either in the same (co-current) or in the opposite (counter-current) direction, again undergoing the sample velocity profile and concomitant dispersion.

The present study reports on a computational fluid dynamics study of the possible sample loss and dispersion occurring in sample loops used in 2D-LC setup. The aim is to develop a model to estimate the fraction of an injection loop the can be filled by sample before sample loss occurs, including the effect of sample shape. By presenting these results in a dimensionless form, the results can be generalized for different experimental conditions. The simulation results were compared with experimental breakthrough profiles where a fixed volume loop was filled at different flow rates and with different mobile phase conditions, showing good quantitative and qualitative agreement. In addition, the effect of sample loop coiling was investigated.

The shape of the sample plug eluting from the loop is of high importance for the overall quality of the 2D-LC separation, as the second dimension columns are typically small, making them more sensitive to peak dispersion due to the injection plug. Understanding injection peak shape also allows to optimize parameters such as solvent modulation or optimal elution direction of the loop. Therefore, peak shapes and variances were determined for different cases (loop volume, flow rate, diffusion coefficient) and for different shapes and parts of the collected first dimension sample plug.

MDLC-04

Deciphering the complex distributions of cellulose ethers by 2D-LC

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Within the UNMATCHED project, universities and companies are jointly developing new analytical methods for the characterization of complex (bio)polymers, such as cellulose ethers (CEs). The ultimate goal is to achieve polymer products with enhanced properties by a better understanding of molecular structures through improved separation and detection. Chemically substituted celluloses find a broad range of application. For example, in the pharmaceutical industry, they are used as excipients for controlled drug release, but they are also widely applied as essential thickening agents in paint and food.

CEs are obtained by the reaction of cellulose with one or more of the following reagents: ethylene oxide, propylene oxide, ethyl chloride and methyl chloride. This results in derivatised celluloses with a highly complex chain structure and heterogeneity. The resulting distributions at the molecular level ultimately determine polymer properties, such as solubility, viscosity, and biodegradability, and thus performance. In order to understand performance differences between CE batches, new analytical methods that reveal these detailed molecular structures are required, with a focus on establishing sequence distributions of the various substituents.

In this work, we aim to probe chemical and size properties of various water-soluble CEs independently by combining reversed-phase liquid and size exclusion chromatography and evaporative light-scattering detection (RPLC-SEC-ELSD). In the first RPLC dimension, CEs are resolved based on the abundance and distribution of substituents, while aqueous SEC provides size distributions in the second dimension. First-dimension resolution is enhanced by using multiple RPLC columns in series, aiming to reveal more details in chemical heterogeneity. The RPLC and SEC methods are well compatible, circumventing the need for active solvent modulation. Effect of sample concentration, injection volume, flow rates, internal column diameters, stationary phase particle sizes and column temperature are studied and optimized to achieve high plate numbers at acceptable pressures.

MDLC-05

A 2D-LC-MS Method for Impurity Profiling of Synthetic Oligonucleotides

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As an important representative of the biopharmaceuticals, several oligonucleotide based products were recently approved or have been clinically tested for a new gene therapy, e.g. Zolgensma for spinal muscular atrophy (SMA). From an analytical point of view, the clinical trials require those products to be of extreme purity i.e. of pharmaceutical quality. In other words, suitable analysis methods are necessary for an effective separation and characterization of possible structurally related impurities.

In this work, we want to present a 2D-LC-MS method to achieve such a workflow of oligonucleotide analysis. In the first dimension, the different oligonucleotides and its impurities can be separated by mixed-mode chromatography with a high selectivity. Until now, MS-incompatible additives like triethylamine and phosphate buffer are still remaining in the eluents. Therefore, the second dimension includes an online desalting procedure performed by a RP chromatography. Consequently, the separated compounds under MS-compatible conditions can be identified via mass spectrometry.

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MDLC-06

Development of peak-tracking algorithms for use in data analysis and method optimization in multi-dimensional liquid chromatography

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Comprehensive two-dimensional liquid chromatography is indispensable for the separation of complex mixtures. In recent years, this latter technique has been maturing rapidly. In principle, the development of an 2D-LC method requires establishing two separation dimensions with vastly different ("orthogonal") selectivities. However, with the advent of state-of-the-art instrumentation for 2D-LC, the number of options to realize and optimize 2D-LC separations is increasing dramatically. Advanced modulation interfaces have significantly reduced the threats of solvent incompatibility and limited detector sensitivity in the comprehensive mode (2D-LC). However, these developments are accompanied by an increase in the complexity of the system and, thus, the time required for method development. The challenge of optimizing tailored 2D-LC separations is daunting, yet it must be rigorously addressed if sophisticated 2D-LC systems are to be utilized to their full potential in an efficient manner.

Our group has been working on the development of algorithms for optimization software, which can be used to dramatically reduce method-development time for two-dimensional LC[1]. As these optimization strategies work with input data which describes the sample, one key element of the optimization algorithms is the tracking of peaks across the input chromatograms which have been recorded using different methods. A first iteration was recently demonstrated for LC-MS[2]. Peaks are tracked across chromatograms using the spectrometric information, the statistical moments of the chromatographic peaks and the relative retention. The algorithm was specifically tailored to process chromatograms as used with method-optimization tools, where mobile-phase composition programs are applied to obtain model parameters to describe the retention of sample components. In that case time-saving pre-selection protocols can be used to narrow down the number of possibilities. Another application of the peak tracking algorithm can be the assessment of repeatability of a method. Since the algorithm is able to determine the change in retention time for each analyte in the sample.

In this poster, the general principles of the peak-tracking algorithm will be explained. Moreover, application of the algorithm to a complex sample of peptides will be shown.

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MDLC-07

Cold-Trap Modulated LC×LC for Polymer Analysis

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Nowadays many of the materials used in a wide range of fields consist of polymers that embody great structural complexity. Examples include amphiphilic block copolymers that are employed as drug delivery systems or many of the block copolymers used in engineering. Often these materials are synthesized using a wide variety of methods and variable conditions so that the desired functional properties may be attained. Because of this, the underlying structure-property relationship is often not clearly understood. The analysis of these materials can aid in elucidating this connection between on one hand the polymer's distributions in e.g. microstructure, chemical composition or molecular weight, and on the other hand the resulting functional properties.

