

# A multiresidue pesticide method using a modified quadrupole-Orbitrap MS for quantitation, screening and confirmation.

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## ABSTRACT

**Purpose:** To present LC-MS HRAM multi-class pesticide residue analysis methods in olive oil that are robust, rapid, easy to use, and have the sensitivity, accuracy, and precision that is required in order to meet regulatory guidelines. All aspects of these methods represent a 'workflow' from sample to final report for food safety laboratories.

**Methods:** Olive oil were processed and analyzed to test the core methodology- from sample preparation using a modified QuEChERS protocol (Quick, Easy, Cheap, Effect, Rugged, and Safe) to analysis, data processing, and reporting with LC-MS HRAM and comprehensive data handling software. A compound database of over 700 compounds with Fragments, Retention Time, Spectra library from mzCloud and liquid chromatography conditions was created, along with a single screening and quantitative method for over 500 residues.

**Results:** Results demonstrate that the methods are fit-for-purpose for both quantitative and broad-spectrum of pesticide residue screening that can be easily implemented in food safety testing labs. Calibrations with matrix matched standards (MMS) were performed. Acceptable results were obtained for the key figures of merit: Limit of Quantitation (LOQs), calibration range/linearity, fragmentation matching scoring and spectra library matching.

## INTRODUCTION

The demand for quick and simple analysis of large numbers of samples in agriculture applications is growing year by year. Throughout the world pesticides are used to control pests that are harmful to crops, humans and animals. These substances can pose a significant health threat and therefore need to be accurately detected at the lowest levels required by the governmental authorities, typically at low part per billion (ppb) levels. A modified Thermo Scientific Orbitrap Exploris quadrupole-Orbitrap mass spectrometer with S-lens interface provides high-resolution, accurate mass data acquisition to unequivocally identify compounds. Utilizing a full scan data dependent ms/ms or fixed window data independent acquisition workflow can provide semi-quantitation and screening for a broad range of analytes, using a curated spectral library for confirmation.

## MATERIALS AND METHODS

### Sample Preparation

Sample preparation involves a protocol that was optimized to be easy to implement and also reduce matrix co-extractives, resulting in enhanced sensitivity and robustness in electrospray ionization LC-MS/MS. The basic elements of the preparation procedures are described in Figure 1.

**Figure 1: Extraction procedures for olive oil samples. No dispersive solid phase extraction clean-up was required. Only 1 µL of the extracts were injected (pure acetonitrile), which provided excellent sensitivity and robustness for the method.**



Add 5 g of olive oil to a 50 mL polypropylene centrifuge tube
Add 10 mL of acetonitrile
Add 6 mL of water
Add 100 µL of internal standard and 50 µL of control standard
Cap and shake vigorously for 5 minute
Add salt pouch: 4000mg MgSO <sub>4</sub> , 1000mg NaCl, 500mg Na <sub>2</sub> Citrate, 1000mg Na <sub>3</sub> Citrate
Shake for approximately 1 minute
Centrifuge at >3300 rpm for 15 minutes
Filter at least 2.0 mL of supernatant through a 0.45 µm filter
Pass 1.5 mL thru lipid removal cartridge
Inject 1 µL for analysis

### Instrument Method

Analytical condition use for the analysis are described here below in Figure 2

**Figure 2: LC gradient, mobile phase, column, and API source conditions for the Vanquish Flex Binary UHPLC pump with Orbitrap Exploris™ 240 mass spectrometer.**

Thermo Scientific™ Vanquish™ Flex Binary UHPLC system:	Thermo Scientific™ Orbitrap Exploris™ 240 MS:
Mobile phase: A: Water + 5mM Ammonium formate & 0.1% Formic Acid B: MeOH + 5mM Ammonium formate & 0.1% Formic Acid Injection volume: 1 µl Column: Thermo Scientific™ Accucore™ aQ 100 x 2.1mm x 2.6µm Column temperature: 25° C Flow rate: 300 µl/min Run time: 15 min	Negative Voltage: 2500V Positive Voltage: 3500V Sheath Gas: 30 units Auxiliary Gas: 6 units Sweep Gas: 1 units Ion transfer tube Temp: 290° C Vaporizer Temp: 350° C Resolution: Full Scan 45,000, DIA 15,000

