

Development of a high-resolution MRM quantitative method for pesticides in apple, honey, olive oil, orange and tomato food matrices

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Overview

- The European Union SANTE 11312/2021 v2 guidelines describe the method validation and analytical quality control requirements for reporting multi-residue pesticide screening in food safety. This document provides regulatory guidance for nominal mass and accurate mass LC-MS/MS acquisition and data processing.
- In this work, a high-resolution LC-MS/MS QTOF was applied to the analysis of a panel of over 300 pesticides using a method which includes an MS full scan and selective fragment ion detection (MRM mode) compared to an untargeted data independent acquisition (DIA) approach.
- The optimized selective fragment ion method resulted in higher reference ion signal intensities and generated lower limits of quantitation.
- For some components and food commodities the MRM mode also generated a greater selectivity for some target pesticides.

1. Introduction

Regulatory organisations such as Environmental Protection Agency (EPA), the European Union (EU), Food and Agricultural Organization (FAO) and World Health Organization (WHO) have defined maximum residue levels (MRLs) to set a common assessment scheme for all agricultural products for food or animal feed. A general default MRL of 10 µg/kg applies where a pesticide is not specifically mentioned. The EU SANTE 11312/2021 v2 guidelines have been applied to the quantitative analysis of a panel of pesticides in different types of food commodity groups using an LC-MS/MS QTOF with an optimized fragment ion method to help enhance sensitivity and selectivity.

2. Materials and Methods

- Sample preparation.** 317 pesticides and 10 deuterated internal standards were spiked into apple, honey, olive oil, orange and tomato food commodity matrices (concentration range 0.1 – 100 µg/kg) and extracted using a standard QuEChERS method.
- Reverse phase LC Separation**
- LC column: Shim-pack Velox Biphenyl (100 x 2.1 mm, 2.7 µm) + guard (2.7 µm 5 x 2.1 mm Biphenyl); column maintained at 45 °C
- Method transfer from a triple quadrupole (TQ) to QTOF.** The ion guides and lab frame collision energy are identical between high sensitivity TQs (for example, LCMS-8060 NX, Shimadzu Corporation, Japan) and the QTOF (for example, LCMS-9050 Shimadzu Corporation, Japan).