One of the techniques applicable for this is comprehensive two-dimensional liquid chromatography (LC×LC) as polymers naturally contain correlated distributions that must be resolved, which can be visualized by LC×LC. For polymers the most often cited example is the coupling of gradient reverse-phase liquid chromatography (RPLC) with size-exclusion chromatography (SEC) which allows one to directly visualize the correlation between chemical composition (CCD) and molecular weight distributions (MWD). The coupling of the first and second dimension may be achieved by utilizing a 10-port 2-position switching valve and alternatingly storing fractions of the first dimension in two sample loops or low volume trap columns. For the RPLC×SEC analysis of polymers the benefits of utilizing a trapping strategy are clear: firstly, due to a lowered injection volume specific narrow diameter, small particle, SEC columns can be used that facilitate fast and high-resolution SEC. Additional advantages include a reduction in band broadening that may occur prior to the SEC separation and a decoupling of the first- and second-dimension flowrates.

For synthetic polymers an alternative trapping strategy, termed cold trapping, has been developed which avoids many of the conventional issues that are present when trapping small molecules. The use of cold trap modulation is demonstrated for the RPLC×SEC analysis of a characteristic styrene/butadiene star-block copolymer. Trapping efficiency is qualitatively evaluated by monitoring the waste coming from the first dimension by means of ELSD detection.

MDLC-08

2D-LC-MS/MS for Bioanalytical Quantitation of Chiral Drugs

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The enantiomers of chiral biologically active compounds often show differences in pharmacokinetic behavior and pharmacological activity. For this reason, each enantiomer of chiral pharmaceuticals should be considered as single active compound according to guidelines published by the FDA and EMA1,2.

Chiral recognition of drugs and their metabolites plays an important role for comprehension of their metabolism. Combining achiral and chiral discrimination in one LC separation is challenging or impossible for complex biological samples. Two-dimensional liquid chromatography (2D-LC) with a chiral dimension allows the enantiomeric discrimination of analytes in complex biological samples3,4.

In this work, 2D-LC-MS/MS is used in the quantitative investigation of the chiral shift in metabolism of propranolol and its hydroxy metabolites 4'-hydroxy propranolol, 5'-hydroxy propranolol and 7'-hydroxy propranolol in human urine5. Separation of propranolol and its hydroxy metabolites as well as separation of their respective enantiomers is achieved combining an achiral separation on a Phenyl-Hexyl column in the first dimension (1D) with a chiral separation on a teicoplanin-based column in the second dimension (2D). The 1D peaks of propranolol and its hydroxy metabolites are transferred to the 2D chiral separation in individual heart-cuts.

Analysis of human urine samples after administration of a single oral dose of 40 mg of racemic propranolol clearly reveals considerable chiral shifts in propranolol as well as its hydroxy metabolites. Urinary excretion rates of the individual (R)- and (S)-enantiomers of propranolol as well as its hydroxy metabolites are monitored for investigation of the enantioselective metabolism of propranolol.

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MDLC-08

Enhancing the Application and Exploitation of Temperature Gradients in Temperature Responsive Liquid Chromatography

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In Temperature Responsive Liquid Chromatography (TRLC) stimuli-responsive polymers are used to drive the separation processes. This type of polymer is an "intelligent material", as it is able to respond to a small variation in its surroundings with a sharp change in its physical properties. In the case of temperature responsive polymers, they possess a unique characteristic that allows them to change their water solubility based on changes in the ambient temperature. Implementing this polymer into a bonded-phase for liquid chromatography, allows for the control of the column polarity through control of the column temperature. This introduces the possibility to perform reversed phased like liquid chromatography in pure water, whereby the polarity is controlled through temperature, therefore eliminating the need for any organic modifiers. Although this concept is promising it has not been that straightforward to develop to its full potential, as it requires rapid heating and cooling of the column. Previously, the possibility of this property was already demonstrated by rapidly cooling the column in a water bath. This external cooling of the column, however, leads to the occurrence of radial temperature gradients inside the column, while not allowing for refocusing effect typically associated with the application of gradients. Thus far, although the possibility of TRLC has extensively been demonstrated through several applications, the combination of increased plate height at lower temperatures and of the additional radial temperature gradients inside the column has detrimentally affected chromatographic performance. One of the reasons for this sub-par performance is the absence of an efficient linear gradient setup, which currently limits these types of separations to less efficient and lengthy isothermal measurements.

In this research we will evaluate the possibility of linear temperature gradients in temperature responsive liquid chromatography. For this, a regular HPLC system will be converted into a system that is able to efficiently deliver both hot and cold aqueous mobile phases whilst retaining all of its basic functionalities. In order to achieve the required fast and efficient temperature gradients, an important focus will be placed on achieving efficient heating and cooling of the temperature responsive column whilst minimizing any unwanted heat transfers. After this, the performance of this new setup will be optimized and evaluated by comparison to the current non-linear gradient setup. To demonstrate the capability of this newly developed linear gradient setup, it will then be employed towards the separation of relevant test mixtures, such as food additives and natural products.

MDLC-09

Enhanced determination of pharmaceutical impurities through a temperature-responsive stationary phase in 2D-LC (TRLCxRPLC)

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Peak purity determination and quantitative analysis of pharmaceutical drug formulations containing multiple active components is a challenging task by one-dimensional LC. Baseline separation of all the active constituents and their related impurities cannot be obtained anymore, while the impurities typically present at very low concentrations require sensitive detection to make their identification and quantification following current regulations possible.[1] Comprehensive two-dimensional LCxLC approaches are more and more frequently used to analyse natural and synthetic samples of high complexity in order to address too low peak capacities in 1D-LC. Nevertheless, many of the current approaches are limited in their robustness, characterized by modulation problems, too low orthogonality and undersampling.[2]Especially the modulation issue often becomes visible in form of solvent immiscibility and too high elution strength, leading to e.g. peak broadening and decreased sensitivity. Only a few comprehensive LCxLC modes, based on aqueous mobile phases in the first dimension are today exempted from these concerns. One of those is the recently introduced combination of temperature-responsive chromatography with reversed phase liquid chromatography (TRLCxRPLC).[3] Such temperature-responsive phases depict an adaptable hydrophobicity and hence retention characteristics as a function of temperature, allowing for a complete forgo of organic mobile phases. This new separation technique also reduces the methical complexity of LCxLC, by allowing trouble-free modulation when using a conventional loop based 10-port valve and a purely aqueous mobile phase in the first dimension. This on the one hand allows for the transfer of high sample volumes from the first to the second dimension with near perfect peak refocussing, and on the other hand can facilitate more sensitive detection compared to conventional LCxLC approaches. The possibilities offered by TRLCxRPLC in terms of selectivity, sensitivity, peak capacities and quantitative potential are assessed for improved separation of pharmaceutical mixtures. Therefore, synthetic mixtures of structurally similar pharmaceutical compounds are investigated, while optimizing the first and second dimension to optimally use the given separation space and the added selectivity.

