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QuEChERS extracted pesticide quantitation by LCMS QTOF using HRAM at high data acquisition speed

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Overview

- Applying high resolution accurate mass (HRAM) QTOF analysis for routine quantitative pesticide monitoring programs in agreement with SANTE/11813/2017.
- A panel of 212 pesticides were quantified using a MS and DIA-MS/MS method with a cycle time of less than 0.8 seconds to acquire 39 mass scans.
- The method detected and quantified the target panel of pesticides at the default maximum residue level (MRL) value of 0.01 mg/kg for all targets and below.

Introduction

Pesticide monitoring programs are designed to identify and quantify pesticides within a defined regulatory framework. To minimize the risk to public health, maximum residue levels (MRL's) have been set for pesticides in food products/groups within the EU. In this work a panel of over 200 pesticides were quantified using high data acquisition speeds and high resolution accurate MS and MS/MS analysis (in agreement with the guidelines stated in SANTE/11813/2017). A number of commodities were considered in this analysis and extracted using a conventional QuEChERS method.

Materials and Methods

The method was designed to meet the regulatory guidelines identified in SANTE/11813/2017 for EU food safety. The criteria for confirmation requires the measurement of 2 ions with a mass accuracy ≤ 5 ppm (for masses below m/z 200 the tolerance is ≤ 1 mDa) preferably including the molecular ion (or de-protonated molecule or adduct ion) and at least one characteristic product ion. The retention time of the analyte in the extract should correspond to that of the calibration standard (may need to be matrix-matched) with a tolerance of ±0.1min. Criteria for HRAM ion ratio confirmation differs from nominal mass guidelines. No generic guidance value for ion ratio is given as the added value of accurate mass measurement results in a different approach for HRAM analysis. As matching ion ratios are less critical in HRAM analysis, they should be considered as indicative and deviations exceeding 30% should be further investigated and judged with care.

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Concept Life Sciences, UK. Matrices included avocado, cocoa, curry leaf and flaxseed. Final extracts were prepared in acetonitrile without any dilution and directly injected into the LC-MS/MS. A water co-injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes in addition to a sub 3 micron particle size column to improve peak capacity.

A panel of pesticides was separated using a Restek Raptor ARC18 (100 x 2.1mm 2.7um) column using a binary gradient of Solvent A (formic acid (0.004%) in 2mM ammonium formate solution) and Solvent B (formic acid (0.004%) and 2mM ammonium formate solution in methanol).

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HRAM QTOF MS and DIA-MS/MS analysis

HRAM LC-MS/MS data (Nexera LC and LCMS-9030 QTOF system, Shimadzu Corporation) was acquired using a MS and DIA-MS/MS method with a total cycle time 0.773 seconds over a MS/MS mass range of 65-900 Da. The initial mass scan acquired full scan precursor ion data over a mass range of 140-900 Da in profile mode (13 msecs) followed by 38 DIA-MS/MS mass scans with a precursor isolation width of 20 Da (the accumulation time for each mass scan was 20 msecs and acquired in centroid mode). The collision energy for DIA-MS/MS mass scan was 0-30V to ensure precursor ion and product ion data were present in each DIA-MS/MS scan.

