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Applikationsbericht

Q-TOF MS and Residue Analysis

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

In this application brief, Q-Tof MS technology is described as a viable MS approach for detection and/or quantification of trace amount of contaminants that may be present in a food, feed, or biological matrices.

Benefits

- It has good sensitivity, selectivity, improved dynamic range, speed, and it can handle virtually an unlimited number of multi-residue analytes in a single run.
- Commission Decision 2002/657/EC and Guide SANCO/10684/2009 provide criteria and minimum requirements for method validation and analytical quality control procedures for residue analysis.

Introduction

Residue analysis for food safety involves detection and/or quantification of trace amount of contaminants that may be present in a food, feed, or biological matrix (***e.g.***, meat, hair, tea, urine). The contaminants may include pesticides, veterinary drugs, growth promoters, and their metabolites. Their presence in food is a potential threat to consumer health, and international organizations have issued regulations on these substances in food products.[1]

Tandem mass spectrometry (MS/MS) coupled to liquid chromatography (LC) is currently the most widely used technique for organic residue analysis. This is mainly because of its highly sensitive and selective multiple reaction monitoring (MRM) mode of measurement. In this mode, pre-selected ions (precursor ions) are fragmented and their characteristic fragment ions (product ions) are detected and quantified. It has high sensitivity, high selectivity, wide detector dynamic range, and it is very suitable for trace level detection and quantification. Multiple residues can be analyzed in a single run by setting up multiple timed MRM windows (or MRM channels). Using this approach, multiple residues of over 400 substances may be screened with one injection and confirmed with two injections by LC-MS/MS analysis.[2]

Although LC-MS/MS is the most used MS technique in residues analysis laboratories, it has its own limitations. First, it is not suitable for non-targeted analysis. Prior knowledge of the characteristic transitions (precursor ions to product ions) for a substance is a prerequisite for MRM measurement. It is impossible to predict the characteristic MRM transitions of the unknown beforehand. Secondly, there are a finite number of MRM transitions that can be monitored by LC-MS/MS technique in a single run, since each transition needs dedicated mass analyzer machine time.

Quadrupole Time-of-Flight (Q-TOF) mass spectrometry is a hybrid technique that combines the benefits of the MS/MS technique and the TOF accurate mass MS technique. It offers an alternative but attractive approach for residue analysis. Modern Q-TOF MS instruments, such as the Waters Xevo QTof mass spectrometers, provide accurate mass determination, high mass resolving power, improved dynamic range, and speed. They are capable of analyzing virtually unlimited numbers of substances simultaneously without any prior knowledge of the analytes. This not only avoids tedious MRM tuning for multiple compounds as a tandem quadrupole instrument requires; but more importantly, it opens the door for non-targeted or unknown analysis. This is especially useful when analytes have no reference standards available. Furthermore, the full scan mass spectrometric data obtained by TOF MS can be analyzed retrospectively when new substances of interest emerge. This kind of "collect first – think later" practice reduces the number of repeated analysis of the original samples.

Results and Discussion

This "collect first – think later" idea is further improved by Waters' patented MS^E technique, which collects not only the accurate mass spectra of all of the molecular ions, but also the fragment ions of the sample during the data acquisition. MS^E does this by switching between low and high collision energy in the collision cell during the data acquisition. This allows all of the accurate masses of molecular ions and fragment ions, as well as their chromatographic retention times, to be collected in a single run. These interrelated retention time-aligned molecular and fragment ion accurate mass spectral data, generated only by Waters patented Q-Tof MS^E technique, provide a unique solution to resolve potential co-eluting isobaric, or even isomeric ions. Other MS instruments, no matter how high its mass resolution is, cannot resolve co-eluting isomeric ions based on mass resolution alone. Figure 1 illustrates the benefits of MS^E for identifying simetryn and desmetryn isomers.[3]