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PHA-01

Assessment of operating conditions affecting the peak capacity of intact protein bioanalysis in hydrophobic interaction liquid-chromatography

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With the current increased demand for chromatographic analysis of intact proteins, the need for an in-depth characterization of proteins and protein-derived macromolecules is inevitable. In this regard, hydrophobic interaction chromatography (HIC), which is based on the interaction between hydrophobic patches of proteins and weakly hydrophobic ligands of the stationary phase while maintaining the protein 3D structure, offers a unique possibility for sample characterization. In recent years, the performance of a gradient separation is frequently curbed by assessing the peak capacity, which is defined as the maximum number of peaks that can be separated within the defined gradient window. This study was performed to assess the effects of gradient conditions on the separation of intact proteins. The effect of mobile-phase velocity on peak capacity was assessed at different gradient steepness. Furthermore, the effect of gradient window (and especially gradient starting conditions) on resulting resolution and selectivity were investigated. Finally, optimized gradient conditions were applied to profile intact protein samples, that vary in sample complexity. This study further highlights the efficiency of HIC as a suitable method for biomolecule analysis.

Keywords: peak capacity, hydrophobic interaction chromatography

<u>PHA-02</u>

HPLC as a PAT tool featuring ballistic separations and direct process sampling

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Applications of HPLC in process analytics come with two key challenges: speed of analysis and sampling interfaces.

Presented here is an example of on an ultra-fast gradient method resolving key reaction components on a timescale of seconds and a nanolitre level, direct process sampling interface designed to ensure effective data density of as little as 2 to 5 points per minute.

Examples are provided where this data rate was short enough to follow and model reaction kinetics in flow chemistry processes.

Discussed are aspects of system dispersion optimisation in Agilent 1290 Infinity II setup as well as hardware limitations related to the lack of injection synchronisation in an external injection valve and resulting retention drift. Data analysis workaround is employed to combat the drift. Data reproducibility and column stability in such challenging conditions are analysed for Waters Cortecs C18+ column.

PHA-03

Optimisation of oligonucleotide separations using ion exchange chromatography - Focusing on the type of mobile phase counter ion

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Nucleic acid therapeutics such as antisense, siRNA and aptamers are expected to be the next-generation pharmaceuticals following antibody drugs. To supply these drugs, purification and separation methods that can recognize slight structural differences after synthesis are important.

Non-porous anion exchange columns are generally said to show good separations for analysis of oligonucleotides. Therefore, we tried to optimise an analytical method for single-stranded unmodified and modified DNA and RNA of about 20 mer, using BioPro IEX QF, a nonporous high performance anion exchange column. For optimisation, some conditions were changed, including the type of salt used for buffer solution, the nature and concentration of the counter ion (Cl, ClO4, SCN, I, Br) added to mobile phase and the column temperature used. The results show good separations could be obtained for the oligonucleotides with a single-base difference in length. For separations of DNA 20 mer and DNA 21 mer, separation conditions were also examined for differences in the 5' terminal base of DNA 21 mer.

In this poster, we will describe further details on the optimisation of separation conditions.

<u>**PHA-04**</u>

Characterization of a GlyCLICK site-specific ADC using complementary middle-up LC-MS analysis

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Antibody-drug conjugates (ADCs) are designed to couple highly cytotoxic small molecules to cell-targeting recombinant monoclonal antibodies (mAbs) [1]. However, currently used non-selective conjugations techniques create a great amount of ADC subpopulations, containing a wide variety of drugs per antibody. The resulting heterogeneity in Drug-Antibody Ratios (DARs) and drug load distribution (DLD) can affect the drug efficacy and patient safety and is therefore an important critical quality attribute (CQA) [2]. Moreover, the addition of chemical heterogeneity to the inherent micro variability of mAbs hampers the characterization at intact protein level (~150 kDa)[3]. An attractive approach to produce homogenous ADCs is by using glycan-mediated conjugation of the drug-linker to the conserved N-glycosylation site of IgG-type mAbs [4]. Recently, Genovis introduced a commercially available kit (GlyCLICK(R)) that uses a two-step enzymatic procedure to transform present Fc-glycans on IgG mAbs into site-specific anchor points for the conjugation of any alkyne-containing payload of choice. In this study, we compared naked trastuzumab and trastuzumab conjugated with MMAE following the GlyCLICK procedure on a middle-up level, to evaluate the conjugation process. RPLC and HILIC coupled to MS detection were used to analyze the protein subunits obtained after enzymatic digestion and chemical reduction. In order to compare both products, the N-glycosylation profile of trastuzumab was first characterized with the same workflow. It was shown that replacement of the glycans by the hydrophobic drug resulted in a strong shift in retention time. This allowed to rapidly identify the site-specific DLD at the chromatographic level. Moreover, based on the accurate mass identification of the ADC subunits, a homogenous DAR of 2.0 was determined. Together, the DLD and DAR confirm the site-specific conjugation of the Fc subunit. To conclude, this work illustrates how middle-up level analysis using LC-MS can provide accurate and complementary information on CQAs of both mAbs and ADCs within a single workflow.

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A comparative study of UniSpray and Electrospray for the ionization of neuropeptides in LC-MS/MS

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LC-MS/MS has manifested itself as a powerful analytical technique for peptide quantification. Although electrospray ionization (ESI) is extensively used as ionization source, poor ionization and transmission efficiency are reported. Therefore, Waters developed a new atmospheric pressure ionization source, UniSpray, which is built up by a high velocity spray arising from a grounded nebulizer that impacts asymmetrically on a cylindrical target held at high voltage. Although ESI and UniSpray display an analogous ionization of analytes, Unispray possesses some additional mechanisms that contribute to enhanced ionization and ion transmission efficiency. The source has already been evaluated for the analysis of a broad range of small molecules. For many of these compounds, sensitivity improvements have been reported with UniSpray compared to ESI.

As earlier research only focused on small molecules, this study aimed to evaluate the LC-MS performance of the UniSpray source in comparison with ESI for seven neuropeptides. In this way, our results may provide better insights into the potential applications and advantages of this source for quantitative peptide analysis.

