Multi-Residue Pesticides Analysis in Herbal Tea Products by GC-MS/MS

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Overview

Purpose

This poster describes the analysis of a large number of pesticide components from herbal and tea products using GC-MS/MS.

These types of dried plant products usually deliver high matrix loaded extracts from the sample preparation, and hence, pose a particular analytical challenge to the chromatographic and mass spectrometer system for a sensitive quantitation. High analyte selectivity and long term robustness are key for the high productivity pesticide monitoring in a routine trace analysis lab.

Methods

The tea or herbal product samples were extracted using accelerated solvent extraction. The collected extracts were cleaned-up by using gel chromatography. The analysis was done by GC-MS/MS using a timed-SRM detection method on the Thermo Scientific TSQ 8000 triple quadrupole GC-MS/MS instrument, employing two SRM transitions for each pesticide compound in a typical MRM method setup. Data processing and reporting are performed by using the Thermo Scientific TraceFinder software with one SRM transition used for quantitation and the second one for ion ratio confirmation of the positively identified pesticide compounds.

Results

The described method has been used for the routine analysis of a wide variety of herbs, teas and dried fruit with challenging heavy matrix impact for the control of the regulated maximum pesticide residue levels. A large number of real life samples have been analyzed demonstrating the robustness and productivity of the applied method.

FIGURE 1. TSQ™ 8000 GC-MS/MS system with Thermo Scientific TriPlus RSH autosampler with automated tool/syringe changer

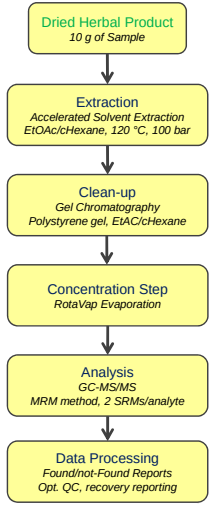
Introduction

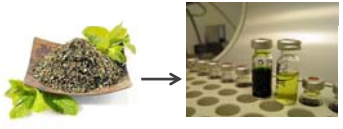
The residue analysis of pesticides has developed in recent years into a comprehensive methodology for the detection of many hundreds of potentially food contaminating compounds. A multi-residue method for teas and herbal products in general is faced with particular challenges with the high number of pesticides due to a worldwide origin of the products and the complex matrix of the dried plant materials.

In the due quality control of raw materials, the unknown or undeclared local plant protection treatment has to be taken into account with a wide variety of potential pesticide contaminations. The dried leaves, fruits or seeds and other medical products deliver highly complex extracts from the sample preparation due to the rich content in active ingredients, essential oils and the typical high boiling natural polymer compounds from broken cells, leaves or fruit skins.

Analytical Workflow

From Sample Preparation to Reporting





Methods

Sample Preparation

ASE Extraction Method

The herbal and tea samples were extracted using the automated accelerated solvent extraction (ASE) with the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor. ASE offers the advantage of a fast extraction for a wide range of pesticides of different chemical nature. Up to 24 samples can be loaded and automatically processed.

FIGURE 2. Dionex ASE™ 350 Accelerated Solvent Extractor.

From the dry herbal or tea samples, up to 10 g are added directly to the extraction cells. The extraction method has been described elsewhere [1]. During the extraction run, the cell is filled with solvent and then heated and pressurized. After the run, the extract is rinsed from the cell into a collection vessel for analysis or further clean-up. As extraction solvent ethylacetate / cyclohexane 1:1 is used (same as for subsequent GPC clean-up). The extraction temperature has been set to 120 °C at a pressure of 100 bar. One extraction cycle of 5 minutes was applied, with solvent rinsing of 60% of the cell volume. In a final step, the cell is flushed with nitrogen for 100s.

GPC Clean-up

The collected extracts get concentrated by a rotary evaporator (RotaVap) and further cleaned up via gel permeation chromatography (GPC). The extracts are separated from the majority of the matrix on a polystyrene gel (Bio-Beads S-X3) with a solvent mixture of ethylacetate / cyclohexane 1:1. After additional concentration by RotaVap the extracts are ready for GC injection using ethylacetate as the main solvent.

TSQ 8000 GC-MS/MS Method Setup

The analytical method comprises the sample handling using the TriPlus™ RSH liquid autosampler, Thermo Scientific TRACE 1300 Series gas chromatograph equipped with a temperature programmable PTV injector, and the TSQ 8000 triple quadrupole MS. The MRM detection method was taken as a proven solution from the long year routinely employed Thermo Scientific TSQ Quantum XLS GC-MS/MS system without any further optimization onto the TSQ 8000 GC-MS/MS system [4].

Triplus RSH Autosampler

Injection volume and type	1 µL, fast liquid band injection, 100 ms inj. time
Washing cycles	3 x 10 µL, solvent ethylacetate

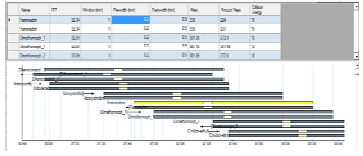
TRACE™ 1310 Gas Chromatograph

Injector PTV	splitless mode
Base temp.	50 °C
Transfer	10 °C/s to 250 °C, until end of run
Flow	const flow, 1.2 mL/min, helium
Analytical column	40 m, ID 0.18 mm, 0.18 um film, 5%-phenyl, 5MS type
Pre-column	5 m, ID 0.18 mm, empty deactivated, no backflush
Column oven	temp. programmed
Start	70 °C, for 1.50 min
Ramp 1	15 °C/min to 190 °C
Ramp 2	7°C/min to 290°C, 12 min
Transfer line	250 °C

TSQ 8000 Mass Spectrometer

Ion source temperature	220 °C
MRM Detection	timed SRM mode, see Figure 3

FIGURE 3. Principle of the Timed-SRM acquisition setup of the TSQ 8000. The white center parts show the peak width, grey the full SRM acquisition window.