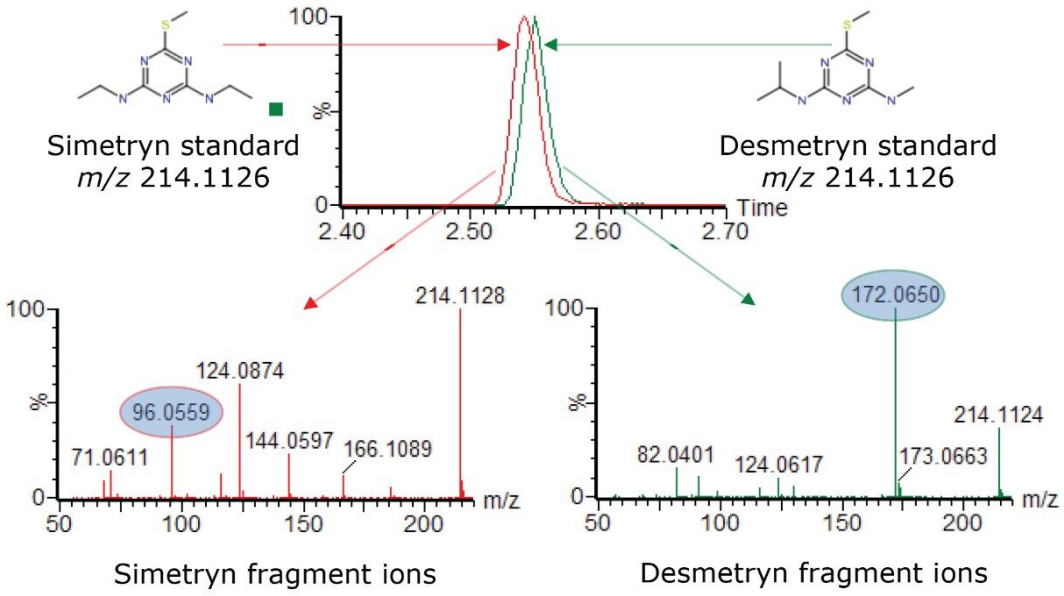

Figure 1. Simetryn and desmetryn are isomeric and co-elute, but have unique MS^E fragment ions (circled) that can be used to unequivocally identify them.[3]

EU regulation

EU Commission Decision 2002/657/EC[4] and Guidance SANCO/10684/2009[5] are important regulations for residue analysis in the European Union community. The document 2002/657/EC provides guidance on performance criteria, requirements, and procedures for analytical methods concerning substances and residues in live animals and animal products, while the SANCO/10684/2009 document provides method validation and quality control procedures for pesticide residue analysis in food and feed. There is a partial overlap in coverage areas for these two guidelines, since some food and feed products are of animal origin.

Decision 2002/657/EC, which was implemented in 2002, specifies performance criteria and requirements for the screening and confirmatory analytical methods and validation procedures. According to this guideline, the analytical screening methods should have less than 5% (beta-error) false compliant rate (or false negative). For the confirmatory methods for organic residues and contaminants, chromatographic analysis coupled to mass spectrometer or other detectors (such as IR, fluorescence, full-scan DAD, etc.) can be used. When MS is used, the minimum requirements for the relative ion intensities (ion ratios), full scan MS spectral data, and non full-scan MS data are specified in the Decision 2002/657/EC. It should be pointed out that a system of identification points was introduced for non full-scan MS data, such as SIM (selected ion monitoring) or MRM, as shown in Table 1. High resolution MS (HRMS) earns more identification points than low resolution MS per diagnostic ion; however, the guideline lacks the necessary mass accuracy requirement

for HRMS.

MS technique	Identification points earned per ion
Low resolution mass spectrometry (LR)	1,0
LR-MS^n precursor ion	1,0
LR-MS^n transition products	1,5
HRMS	2,0
HR-MS^n precursor ion	2,0
HR-MS^n transition products	2,5

Table 1. The relationship between a range of classes of mass fragment and identification points earned in Commission Decision 2002/657/EC.