Initially, optimal MS parameters were determined for both sources by direct infusion of neurotensin. During this step, the position of the spray emitter was also optimized based on visual observations, as it may affect source stability and sensitivity. The highest signal intensity with the UniSpray source was indeed obtained when the spray impacted on the upper right quadrant of the rod. Next, the best overall capillary/impactor voltage was determined by injection of a mix containing all seven neuropeptides on the LC-MS/MS system. An optimal capillary/impactor voltage of 2 kV was chosen and applied in the subsequent experiments. Furthermore, higher total signal intensities were observed with UniSpray for all seven neuropeptides. Limit of quantification and linear range were determined and compared between both sources by measuring calibration curves in the range 10 – 4000 nM. Moreover, as matrix effects are regularly seen with ESI, it was investigated whether this is also observed for UniSpray. For this purpose, a post-column infusion method was applied to evaluate the matrix effects of protein-precipitated human plasma and microdialysate on the ionization of neuromedin N and neurotensin. While for neurotensin an important difference in matrix effects is observed between ESI and UniSpray, this is not the case for neuromedin N. Finally, the effect of the supercharging agents m-nitrobenzyl alcohol, dimethyl sulfoxide and sulfolane on the ionization of the neuropeptides was examined with both sources. Although the use of supercharging agents can be advantageous to modify the charge state distribution, an individual approach for each peptide is demanded.

PHA-06

Advances in Biomolecule separation on small ID silica monoliths

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With increasing interest in using biomolecules as therapeutic entities, there is growing need in their separation, in specific in combination with Mass Spectrometry. Due to molecule size and further physical properties, stationary phases with larger mesopores and adapted surface bonding are necessary, to enable effective separation.

In this work the separation of monoclonal antibodies on Protein A carrying widepore, small ID silica monoliths is presented. Applications with antibodies, proteins and peptides are shown on RP4, Phenyl and RP18 modified widepore, small ID silica monoliths. Stability, reproducibility and linearity data are shown as well as the influence of temperature, mobile phase conditions and surface bonding on retention behavior, peak shape and separation efficiency.

Furthermore, it is explained how ligands can be attached to monolithic small ID silica gel via different immobilization techniques, e.g. epoxy-coupling and reductive amination. It is shown that surface bonding, its modification and the immobilization procedure can be varied for different ligands and their applications. Immobilization examples of peptides, proteins and enzymes onto silica monoliths, carrying epoxy-groups, are presented as well as applications of these columns e.g. HILIC and affinity chromatography.

PHA-07

Optimisation and standardisation of a breath biomarker discovery platform

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The analysis of volatile organic compounds (VOCs) in exhaled breath offers the attractive prospect to diagnose life-threatening diseases in a non-invasive and inexpensive manner. In a clinical setting, these tests could screen large populations, improving patient survival chances and reduce associated medical costs.

The aim of this study was to develop a robust method for the collection and analysis of breath volatiles. Optimisation of the system will be demonstrated - from the sampling of breath onto sorbent tubes for thermal desorption (TD), through to the data analysis workflows.

A key challenge is that breath can contain hundreds of different VOCs, often in trace levels - making it difficult to isolate and identify biomarkers of disease. During the biomarker discovery phase, an incorrect identification can compromise the validity of an entire trial, meaning robust analytical techniques are required.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) has been proposed as a powerful technique for the analysis of such complex biological samples. The commercialisation of consumable-free modulators has only strengthened its position for breath profiling – where routine analysis of large sample batches and efficient modulation of volatiles are essential.

The enhanced separation and high sensitivity of TD-GC×GC-TOF MS ensures that trace metabolites are not masked or overlooked and provides cleaner spectra for confident identification of potential biomarkers.

We will demonstrate this enhanced performance through the analysis of sorbent tubes spiked with a suite of known biomarkers. The repeatability and linearity will be shown, prior to the analysis of real breath samples. To simulate a real-world scenario, participants' breath will be measured before and after ingestion of a peppermint capsule to produce a controlled, artificial change in the breath profile and mimic a change in metabolism due to disease.

PHA-08

Optimization of innovative MS-compatible mobile phases for the characterization of monoclonal antibody charge variants by CEX

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Monoclonal antibodies (mAbs) are produced via recombinant DNA technology and their complexity is far exceeding those of small molecules. MAbs are inherently heterogeneous due to the post-translational modifications (PTMs) in the biological production process and storage conditions. Different PTMs can result in charge variants that affect both efficacy and safety of the mAb product. For this reason, regulatory agencies require a detailed analytical characterization of the critical quality of attributes of novel mAbs [1]. To assess these charge variants, one of the commonly used technique is cation exchange chromatography (CEX). This technique can separate the main isoform from acidic and basic charge variants, through ionic interactions between the negatively charged stationary phase and the positively charged mAbs. Despite good reproducibility, the disadvantage of this technique is the limited information on characterization of charge variants. This issue could be solved by the use of mass spectrometric (MS) detection, thanks to the accurate mass identification of chromatographically resolved peaks. Nowadays, the hyphenation of CEX to mass spectrometry (MS) has become more widespread in research and development laboratories.

CEX is inherently incompatible with MS, because classical separation of charge variants is achieved by using non-volatile salts (e.g., sodium chloride), which could lead to adducts formation and signal suppression, hampering the mass resolution and signal. For these reasons, multi-dimensional chromatography could be used. However, this technique is tedious and complex and could be circumvent by the development of online CEX-MS. Direct CEX-MS could be performed by using volatile salts in the CEX mobile phase, such as ammonium acetate, ammonium formate and/or ammonium carbonate. During the past few years, advances in volatile recipes were realized. However, many mobile phase systems have not enough buffering capacity or appropriate ionic strength, resulting in a system suitable for only a few specific mAbs. The goal of this work was to systematically study the mobile phase composition, to understand the impact of ionic strength, buffer capacity and pH-response on retention behaviors, peak shape and selectivity.

After preliminary tests, ammonium acetate and ammonium carbonate were selected as suitable buffer components. Then, buffer capacity and pH-response of different buffer compositions were calculated and modelling of the retention was performed to give a concentration range for each volatile salt. Lastly, chromatographic experiments were performed on mAbs possessing a wide isoelectric points (pI) range from 6.8 to 9.2. Here, we show the development of robust MS-compatible mobile phase system for CEX-MS with salt mediated pH gradient elution, suitable for the charge characterization of mAbs with a wide range of pI.