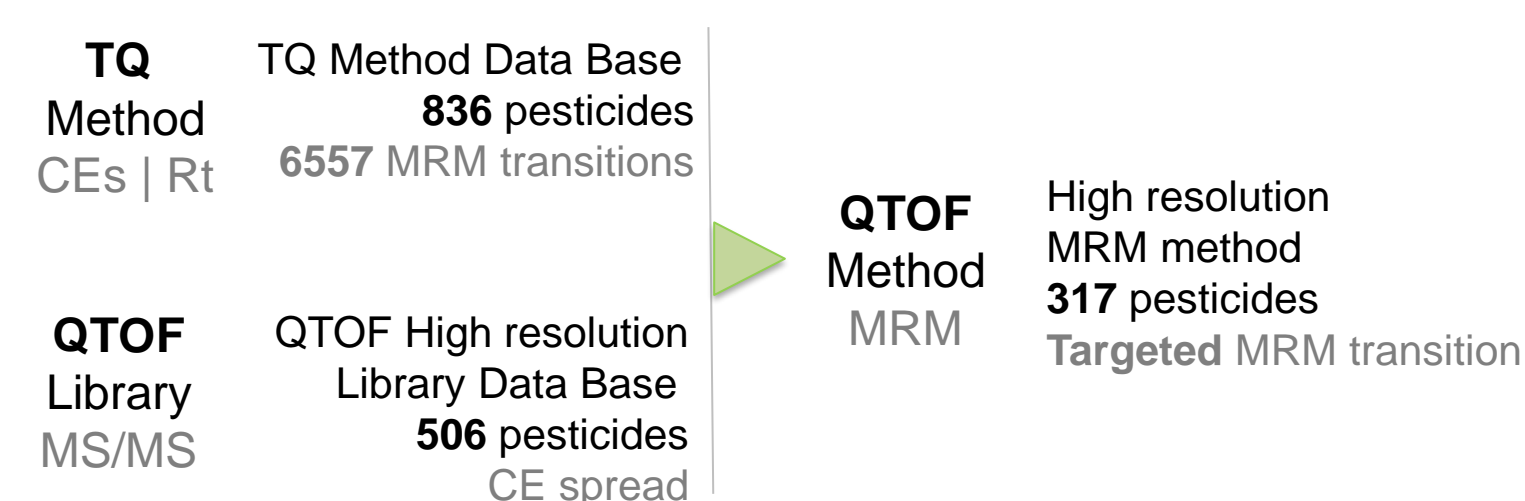


Figure 1. Optimized collision energies for each target pesticide acquired using a triple quadrupole method were transferred to a QTOF MRM acquisition method. The optimized collision energy matched the most intense fragment ion detected in the high-resolution LC-MS/MS pesticide library (Shimadzu Corporation, Japan).