Data Analysis

The data processing and reporting is achieved by using the TraceFinder™ quantitation and reporting software suite.

Results

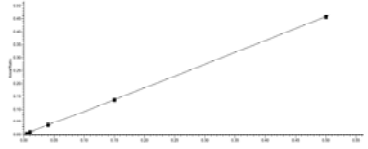
This method describes the methodology used for the multi-residue pesticides analysis of herbal products using the quick ASE sample preparation with GC-MS/MS detection employing the TSQ 8000 GC-MS/MS system for routine target analyte detection and quantitation. More than 200 pesticide compounds have been taken until today into a routine screening method which is applied for a wide variety of different sample types ranging from regular black tea or sage leaves, to seeds like fennel, and herbs of medical and fragrance use like thyme and chamomile.

Calibration and Linearity

The quantitative calibration and linearity check has been performed by using six calibration levels in the range of 0.004 µg/mL to 1.0 µg/mL. This range represents an analyte concentration of 0.01 to 2.5 mg/kg in the samples (10 – 2,500 ppb) being compliant with the above mentioned regulations.

The calibration solutions have been prepared in a standard matrix prepared from lemon peel with a matrix load equivalent to the typical herbal extracts applied. The standard matrix blank consisted of pure lemon peel extracted as of the standard procedure. The pesticide blank level was tested before applying as blank standard matrix. Solutions have been prepared containing lemon peel extract dissolved 1:1 with ethyl acetate. The correlation coefficients R^2 had been achieved for all pesticides compounds better than 0.99, see an example for cyfluthrine in Figure 4.

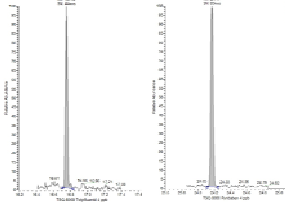
FIGURE 4. Quantitative calibration for Cyfluthrine, range 5 ppb to 500 ppb



Sensitivity

Using the standard pool of pesticides, the detection limits in the standard lemon peel have been determined. At the level of 4 ppb (equivalent of 0.01 mg/kg in the sample), the achievable S/N values have been calculated to estimate the limits of detection (LOD). The S/N values in matrix range from 12 for Alachlor to 83 for Pyridaben being representative for compounds eluting at retention times that are affected most from the eluting matrix. Although these compounds are eluting in heavily impacted matrix regions of the chromatogram, the high selectivity of the TSQ 8000 system for the target pesticides against a multifold-more intense matrix load is demonstrated with compelling S/N values in Figure 5.

FIGURE 5. Pesticide peaks at 4 ppb in lemon peel matrix for Tolylfluanid (238.1 > 137.1, CE 15 V) and Pyridaben (309.1 > 147.1, CE 15 V)



Analytical Precision

Within a routine series of commercial samples, the quality control calibration samples have been measured with replicate injections. The effect of the injected matrix on known sensitive pesticide compounds can be seen, while coefficients of variation (CV %) for unaffected compounds all stay well below 10% even within a long series of injections.

Results from Real Life Samples

The described method has been used for the routine analysis of a wide variety of herbs, teas and dried fruit known to be of the most challenging analytical task for controlling the pesticide maximum residue levels due to the heavy matrix impact. All compounds had been detected by using at least two SRM traces for a solid confirmation of positive findings by checking the known ion ratios. The concentration ranges covered are from below the MRL level of below 10 mg/kg to very high levels of up to 50x above the regulated maximum.

Conclusion

The analysis of pesticide residues in tea and herbal products follows the regulations by the European Directorate General for Health and Consumer Affairs (SANCO) for "Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed" [2], and of the Codex Alimentarius [3], with the maximum residue levels of 0.01 mg/kg for most of the pesticide compounds.

The TSQ 8000 system provided high selectivity and robustness for pesticide residue analysis in tea and herbal product samples, especially for those samples with high matrix load seen with the often dark colored extracts even after GPC clean-up. More than 200 of the most critical matrix samples have been measured so far without requiring any preventive maintenance on the mass spectrometer.

The sensitivity of the TSQ 8000 system reached significantly below the regulated levels even in matrix samples, offering a safe monitoring and control of the regulated pesticide maximum levels for tea and other dried herbal products. Confirmation of positive findings has been provided by checking the pesticide characteristic ion ratio.

The precision of quantitative results stayed within 10% for a series of commercial and quality control samples. The calibration has been performed as standard matrix spike and showed excellent linearity from below the MRL levels.

Due to the high matrix selectivity and low baseline noise of the TSQ 8000 system, the pesticide peak integration in matrix delivered very reliable area results, reducing significantly the manual quality control reducing a typical bottleneck in trace analysis laboratories and increasing the productivity for the final sample report processing.

References

1. Accelerated Solvent Extraction (ASE) of Pesticide Residues in Food Products, Dionex Application Note 332, 2011.
2. SANCO Document N° SANCO/12495/2011, Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Implemented by 01/01/2012.
3. Codex Alimentarius (www.codexalimentarius.net/mrls/pesticides/jsp/pesq-q-e.jsp)
4. Pesticide Method Reference 2nd Edition, Thermo Fisher Scientific, p/n 120390.