Footnotes:

1. Each ion may only be counted once.

2. GC-MS using electron impact ionization is regarded as being a different technique to GC-MS using chemical ionization.

3. Different analytes can be used to increase the number of different points only if the derivatives employ different reaction chemistries.

4. For substances in Group A of Annex 1 to Directive 96/23/EC, if one of the following techniques are used in the analytical procedure: HPLC coupled with full-scan diode array spectrophototometry (DAD); HPLC coupled with fluorescence detection; HPLC coupled to an immunogram; two-dimensional TLC coupled to spectrometric detection; a maximum of one identification
point may be contributed, providing that the relevant criteria for these techniques are fulfilled.

5. Transition products include both daughter and granddaughter products.

Guide SANCO/10684/2009, which was implemented in 2010, provides guidelines for method validation and quality control procedures, from sampling, transport, processing and storage of samples, to reporting of results. Its criterion for screening methods is also less than 5% beta-error in false negative rate, the same as 2002/657/EC. It contains more specific requirements on the MS data for the confirmation or identification of potential positive results, described in Table 2, than the decision 2002/657/EC does. The benefits of high resolution full scan MS data is also recognized in that it allows fewer HRMS diagnostic ions for confirmation. In addition, the mass accuracy requirement (< 5 ppm) is specified for HRMS. No identification point system is used, however, in SANCO/10684/2009.

MS mode	Single MS (standard mass resolution)	Single MS (high resolution/high mass accuracy	MS/MS
Typical systems (examples)	quadrupole, ion trap, time-of-flight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole ion trap, hybrid MS (*e.g.* Q-TOF, Q-trap)
Acquisition	Full scan, limited *m/z* range, Selected ion monitoring (SIM)	Full scan, limited *m/z* range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for identification	≥ 3 diagnostic ions, (preferably including the quasi molecular ion)	≥ 2 diagnostic ions (preferably including the quasi molecular ion). Mass accuracy < 5 ppm. At least one fragment ion.	≥ 2 product ions

Table 2. Identification requirements for different types of mass spectrometers in SANCO/10684/2009.[5]

High mass resolution in MS

In both regulations, the benefit of high resolution MS for confirmation is recognized. However, the definitions of HRMS in both regulations are not consistent. The earlier regulation (2002/657/EC) defines HRMS as the resolution typically > 10,000 at 10% valley, which is commonly believed equivalent to 20,000 FWHM (full width at half maximum), while the newer regulation (SANCO/10684/2009) defines it as > 10,000 FWHM. In spite of the ambiguity of the HRMS definition, Q-TOF MS instruments of various mass resolving power have been successfully applied to residue analysis of veterinary drugs and pesticides in food matrices.[6-9]

Conclusion

A tandem quadrupole mass spectrometer coupled to an LC is the most popular technique in targeted organic residue analysis. The development of Q-TOF MS technology, however, provides an attractive alternative MS approach in organic residue analysis. It has good sensitivity, selectivity, improved dynamic range, speed, and it can handle virtually an unlimited number of multi-residue analytes in a single run. Its accurate mass spectral data can be retrospectively processed for new emerging compounds of interest, and

its non-targeted analysis capability can not be matched by a tandem quadrupole LC-MS/MS approach.

Commission Decision 2002/657/EC and Guide SANCO/10684/2009 provide criteria and minimum requirements for method validation and analytical quality control procedures for residue analysis. These guidelines recognize the benefits of high resolution and accurate mass MS data for residue analysis, although the definitions of high resolution MS are not unified.

Besides the high mass resolution and accurate mass, it is worth pointing out that other important factors, such as ion fragmentation, dynamic range, sensitivity, acquisition speed, data processing software, sample extraction, and LC separation, all play important roles in the screening and confirmation in residue analysis. Instead of relying mainly on the high mass resolution of the mass spectrometer, a good analyst should take all these factors into consideration and adopt a systematic approach to achieve the best overall performance in the residue analysis.

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