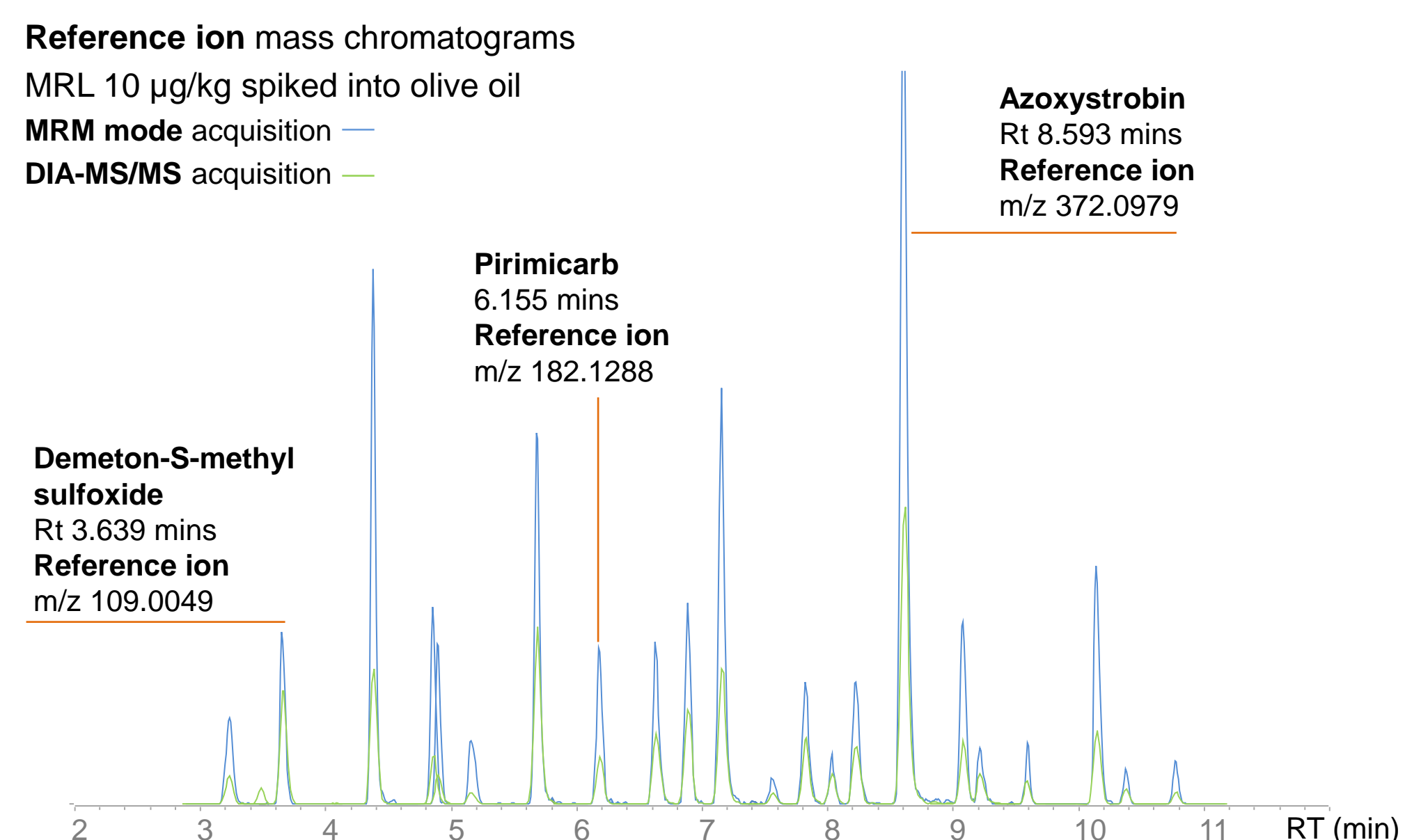


Figure 2. Reference ion mass chromatograms for a panel of pesticides highlighting the difference in ion signal intensity using an optimized MRM mode acquisition or a DIA-MS/MS data acquisition mode. For all target pesticides, acquiring the reference ion with a collision energy specific to each target and a small mass extraction window resulted in a higher signal intensity compared to a DIA-MS/MS method.

LC-MS/MS Mass Spectrometry Detection. High resolution QTOF LC-MS/MS analysis (LCMS-9050, Shimadzu Corporation, Japan) using external mass calibration for positive ESI. The targeted pesticide method used a TOF MS mass scan followed by a series of MS/MS mass scans with an optimized fragment ion energy for each pesticide. Data was acquired with external mass calibration from the same data file using 1 min mass calibrant infusion during column equilibration for each sample, both methods with a cycle time of 1 second.

- High resolution scanning MRM mode acquisition**
 - Quantifier ion** TOF MS mass scan m/z 110-950; 100 msecs
 - Reference ions** The signal intensity for a target pesticide reference ion was optimized using the same collision energy (CE) as a validated triple quadrupole LC-MS/MS method and a narrow reference ion mass window (60 Da). For example;
 - Demeton-S-methyl sulfoxide** | Precursor m/z 247.0222 (Rt 3.345-4.077 mins)
 - Reference ion m/z 108.9749-109.0349 **Fixed CE 26 V**; 10 msecs
 - Primicarb** | Precursor m/z 239.1503 (Rt 3.345-4.077 mins)
 - Reference ion m/z 182.0988-182.1588 **Fixed CE 16 V**; 10 msecs
 - Azoxystrobin** | Precursor m/z 404.1241 (Rt 8.158- 9.131 mins)
 - Reference ion m/z 372.0679-372.1279 **Fixed CE 15 V**; 20 msecs
- High resolution DIA-MS/MS mode acquisition**
 - Quantifier ion** TOF MS mass scan m/z 110-950; 100 msecs
 - Reference ions** DIA-MS/MS mass range was m/z 40-1000. Collision energy ramp 5-55 V. Scan time for each mass scan event was 29 msecs
 - m/z 140-540; precursor isolation window 20 Da
 - m/z 540-925; precursor isolation window 35 Da

3. Results

Advantages of high-resolution scanning MRM mode acquisition

- Reference ions have higher ion signal intensities and greater selectivity as the collision energy and mass scan time is specific to each target compared to a DIA-MS/MS method.
- Provides an option for lowering the detection limit in pesticide analysis.