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PHA-09

Secondary column interactions between biopharmaceutical proteins and some recent size exclusion chromatography (SEC) columns in native SEC

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Native size exclusion chromatography (SEC) is often used for qualification of protein biopharmaceuticals. Ideally native SEC should separate molecules only based on size and with no interaction with the column material. Many biopharmaceuticals naturally contain hydrophobic or charged domains and other protein drugs have fatty-acids attached to increase their half-life in-vivo. These domains might interact with the column material causing secondary column interactions. To suppress hydrophobic column interactions organic modifier can be added and to suppress electrostatic column interactions high amounts of salt can be added to the mobile phase. When organic modifier and excess salt is added there is a risk that the protein is no longer in its native state and thus it is beneficial to find inert columns that are less prone to interact with the desired protein at native conditions.

The goal of this work was to find inert columns with minimal secondary column interactions. >10 columns were compared with respect to hydrophobic and electrostatic column interactions. Due to the recent trend in smaller particle size columns for native SEC a number of sub-3-μm columns from various column manufacturers were chosen, the majority of the columns recently released. As a tool for the study two compounds were used; one with an attached fatty acid and the related molecule without fatty acid. Due to their similar size the two molecules should have the same retention in the SEC system if no hydrophobic interaction occurs. The retention of the two proteins was studied as a function of isopropanol concentration and NaCl concentration, using a set-up with on-line mixing and FusionDoE software, showing differences between the columns in secondary column interactions. Additionally, the columns were compared with respect to separated aggregates, recovery and priming.

PHA-10

Factors that Influence the Recovery of Hydrophobic Peptides During LC-MS Sample Handling

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A sensitive and reproducible LC-MS analysis of biomolecules requires a fully optimized workflow from sample preparation to chromatographic separation and mass spectrometry detection. One frequently overlooked fact is that the analytes can be lost before LC-MS injections, especially in the sample container. Some proteins and peptides in solution stick to the wetted surfaces of the container and may be permanently lost. This non-specific binding (NSB) can lead to inaccurate quantitative results, and thus can limit the LC-MS assay capability. Using a blocking agent has been the most common workaround to mitigate NSB problems, but it may not be fully compatible with the downstream LC-MS analysis.

Losses of hydrophobic peptides in sample containers could be mitigated by weakening the hydrophobic interaction, e.g., by increasing the percentage of organic solvent in the sample matrix. While effective, there is a practical limit in this approach because highly organic sample matrices disrupt the retention of the peptides on the chromatographic column. The types and concentrations of the acidic additives in the sample matrix also influence the peptide recoveries as well as the peak shape. Peptide losses depended on the storage time and temperature. Overall it is challenging to find the optimum conditions to achieve good peptide recoveries and, at the same time, maintain compatibility with downstream LC-MS analyses.

Using a container that is specifically designed to exhibit low surface binding is an effective starting point. By providing a more inert surface, these containers show good protection against peptide losses in sample matrices that result in significant losses in standard containers. Using these containers and an LC-MS compatible sample matrix greatly simplifies the process of selecting the optimal storage conditions without compromising peptide recovery.

PHA-11

Optimization of an untargeted LC-MS method for metabolite profiling of restricted-volume plasma samples

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Untargeted metabolomic studies provide the opportunity for innovative diagnostic approaches. Different analytical techniques are available to perform metabolomic studies. Nowadays, the most applied technique is reversed-phase liquid chromatography coupled with high-resolution mass spectrometry. Still, interpretation of the obtained data remains challenging. Therefore, a robust, reproducible and reliable analytical approach is required to translate the obtained fingerprints and markers into a biological relevant outcome.

In this study, a reversed-phase LC-MS method is optimized using an experimental design approach by analyzing 10 µL of ½ and ¼ diluted human plasma samples. In a first step, the influences of specific analytical conditions on the responses (total number of peaks and the number of reliable peaks) are estimated by a Plackett-Burman screening design. This type of design contains a number of experiments equal to a multiple of four, resulting in a feasible number of experiments enabling to draw relevant conclusions and to perform an error estimation. In this study, a 15 factor design is applied to evaluate 12 factors. The design contains also 3 dummy factors, which are imaginary factors, applicable for the statistical evaluation to determine the significant effects.

The factors include the column temperature, sample cone voltage, source temperature, desolvation temperature and gas flow rate, cone gas flow, LC flow rate, type of extraction solvent, scan time, low- and high-energy CID and capillary voltage. All factors are evaluated at a high and low level, based on literature information. A total of 16 experiments was executed for the 15 selected factors. Moreover, 3 replicates at center-point level were added for the critical-effect estimation. The experimental error is thus estimated based on 1) the center-point replicates; 2) dummy-factor effects and 3) Dong's Algorithm. The design experiments were performed in a randomized sequence with a center-point experiment in the beginning, middle and end of the design experiments, to include some time effect knowledge.

Not all screened factors show a significant difference between their high and low investigated levels. This can be the result of a too narrow range or from the fact that the factor does not influence the response. The overall outcome suggests significant effects of the column temperature, sample cone voltage, desolvation temperature and gas flow rate, extraction solvent, scan time, low-energy CID and the capillary voltage on both responses. These are too many factors to include in an optimization design and therefore the effects will be further investigated in order to select the factors providing the largest effects. These selected factors will then further be optimized using an optimization design.

SAM-01

Advanced Automated Sample Preparation workflows for challenging GC-MS analyses

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For many challenging GC-MS applications, the key for reliable and high-quality results lies in the way the sample is prepared prior to the injection. Whether it is the extraction protocol for target analytes or a derivatization procedure to make them amenable to GC analysis, or both, sample preparation normally involves multiple time-consuming and error-prone manual steps, with significant impact on data accuracy and precision as well as on laboratory productivity and cost of operation.

Advanced robotic autosampler platforms are capable of combining highly versatile sample injection with automated sample preparation procedures. The typical bottlenecks of GC-MS analytical workflows can be eliminated through higher degree of automation of front-end solutions, for extended unattended operations, reduced overall costs and improved analytical data quality.

This talk aims to illustrate real world cases of time-saving automated sample preparation and cost-saving approaches relevant to environmental and food safety laboratories dealing with high-throughput demand.

The first example shows how automation allowed a water testing laboratory to scale down liquid-liquid extraction for the analysis of semi-volatile compounds in surface water samples, i.e. non-target screening of OCB/PCB/PAH, Halo Acetic acids, Herbicides (N, P – pesticides), Chlorophenols.

Before implementing automated workflow, the laboratory protocol involved a total sample volume of 10L, a total solvent volume >600 mL, and the use of 8 GC/GC-MS plus 1 HPLC systems.