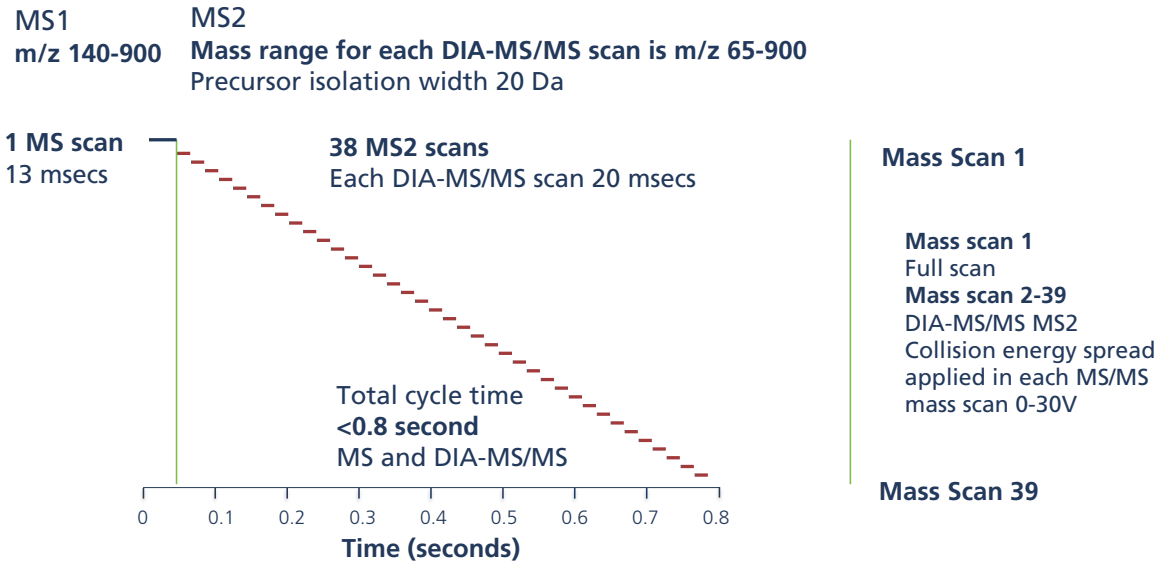

Figure 1. LCMS-9030 QTOF MS and DIA-MS/MS method for targeted and untargeted workflows for pesticide monitoring programs with a cycle time of 0.8 seconds for a mass range of m/z 65-900.

The SANTE/11813/2017 guidelines for the analysis of pesticide residues and analysis in food and feed require 2 ions with a mass accuracy ≤ 5 ppm (for masses below m/z 200 the tolerance is ≤ 1 mDa). To meet this need an accurate mass database of molecular ion and fragment ion data was created using a research application software (Figure 2).

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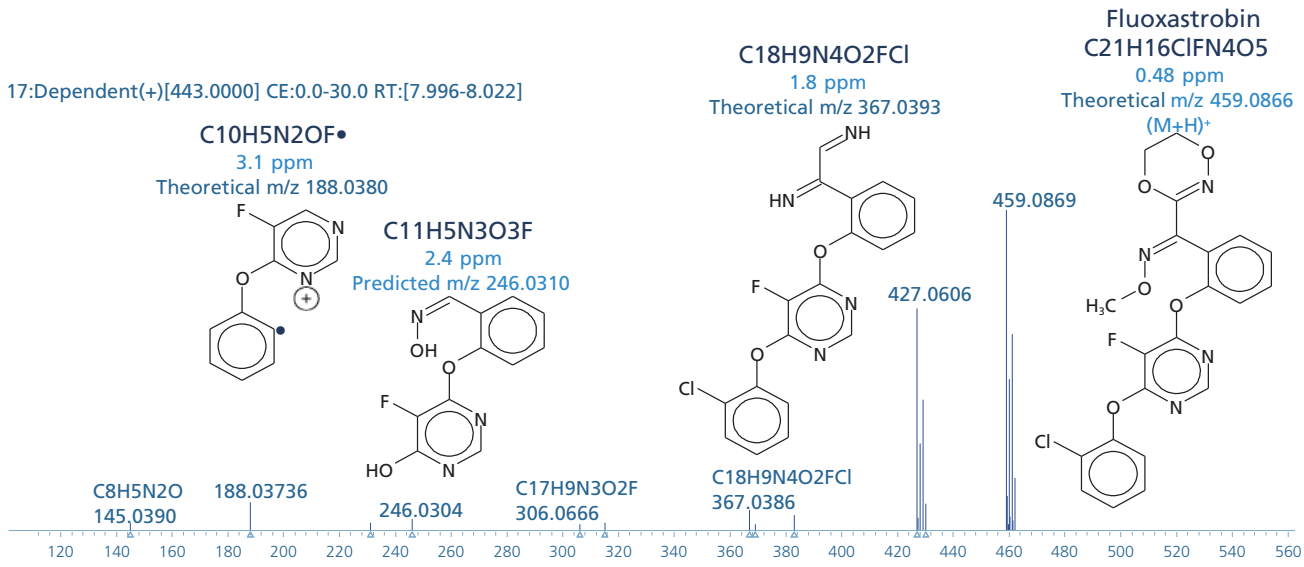

Figure 2. An accurate mass database of molecular ion and fragment ion data was developed for routine pesticide screening using a research application to assign structure and formula for each fragment ion detected in DIA-MS/MS and DDA-MS/MS. The database has over 220 pesticides.