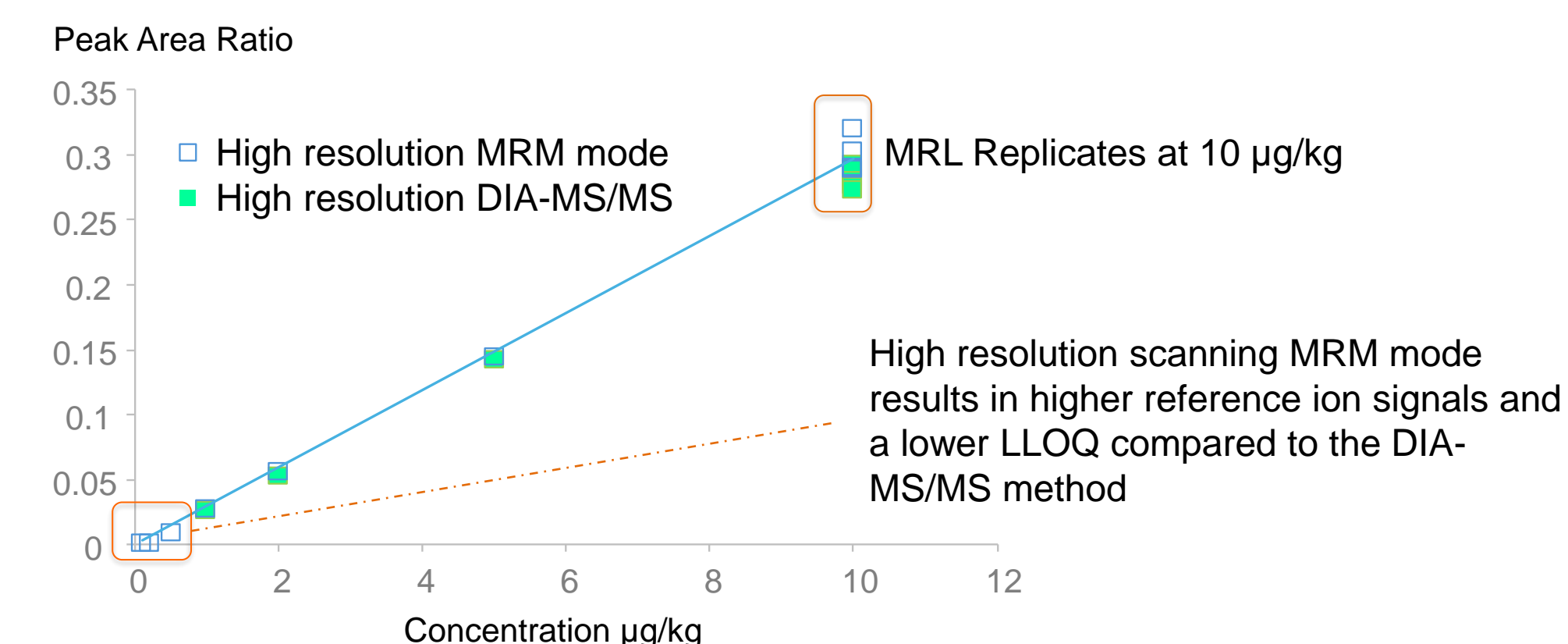


Figure 3. As one example of a lower LLOQ using high-resolution scanning MRM mode acquisition with the quantitation of methiocarb-sulfoxide in an olive oil food commodity. Both MRM and DIA-MS/MS methods use the same TOF MS scan data resulting in the same precursor ion response, however, the MRM method generated higher ion signal intensities for m/z 242.0845 > 185.0631 and concentrations below 1 µg/kg met the SANTE reporting criteria.

Data acquisition for methiocarb-sulfoxide

- MRM mode;** m/z 242.0845 precursor ion isolation width 0.8 Da (fixed retention time window 3.590-4.831 mins), reference ion window m/z 185.0331-185.0931, mass scan time 10 msecs, fixed collision energy 21 V.
- DIA-MS/MS mode;** m/z 242.0845 precursor ion isolation window 20 Da, mass scan time 29 msecs; collision energy spread 5-55 V.

System repeatability (%RSD n=6; tomato food commodity)

- %RSD ≤ 20%;** 312 pesticide targets at the MRL level of 10 µg/kg (at 1 µg/kg 272 pesticide targets met the SANTE reporting guidelines).

