Overview

- Applying high resolution accurate mass (HRAM) QTOF analysis for routine quantitative pesticide monitoring programs to meet EU SANTE/12682/2019 validation guidance.
- The analytical method was based upon a validated triple quadrupole LC-MS/MS analysis (LCMS-8060) and transferred to a QTOF (LCMS-9030).
- A HRAM QTOF method was applied to the analysis of a panel of over 200 pesticides quantified using a TOF MS mass scan and DIA-MS/MS ion ratio confirmation with 31 DIA-MS/MS mass scan events. The cycle time was less than 0.875 seconds to acquire 32 mass scans for MS and DIA-MS/MS.

1. Introduction

The EU SANTE/12682/2019 guidelines for the Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed identifies the following criteria;

- 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 deprotonated molecule or adduct ion) and at least one characteristic product ion.
- Precision (expressed as repeatability RSD) ≤ 20% for each spike level.
- Rt variance ± 0.1 minute.

2. Methods

Sample Preparation

Pesticide spiked samples, extracted using established QECHEERS based methods, were provided by Concept Life Sciences, UK. Matrices included apple, tomato and orange. Final extracts were prepared in acetonitrile without 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.

LC separation

The panel of pesticides were separated using a Restek Raptor Bighplex (100 x 2 mm, 2.7um) column using a binary gradient of Solvent A (formic acid (0.004%) and 2mM ammonium formate) and Solvent B (formic acid (0.004%) and 2mM ammonium formate solution in methanol). Flow rate 0.4 mL/min.

Mass Spectrometry

The LCMS-9030 HRAM QTOF (Shimadzu Corporation, Japan) system with Electrospray ionization (ESI) was used in positive ion mode. TOF-MS mass scan 140-900 Da; 100 msec.

- 31 dependent MS/MS mass scans 65-900 Da; each DIA-MS/MS mass scan was acquired for 25 msec; isolation width of 20 Da up to m/z 540, above m/z 540 variable isolation windows were used; CIE spread of 5-55 V. The total cycle time for all 32 mass scans was 0.875 seconds.
- All data were acquired using external mass calibration only (a TOF mass calibration was typically performed after 3-4 days of continuous analysis). LabSolutions Insight software was used for data review.

3. Results

3.1 Target workflows for pesticide screening

- Mass chromatograms for a panel of over 200 pesticides at 0.010 mg/kg (± 5 ppm). All compounds were detected at the default MRL of 0.010 mg/kg in the TOF Survey mass scan. As the cycle time was 0.875 seconds for all MS and DIA-MS/MS mass scans it was possible to acquire between 10-20 data points across a target peak generating reproducible peak integration and robust quantitation.
- Precision (expressed as repeatability-RSD%); ms. The SANTE/12682/2019 guidance states that the variability of (at least 5) replicate injections (expressed as repeatability-RSD%) should be taken into account. In the batch analysis of 92 samples, calibration standards spiked into an apple matrix over a concentration range of 0.002-1.0 mg/kg were repeatedly injected (n=6). At the lowest calibration standard of 0.002 mg/kg, 183 target compounds resulted in a variance less than 20%.
- Precision (expressed as repeatability-RSD%); ns50 at the MRL level. To assess the system robustness the default MRL sample at a concentration of 0.010 mg/kg sample was repeatedly injected (n=50) and automatically processed using default peak integration parameters (PeakFinder algorithm). 194 target compounds resulted in a peak area variance (RSD%) less than 15%: 203 target compounds were found with a variance less than 20%.

3.2 Suspect screening workflows for pesticide screening.

A key advantage of acquiring TOF Survey scan and DIA-MS/MS mass scans is the capability to analyze the data retrospectively as every data point has precursor and product ion information.

To meet the needs of quantitative pesticide monitoring programs to meet EU SANTE/12682/2019 validation guidance, LabSolutions Insight software was set up to highlight mass accuracy error of the quantitative precursor (TOF Survey scan) and product ion (DIA-MS/MS mass scan), precursor and product ion mass chromatograms and Rt variance.

- Figure 1. To meet the needs of EU SANTE/12682/2019 validation guidance, LabSolutions Insight software was set up to highlight mass accuracy error of the quantitative precursor (TOF Survey scan) and product ion (DIA-MS/MS mass scan), precursor and product ion mass chromatograms and Rt variance.

- Figure 2. In suspect screening workflows Insight Analyze algorithms are used to detect components and match to a list of suspected target compounds. Using the same data file and same compound search list as shown in figure 1, the resultant matches from the suspect screening workflow agree with the targeted data presented in figure 1. Key steps;
  - Component detection. The first step is to locate components in the raw data file. The algorithm locates ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner) and applies several grouping and filtering steps to give a single component for grouped ions.
  - Suspect search list. A search list of target compounds (a spreadsheet with compound name, target m/z, target formula and target retention time together with a mass tolerance and retention time window) is then used to match detected components with the search list. If the match is within the expected mass tolerance and retention time tolerance the target compound is reported. In figure 2, the resultant target compound matches are reported as mass chromatograms which agree with the targeted workflow for the same data file.

4. Conclusion

A HRAM DIA-MS/MS method was applied to the quantitation of over 200 pesticides using high data acquisition speeds (in agreement with the SANTE/12682/2019 guidelines). This approach results in a robust quantitative method which can be used for targeted and untargeted data processing.