Accurate mass precursor and product ions

The MS/MS fragment ion annotation algorithm was used to assign structures and chemical formula for all target pesticides. A HRAM method was created for over 200 pesticides with theoretical accurate mass data for precursor and product ions.

Table 1. The target compound list in the DIA-MS/MS method included theoretical accurate mass values for both the precursor ion (quantitative molecular ion) and product ions (confirmatory ion for identification and verification to meet the needs of the SANTE/11813/2017 guideline). A database of over 200 pesticides was generated with accurate mass molecular and fragment formula. The table shows an extract of the method for 10 target compounds and product ion confirmation for 2 ions (the data base has product ion accurate mass for the 4 most intense fragment ions).

Rt (mins)	Compound Name	Quantitation Ion		Product ion confirmation		Product ion confirmation	
		Formula	Theoretical m/z	Formula	Theoretical m/z	Formula	Theoretical m/z
7.7	Benthiavalicarb isopropyl	C18H24FN3O3S	382.1595	C9H7NFS	180.0278	C9H10N2FS	197.0543
7.8	Methoxyfenozide	C22H28N2O3	369.2173	C9H9O2	149.0597	C7H7	91.0542
7.8	Bifenazate	C17H20N2O3	301.1547	C13H12NO	198.0913	C12H12N	170.0964
7.9	Fenpyrazamine	C17H21N3O2S	332.1427	C13H16N3O	230.1288	C13H17N3O	231.1366
7.9	Mefenacet	C16H14N2O2S	299.0849	C9H10NO	148.0757	C8H10N	120.0808
7.9	Dichlofluanid	C9H11Cl2FN2O2S2	349.9961	C6H5NS	123.0137	C7H5NFSCl2	223.9498
7.9	Fenhexamid	C14H17Cl2NO2	302.0709	C7H13	97.1012	C4H7	55.0542
7.9	Iprovalicarb	C18H28N2O3	321.2173	C9H11	119.0855	C7H7	91.0542
7.9	Azinphos-ethyl	C12H16N3O3PS2	346.0444	H2O2PS	96.9508	C3H8NOPS	137.0059
7.9	Fluoxastrobin	C21H16ClFN4O5	459.0866	C20H13ClFN4O4	427.0604	C10H5N2OF	188.0380

Results

HRAM DIA-MS/MS analysis

HRAM DIA-MS/MS was used to quantitate a panel of pesticides over a concentration range of 0.002 – 0.2 mg/kg in an apple matrix. The exact mass of the most abundant ion (typically the protonated molecule, in some cases the ammonium adduct ion) was used to quantitate each target compound with a mass extraction window of ≤ 5 ppm.

Precursor ion quantitation was processed using the DIA-MS/MS mass scan specific to each target pesticide (all DIA-MS/MS mass scan's had a collision energy spread of 0-30V to acquire both precursor and product ion data at the same time).

QuEChERS extract spiked with over 200 target pesticides
Untargeted MS and DIA-MS/MS; QTOF Data Acquisition LCMS-9030
Cycle time <0.8 second for all MS and DIA-MS/MS mass scans
Mass chromatograms for the panel of pesticides at the MRL of 0.01 mg/kg
Molecular ion (typically the protonated molecule)
≤ 5 ppm (for masses below m/z 200 the tolerance is ≤ 1 mDa)