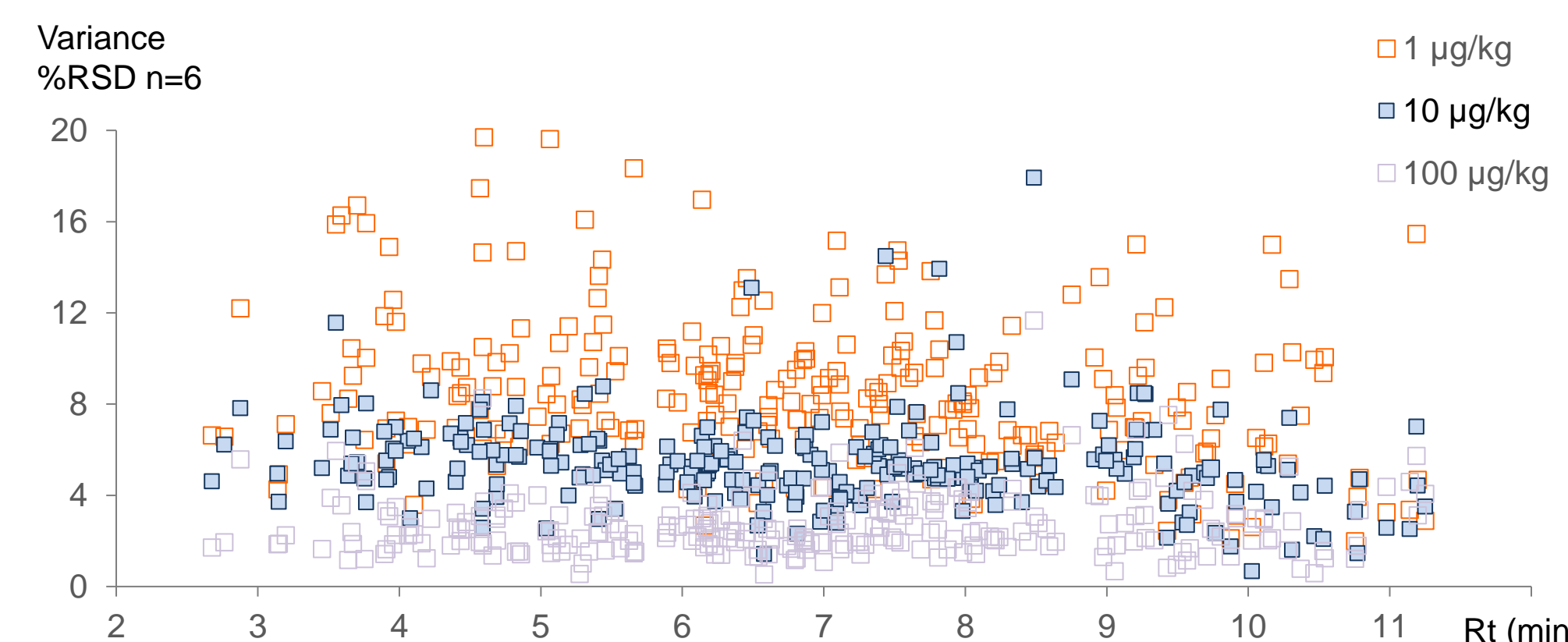


Figure 4. System repeatability at 1, 10 and 100 µg/kg with 6 repeated injections (target pesticide panel spiked into a tomato matrix).

Typical mass accuracy at the MRL level of 10 µg/kg

High resolution scanning MS and MRM data were acquired in positive ion mode using external mass calibration during the analysis. Mass correction was applied using a mass calibrant infusion during column equilibration (15.0-16.2 minutes)

