Applying ‘MRM Spectrum Mode’ and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

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1. Introduction

To help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ions to increase specificity and reporting confidence. In this work, typically 6–10 fragment ion transitions were monitored for each target pesticide as opposed to a traditional approach using 2–3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation pattern that could be used in routine library searching and compound verification using reference library match scores. This MRM Spectrum mode was applied to quantify and identify 193 pesticides using 1,291 MRM transitions without compromising limits of detection, linearity or repeatability using a high speed data acquisition triple quadrupole MS/MS.

2. Materials and Methods

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Scientific Analysis Laboratories, UK. Matrices included turmeric, plum, pepper, pumpkin, cherry, lime, pumpkin, apple, cucumber, and potato. Final extracts were prepared in acetonitrile without any dilution and directly injected into the LC-MS/MS. A water injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes in addition to a sub 2 µL minute particle size column to improve peak capacity (Table 1).

3. Results

3.1 MRM Spectrum based identification

Figure 1. Using a high speed triple quadrupole mass analyser a higher number of fragment ions were acquired in MRM increasing the specificity of detection and reducing false negative and false positive reporting. However in turmeric, no precursor-ion fragment ions were used to increase confidence in assay specificity. There is no compression in data quality between methods despite a higher number of fragment ions monitored. Signal intensity, reproducibility are in good agreement between both methods.

Table 1. LC and MS/MS acquisition parameters used to create the LC-MS/MS method

Table 4. MRM product ion spectra for 12 pesticides with a similarity score of >90. Library searchable fragment data is used in target compound identification without compromising sensitivity, accuracy and robustness in quantitative data reporting.

3.2. MRM Spectrum Quantitation and Library Searching

Figure 5. The limit on the number of MRM transitions used to generate a product ion spectrum is dependent on the chemical structure of the pesticide molecule. In the case of carbamates, several bonds could be broken using collision energies between 10-60V resulting in a product ion spectrum of 12 fragment ions. The product ion spectrum can then be used for library search and analysis confirmation as shown above. For each calibration level ranging from 0.010-0.300mg/kg the library similarity score was greater than 90 confidence confirming the target compound identity.

4. Conclusions

- False positive results are a major issue for all pesticide residue monitoring laboratories. EU regulations require robust data and the ion ratio between MRM transitions are within a set threshold. However, even applying this criteria false positives may occur for certain pesticide matrices.
- We have applied MRM Spectrum mode to identify and quantify 193 target pesticides in a number of different sample matrices. This library has been verified by the library supplier and is used as an additional identification criterion in order to improve confidence when reporting results.
- Acquisition of the MRM Spectrum method (1,291 MRM transitions) did not compromise data quality when compared to a conventional 2 MRM per compound method (384 MRM transitions) with consistent signal response and repeatability in both methods. The MRM pesticide ion transitions were consistent across the linear range and between different matrices. This method acquired data in both positive and negative ion modes with a promise switching time of 5 ms enabling fast cycle times and a high data collection rate.