Restek ARC separation
To minimize matrix effects the injection volume was 2 uL

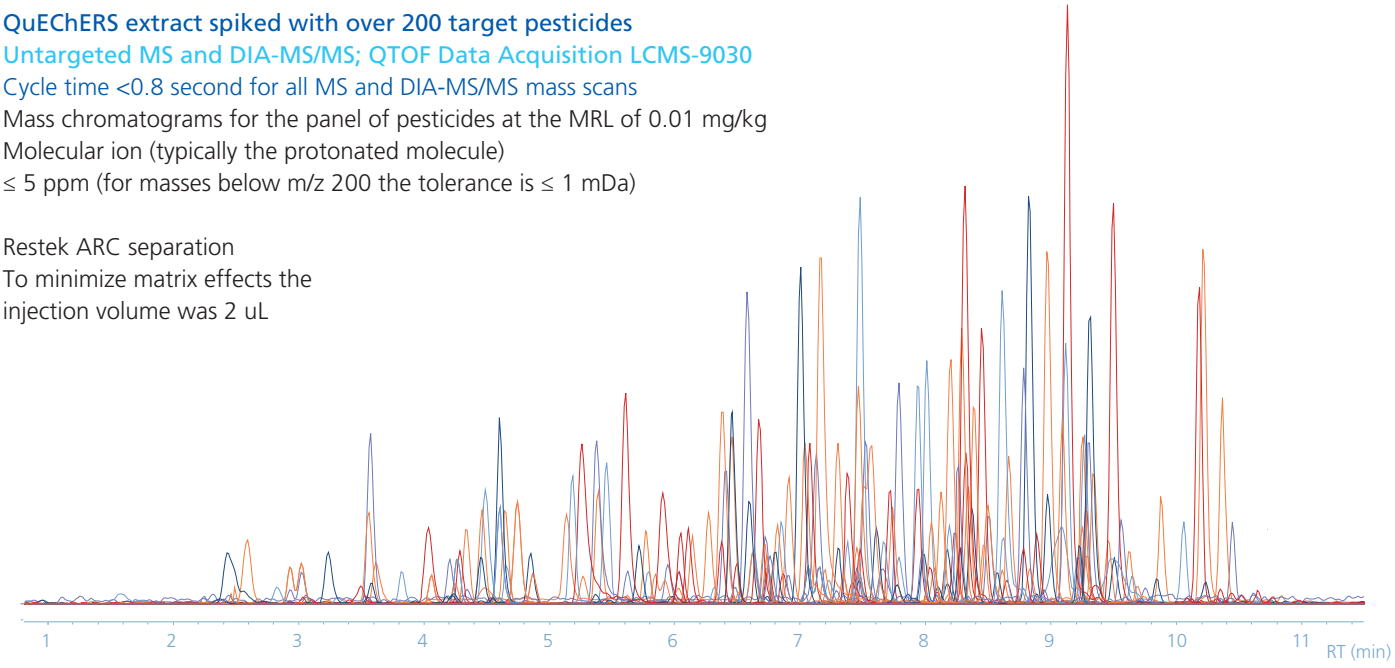

Figure 3. An accurate mass database of molecular ion and fragment ion data was developed for routine pesticide screening using a research application to assign structure and formula for each fragment ion detected in DIA-MS/MS and DDA-MS/MS. The database has over 220 pesticides.

HRAM DIA-MS/MS quantitative analysis

Example calibration data of 3 selected pesticides is shown in Figure 4. Calibration curves were generated for 212 target pesticides using either a linear or quadratic fit (typical R2>0.99). 4 deuterated internal standards were used.in the quantitative data processing.

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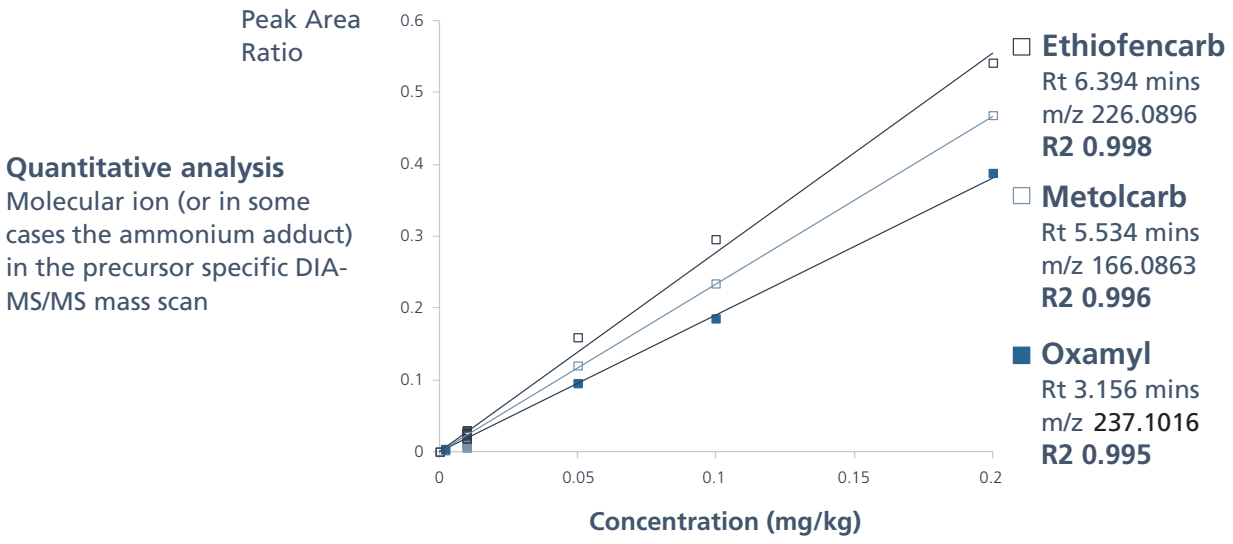

Figure 4. Calibration curves for 3 selected pesticides (ethiofencarb, metolcarb and oxamyl) from 0.002-0.2 mg/kg spiked into a QuEChERS extract.