The implementation of automated liquid-liquid extraction combined with large volume injection in a highly sensitive triple quadrupole GC-MS/MS, allowed to reduce the total sample volume to 250 mL, the total solvent volume to 15 mL and limited the instrumentation to 3 GC-QQQ.

The second example shows how automated solution can streamline the challenging determination of monochloropropandiol (MCPD) and glycidyl-esters (GE) in edible oils. The fully automated workflow involves a fast alkaline transesterification, including automated calibration curves and IS addition. With an achievable on-line throughput of 40 real samples /24 hr, this method is a perfect fit for ambitious laboratories with high throughput requirements.

SAM-02

Direct-connected GC headspace autosampler to extend the applicability of a clean, on-line solventless extraction technique for volatile impurities

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Static headspace sampling technique for gas chromatography is very popular for the analysis of volatiles compounds in non-volatile matrices, for its ease of use, its cleanness and for the minimum or no sample preparation required. Different approaches are available for transferring the headspace into the GC, but the valve and loop technique is one of the preferred methods to assure the highest precision of the injection and sample integrity during transfer.

A new design for a valve and loop headspace autosampler featuring a direct column connection to the valve manifold offers enhanced analytical performance in terms of injection repeatability, inertness of the sample path and elimination of carryover effects, while maintaining the column efficiency. Additionally, the recovery for less volatile compounds is significantly improved, expanding the applicability of this simple and affordable technique.

This talk illustrates the technological advances embedded in the new platform, and the benefits for routine testing laboratories. Key applications in different analytical fields will be covered to show novel uses of static headspace sampling technique, expanding to high temperature operations.

STA-01

Novel Polymer-based Stationary Phases for Temperature Responsive Liquid Chromatography

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The use of polymer derived stationary phases in liquid chromatography, either as a replacement for or as a hybrid silica based stationary phase, has been expanding in last decades. An interesting discovery in this field has been the development of temperature responsive stationary phases, wherein a temperature responsive polymer is used to achieve separation.[1] This type of polymer is an "intelligent material", as it is able to respond to a small variation in its surroundings with a sharp change in its physical properties. In the case of temperature responsive polymers, they possess a unique characteristic that allows them to change their water solubility based on changes in the ambient temperature. Implementing this polymer into a bonded-phase for liquid chromatography, allows for the control of the column polarity through control of the column temperature. This introduces the possibility to perform reversed phase like liquid chromatography in pure water, whereby the polarity is controlled through temperature, therefore eliminating the need for any organic modifiers.[2] Although successful implementation of this technique has already been demonstrated for several applications, the technique is still in its developmental stage. As a result, this strategy has not yet reached its full potential and is still being plagued by several shortcomings. Examples of this are, the often too low polymer coupling efficiencies resulting in low carbon loading, the questionable stability of the silica base at elevated temperature in fully aqueous conditions, or the less than optimal peak capacities which have been reached thus far.[3,4] This contribution will focus on the development of temperature responsive liquid chromatography. For this, the effect of the polymer properties and polymer side chain on the retention behaviour will be evaluated by both direct comparison of the retention and selectivity by way of van 't Hoff plots. These variations to the polymer structure have an effect on the polymer polarity and consequently on the lower critical solution temperature, which is the temperature above which the polymer becomes water immiscible. These changes polymer structure and polymer properties lead to interesting differences in selectivity and retention between the different stationary phases and lead to a better understanding of the retention mechanism in TRLC. These new temperature responsive columns will then be employed towards the separation of relevant test mixtures, such as food additives and steroids.

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STA-02

Fabrication of polymer monoliths in non-transparent 3D-printed polymer housings

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3D-printing has gained popularity as a fabrication tool in many fields. It is now commonly viewed as not only a prototyping tool, but as an approach to obtain a final, customized product. Fused-deposition modeling (FDM) is one of the popular techniques that offers a number of significant advantages over other 3D-printing types. These include the ability to create objects with various shapes against low material and equipment costs, no chemical post-processing required, no resins to cure, and a broad range of materials. The result is a cost-effective process. Therefore, this technique could not stay unnoticed by the field of Analytical Sciences.

Separation and characterization of complex mixtures are of crucial importance in many fields, such as proteomics, metabolomics and lipidomics, which require extremely high separation powers. This growing need is spurring the quest for high peak capacities in separation procedures. Spatial three-dimensional liquid chromatography can offer a solution to achieve peak capacities in the order of a million. A novel way to produce efficient three-dimensional devices for this approach, may be established through the use of 3D-printing technologies.

RPLC separations are achieved by in-situ fabrication of porous polymer monoliths, directly within the 3D-printed channels. AIBN was explored as catalyst for the fabrication of monolithic columns in various polypropylene materials in 3D-printed column housings. Two approaches were used for thermal monolith fabrication, viz. (i) a two-step modification reaction for a standard polypropylene filament using a short reaction time for AIBN and the crosslinker, which was followed by a second polymerization step to generate the monolith using thermal initiation; (ii) a silanization step, followed by a one-pot approach for a glass-reinforced polypropylene was.

The success of wall attachment was investigated, along with several other aspects, such as morphology, using Scanning Electron Microscopy (SEM). To evaluate the mechanical stability of the monolithic stationary phase in contact with organic solvents, the pressure drop across the housing was measured upon flushing it with various solvents. This will indicate the mechanical stability and permeability of the channels. Here, we present proof-of-principle separations using RPLC to demonstrate the feasibility of the polypropylene channels.

<u>STA-03</u>

Advances in HPLC Separations using Graphitized Carbon Particles

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A porous, graphitic carbon (PGC) has been synthesized for use as a stationary phase in high performance liquid chromatography (HPLC). The graphitic carbon is manufactured by impregnating a porous silica template with a proprietary copolymer. The polymer is then carbonized and the silica template removed prior to graphitization. It has exhibited strong HPLC retentive properties, including a unique selectivity for small, polar analytes. PGC is stable throughout the entire pH range of 0-14, exhibits excellent mechanical strength, and is compatible with all solvent systems. With this material, the existing PGC technology has been improved upon in a few ways. Most notably, particle size distribution has been tightened and theoretical plate count has been increased, while maintaining good separation characteristics. This poster will highlight the capabilities of PGC and its ability to separate compounds classified as difficult to separate by conventional silica-based HPLC, as well as its use in translating problematic, non-reproducible, or non-mass spectrometry (MS) compatible methods into much more user-friendly methods.