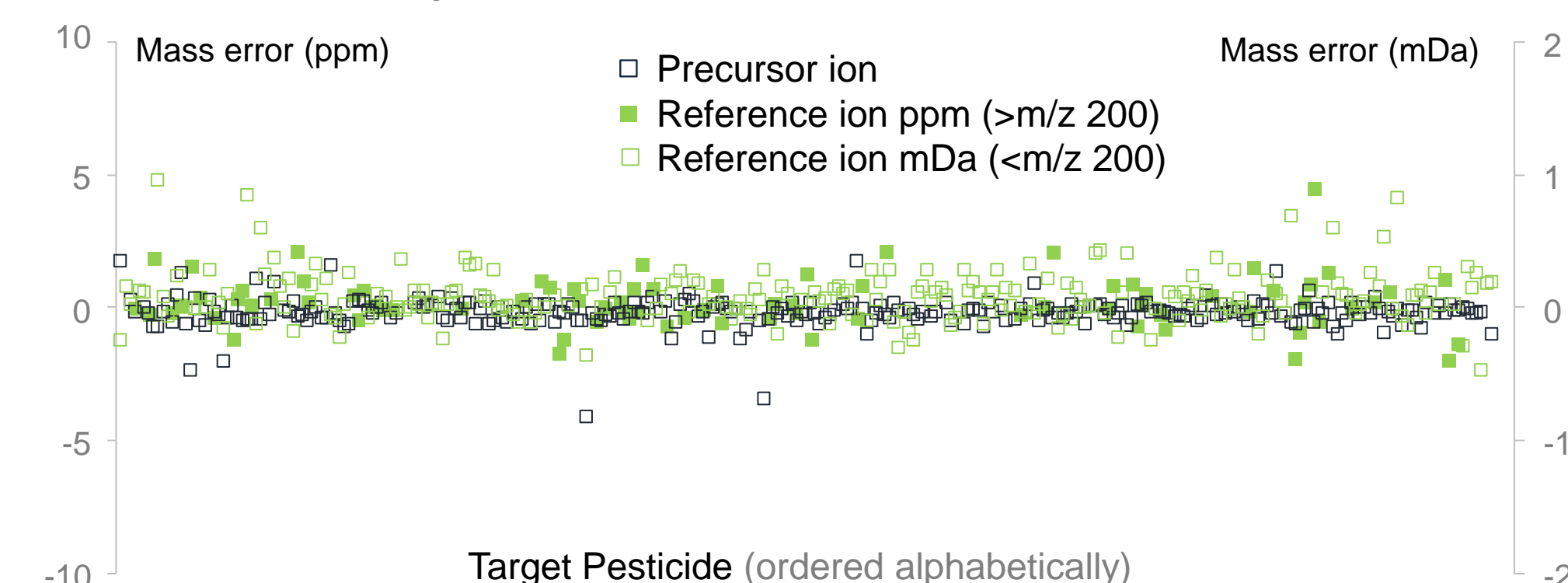


Figure 5. Mass error variation for all compounds (ordered alphabetically) detected at the MRL level at 10 µg/kg in tomato matrix together with the MRM mode reference ion (ppm and mDa).

High resolution scanning MRM mode

6-benzyladenine
m/z 226.1087>91.0542
Rt 4.687 mins

Bendiocarb
m/z 224.0917>109.0284
Rt 5.129 mins

Metsulfuron-methyl
m/z 382.0816>167.0564
Rt 6.037 mins

Spinetoram J
748.4994>142.1226
Rt 10.111 mins

High resolution DIA-MS/MS mode

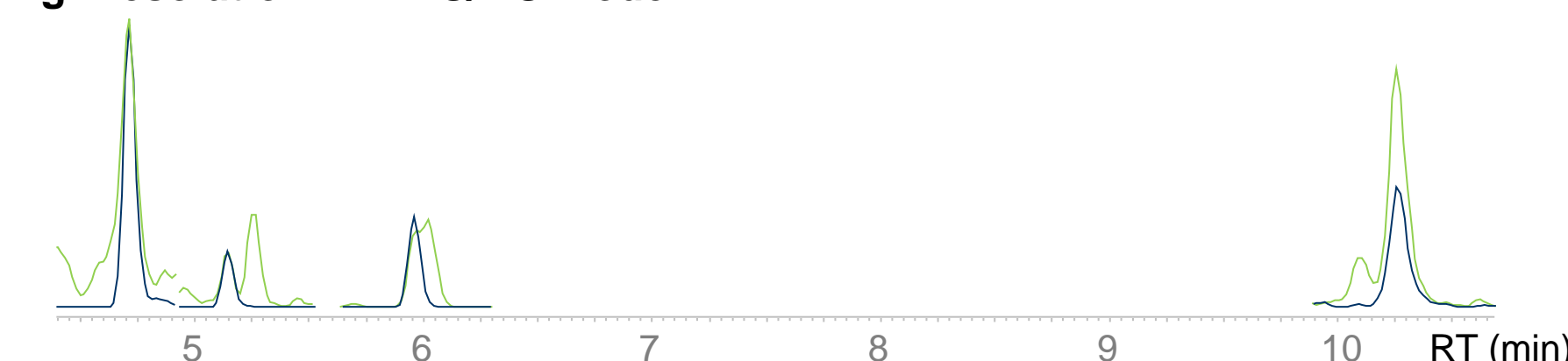


Figure 6. High resolution scanning MRM mode delivered a higher selectivity for 4 compounds in an olive food commodity (10 µg/kg) compared to DIA-MS/MS with a wider precursor ion isolation window.

4. Conclusions

- High resolution scanning MRM mode generates higher fragment ion intensities compared to a DIA-MS/MS method resulting in lower limits of detection in a panel of over 300 pesticides. For a limited number of target pesticides in certain food commodities the MRM mode also enhanced selectivity.
- The MRM mode met the reporting EU SANTE guidelines.

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