Regulatory requirements for identification

For nominal mass triple quadrupole platforms the guidance for identification is to achieve ion ratio from sample extracts should be within ±30% (relative) of average of calibration standards from same sequence. However, for accurate mass the guidelines consider the variability of ion ratios which may be affected by several confounding factors such as the S/N of the peaks in the extracted ion chromatograms and matrix effects. It recommends deviations exceeding 30% should be further investigated and judged with care.

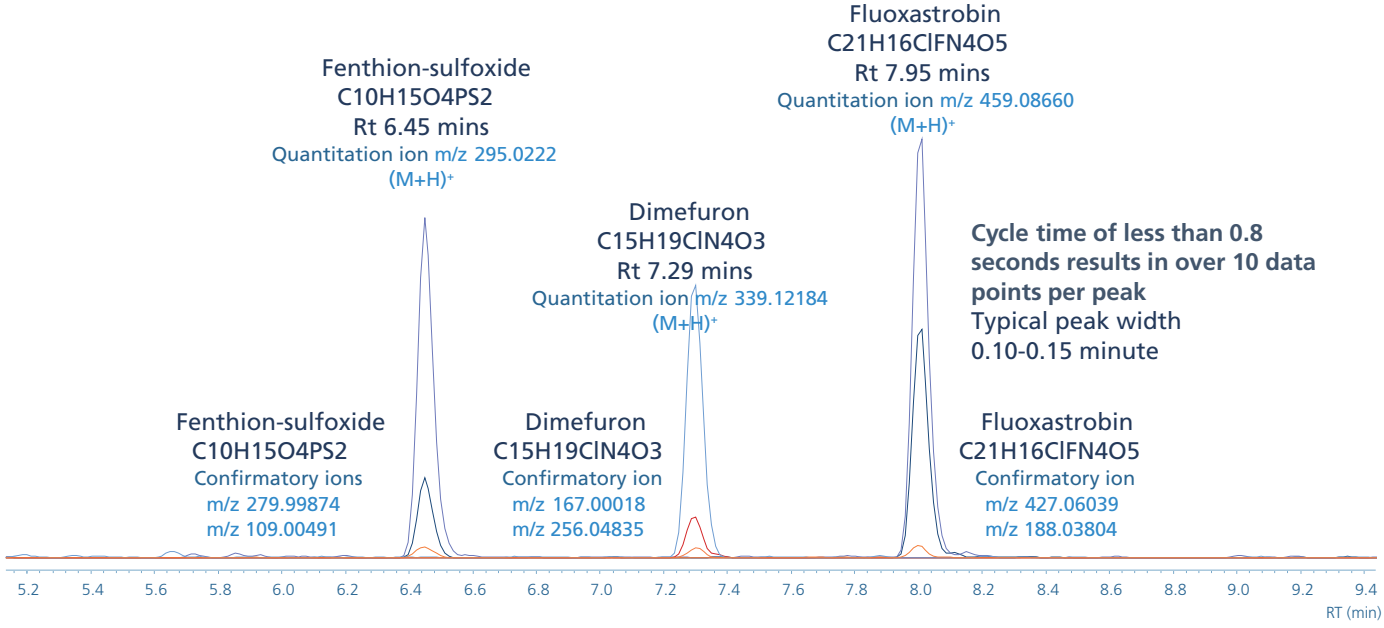

Figure 5. Confirmatory ions used to identify each target pesticide. In this example 3 pesticides are shown with 3 confirmatory ions with a mass accuracy ≤ 5 ppm (for masses below m/z 200 the tolerance is ≤ 1 mDa) for the 0.01 mg/kg MRL standard.

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Conclusion

A HRAM DIA-MS/MS method has been applied to the quantitation of 212 pesticides using high data acquisition speeds (in agreement with the SANTE/11813/2017 guidelines). This approach results in a robust quantitative method which can be used for targeted and untargeted data processing.

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