STA-04

Evaluation of New MS-Compatible Mixed-Mode RP/AX UPLC Columns

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Amit V. Patel

Waters Corp, United States of America

The separation of polar acids using reversed-phase (RP) HPLC poses significant challenges for currently available columns. Many acidic analytes of interest are poorly retained on conventional RP columns. Solutions to this challenge include the use of ion-pairing reagents or mixed-mode RP/anion exchange (AX) columns. However, most existing approaches are not compatible with mass spectrometry (MS) detection. In addition, binding of acidic analytes to metal surfaces in columns is a common problem. To overcome these challenges, we developed a new family of columns employing several novel technologies. The stationary phase is based on a new high phase-ratio hybrid organic/inorganic particle, bonded with C18 groups and a controlled low concentration of anion-exchange groups. The use of a bridged-ethyl hybrid composition allows the columns to be used with a broad range of mobile phase pH values. The material does not exhibit retention losses when used with 100% aqueous mobile phases. The anion-exchange groups have a pKa of approximately 7.5. Consequently, the columns exhibit anion-exchange retention at mobile phase pH values up to 8. The columns are compatible with mass spectrometry detection, exhibiting minimal ion suppression from column bleed. New column hardware was used to provide improved recovery of analytes containing phosphate and carboxylate groups that bind to metal surfaces in conventional columns. The chromatographic properties of the new columns have been evaluated and compared to existing RP columns designed for separating polar analytes.

STA-05

A novel unique separation column for underivatized direct LC-MS analysis of amino acids and related compounds

Itaru Yazawa

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Current LC-MS analysis strategies of intact amino acids are quite challenging due to the compound's polarity and ionic structures, often involving the use of complex derivatization steps and/or the use of non-volatile ion-pairing reagents.

We have succeeded to develop a specialized amino acid analysis column for use in LC-MS applications, which addresses these challenges. This column has both normal-phase (NP) and ion-exchange (IEX) mixed-mode technology built into the column, with the aim of providing the best possible retention and separation of amino acids without the need for derivatization or ion-pairing.

Recently, we have also found that this mixed-mode column can analyze not only amino acids but also derived metabolites. In this study, we show analysis of both amino acids and their various metabolites or related synthetic pathway compounds, using an underivatized LC-MS method.

Also another mixed-mode (reversed-phase and ion-echange) column for polar compounds will be presented and discussed.

SFC-01

Polyethylene Glycol (PEG) Characterisation by Supercritical Fluid Chromatography (SFC) with Evaporative Light Scattering and Mass Spectrometry Detection

Rebecca Mott

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Due to recent advances in instrumentation, there has been a resurgence of interest into SFC over the last couple of decades. In turn, its popularity as an analytical tool has steadily risen, with many advantages over LC increasingly being reported. Enhanced further with the ability to hyphenate SFC with a variety of detectors, the application of SFC as a separation technique for both chiral and achiral analysis in the pharmaceutical industry is an emerging area. In a time where synthetic routes to small molecule active pharmaceutical ingredients are becoming increasingly complex and the use of polymers in the development of new modality pharmaceuticals is growing, the establishment of fast and reliable analytical techniques for their characterisation is becoming of greater importance. To enable the detection and identification of non-UV absorbing achiral polymeric species (i.e., PEG) an ELSD and MS were hyphenated to a SFC instrument. Research work initially focused on investigating critical parameters and suitable chromatographic conditions in the analysis of mPEG acid. SFC successfully separated the polymers differing by one monomer unit, ELSD gave detection of the polymers with sufficient resolution and sensitivity and identification was obtained using the MS. Subsequently, the technique has shown versatility in the characterisation of other polymers and determining Mn, Mw and polydispersity values.

SFC-02

Retention Modeling of Supercritical Fluid Chromatography Separations

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Supercritical fluid chromatography (SFC) is a technique which has experienced variable interest since its introduction. Recently the interest in the technique has increased due to recent advances in instrumentation. Yet, in contrast to the pioneering SFC work, nowadays working under supercritical conditions is less and less common. Instead, mixtures of CO_2-modifier are used at the mobile phase under subcritical conditions. As a result, SFC is no longer a niche technique but is applied in diverse range of areas including environmental, food and bio-analysis. Whereas, the majority of the research groups focus on further expansion of SFC applicability, the important area of retention modelling remains relatively unexplored. Being able to predict retention under super- and sub-critical conditions would speed up method development and boost the growth in the field even further. Whereas, the retention in pure-CO_2 systems is relatively well-studied, that is not the case for mixtures comprising of CO_2 and a modifier, especially for mobile phases utilizing low amount of modifier. Meanwhile, literature focusing on retention modelling of both sub- and supercritical systems is rather scare. Therefore, the aim of this on-going research, is to find empirical equations that describe the retention under supercritical (pure CO_2 mobile phase) and sub-critical (CO_2-methanol mobile phase) conditions. To achieve this, the effect of pressure, density and modifier-percentage on the retention are considered.

SFC-03

Orthogonal Separation under Improved Sensitivity for Low Level Impurity detection by the Agilent 1260 Infinity II SFC/UHPLC Hybrid System Featuring an Agilent 1260 Infinity II Variable Wavelength Detector

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The detection of low-level pharmaceutical impurities in an active pharmaceutical ingredient (API) using a combination of the Agilent 1260 Infinity II SFC/UHPLC Hybrid System with an Agilent 1260 Infinity II Variable Wavelength Detector (VWD) is demonstrated. It is shown that the application of SFC and UHPLC separations in one system enables higher possibility to detect impurities in pharmaceutical APIs. Especially at low SFC flow rates with elution gradients at elevated temperature and smaller id columns, the VWD shows lower noise and therefore higher signal-to-noise (S/N) ratios than the Agilent 1260 Infinity II Diode Array Detector (DAD). These characteristics enable the determination of API impurities at the lowest levels.

Exemplified through a test mixture of the API metoclopramide and a selection of its impurities, it is demonstrated that at lower flow rates and the use of 3.0 mm id columns the detection of trace level impurities with the VWD is approximately four to five times more sensitive than with the DAD when comparing the respective S/N values. At elevated flow rates and with use of a standard 4.6 mm id column, the S/N values obtained from detection by VWD and DAD are in a comparable range [1]. For the detection of trace level compounds with lower flow rates and narrower ID columns, the VWD is the detector of choice. Using the VWD, it is possible to detect impurities at a very low level to fulfill the regulations described in the guideline Q3B(R2) published by the International Conference of Harmonization (ICH) [2]. Furthermore, the influence of different selectivities in SFC mode or UHPLC mode on the separation will be shown. Operating in two orthogonal separation modes enables intelligent screening for the best-suitable method. This capability, provided by the 1260 Infinity II SFC/UHPLC Hybrid System [3], delivers comprehensive information on complex mixtures for higher productivity and confidence in results.

References:

[1] E. Naegele, D. Kutscher, Agilent Application Note, publication number 5994-1351EN

[2] ICH Harmonized Tripartite Guideline: Impurities in New Drug Products Q3B(R2)

[3] E. Naegele, Agilent Technical Overview, publication number 5991-8276EN

SFC-04

Development of a generic method to analyze flavonoids with unified chromatography-electrospray ionization mass spectrometry

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In this project, we aimed at analysing flavonoid-type compounds employing carbon dioxide and methanol as major mobile phase components with unified chromatography (joining supercritical fluid chromatography and enhanced-fluidity liquid chromatography with carbon dioxide-methanol mobile phases) hyphenated to diode-array detection and electrospray ionization mass spectrometric detection (UC-DAD-ESI-MS). First, the chromatographic method was developed for 9 standard flavonoid molecules from three different families (flavanones, flavanols and flavonols, glycosylated or not), with a strong focus on mobile phase composition to achieve the elution of a wide range of flavonoids with satisfying peak shapes. For this purpose, two stationary phases were selected (ACQUITY UPC2 DEA and Diol), and different additives (formic acid, trifluoroacetic acid, methanesulfonic acid and ammonium hydroxide) were successively introduced in the methanol co-solvent. Methanesulfonic acid was retained as it provided the best peak shapes together with the possibility of hyphenating the chromatography to mass spectrometry. The gradient method was then optimized to achieve a fast analysis, which involved elution with a wide range of mobile phase compositions (from 20 to 100% co-solvent in carbon dioxide) together with reversed flow-rate and reversed pressure gradients at fixed temperatures. The final method was 10 min elution, followed by 2.5 minutes re-equilibration. Then ESI-MS detection was optimized. Because the single-quadrupole mass spectrometer employed (ACQUITY UPC2 QDa) allows the variation of only a few parameters, a design of experiment was used to define the best compromise for three parameters (probe temperature, cone voltage and capillary voltage). The make-up fluid introduced after the diode-array detector and before splitting the flow between the backpressure regulator and the MS was also varied: different compositions of methanol-water containing either formic acid, methanesulfonic acid, ammonium hydroxide or sodium chloride were tested. The best results, in terms of signal-to-noise ratio, were obtained with 20 mM ammonium hydroxide and 2% water.

The optimal UC-DAD-ESI-MS method was then applied to two different flavonoid formulation ingredients. The first one, Hidrosmin, is known for its vasoprotective properties and therefore employed in pharmaceutical formulations. The second one, α-Glucosyl-Hesperidin (sometimes referred to as Vitamin P) is employed in cosmetic formulations.

SFC-05

Determination of mineral oil saturated and aromatic hydrocarbons in consumer products by supercritical fluid chromatography with flame ionization and UV detection

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Mineral Oil Hydrocarbons (MOH) are a complex mixture consisting mainly of two classes of compounds, Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH). These compounds, especially MOAH, are potentially harmful for people and hence their content in commercial personal care products and food is regulated. Due to this, quantification and characterization of the different compounds present in mineral oil containing consumer products is needed.

Most of the methods published so far for mineral oil analysis are based on a pre-separation of MOSH and MOAH followed by quantification using GC-FID analysis for quantification or GC-MS for characterization. Unfortunately, these methods are highly time consuming and a complicated and expensive setup is needed. For these reasons, the development of a fast and simple method is essential.

Supercritical Fluid Chromatography (SFC) is a good alternative for mineral oil analysis. The selectivity of the stationary phases used in SFC allows a complete MOSH/MOAH separation while its hyphenation with the flame ionization detector allows a quantification of both fractions. Therefore, direct quantification of the MOSH and MOAH fractions without involving a two-step analysis is achieved.

In this work, a fast and simple SFC-FID/UV method for the analysis of MOSH and MOAH in consumer products is described. The separation of the two mineral oil fractions was performed in a bare silica column using CO_2 at supercritical conditions as a mobile phase. The SFC system was successfully coupled to the FID for quantification of MOSH and MOAH and the method was used for the analysis of different mineral oil samples.

References:

[1] M. Biederman et al., Aromatic hydrocarbons of mineral oil origin in foods: method for determining the total concentration and first result, J. Agric. Food Chem. 57 (2009) 8711.

SPECIAL ISSUE PUBLICATION – JOURNAL OF CHROMATOGRAPHY A

All authors of both oral and poster presentations are kindly invited to submit manuscripts based on their presentation(s) at the HTC-16 meeting for publication in Journal of Chromatography A with the intention of publishing in a Special Issue that is dedicated to this symposium.

The Special Issue essentially rules out possible delays in publication for contributors to the special issue. Please see below the publication process:

- All papers will go through the normal peer review process per journal standard;
- Papers will be published as soon as they are accepted in earliest available regular journal volumes at ScienceDirect, which ensures very fast publication for individual authors;
- There will be a Footnote included in each accepted paper, indicating at which conference it was presented;
- The collection of finally accepted papers will be prepared and hosted on a dedicated Special Issue site - with links to the papers on Science Direct, retaining all original citation details.
- Submission instructions:
- Submission link: JCA: https://ees.elsevier.com/chroma
- First-time users will need to register;
- Please select Special Issue short title " VSI: HTC-16 conference" during the submission process;
- Please follow the step-by-step guide in completing the submission procedure;
- Submission deadline: 30th April 2020

When preparing your manuscript(s), carefully follow the Guide to Authors of the journal, which you can find at the journal's homepage site. In the cover letter please mention that your manuscript is intended for the HTC-16 Special Issue.

We would like to specifically invite contributions of Concept Papers for Journal of Chromatography A - This new article type provides an opportunity to disclose new ideas early in their gestation period and before full validation. They should be brief and contain a maximum of 2500 words and 1-3 figures and/or tables. The format is similar to regular papers, and must contain an abstract and keywords section, with the length and detail of the remaining sections adapted to focus on the novelty of the contribution. This can best be achieved by writing in a direct style and only incorporating background and experimental details as seems sufficient to disclose the concept to readers familiar with the general subject matter.

Please note that all manuscripts will be subjected to the mandatory selection process for the journal selected, including the strict peer review procedure; therefore, acceptance for presentation at the conference is not a guarantee for publication in the journals.

Thanks for your attention and looking forward to your contribution!

Paola Dugo and Deirdre Cabooter

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