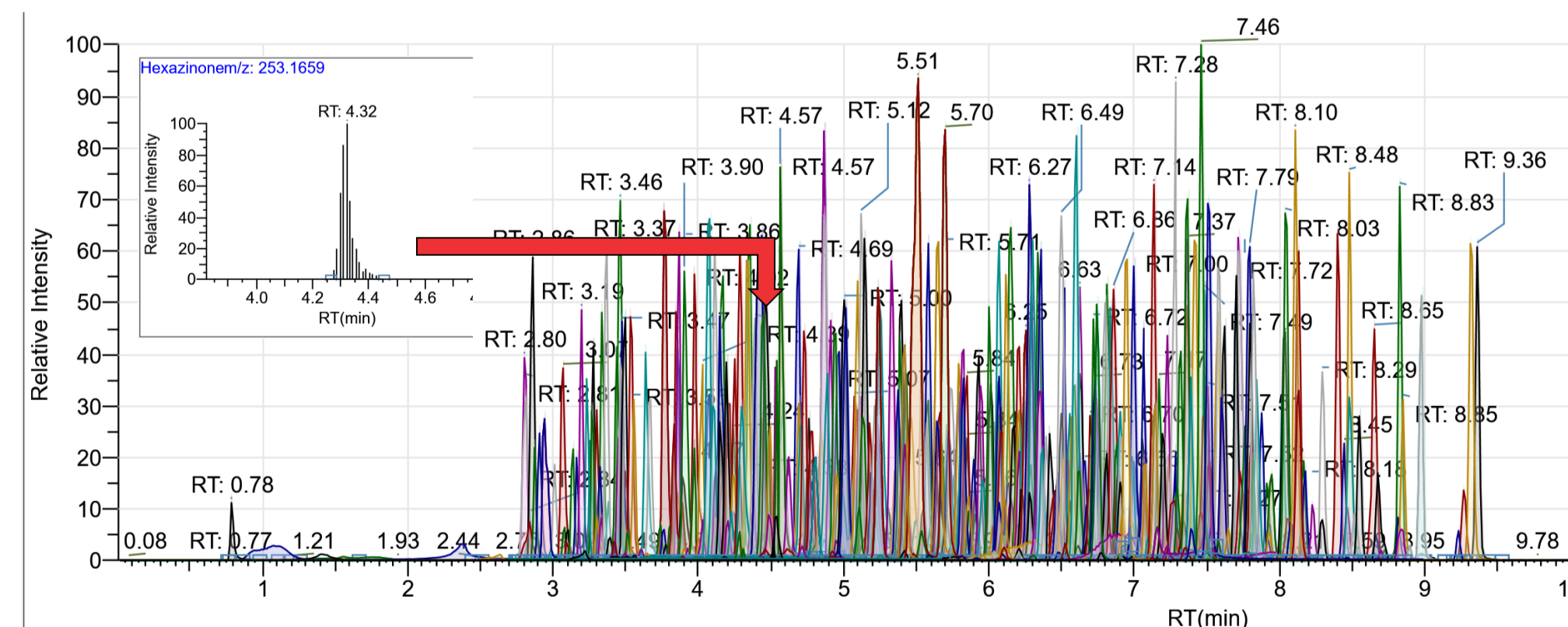
### Data Analysis

Individual standards for 720 pesticides were purchased from Ultra Scientific, Inc., and solutions of each were prepared at 1 ppm in acetonitrile or methanol. Up to five fragments were obtained for each pesticide using an automated routine, in which large numbers of the standards are simply placed into the autosampler and infused unattended into the LC-HRAM. This information combined with compound retention times and other meta-data was then used to create a comprehensive compound data base in Thermo Scientific™ TraceFinder™ Software. A quantitative method containing more than 500 pesticides and a screening method were easily created from the database and used for method development and validation.

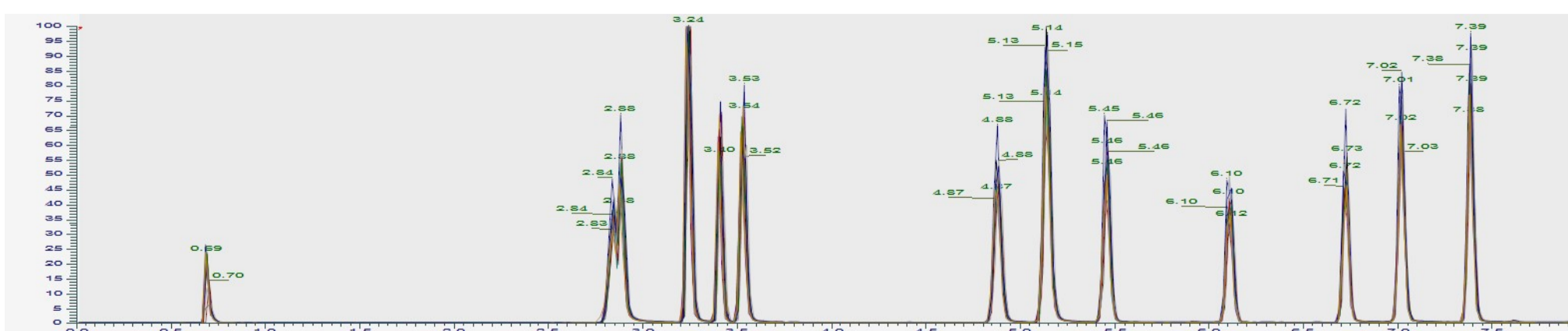
## RESULTS

Olive oil sample was obtained for method validation. Typically, matrix matched standards (MMS) are required for calibration, and matrix extracted spikes (MES) are used to assess recovery. For excellent quantitation, there must be an adequate number of scans across the target quantitation peak. At least one or two fragment ions must be used for confirmation. Figure 4 shows a 10 ppb MMS for a method containing over 500 pesticides with positive and negative polarity switching occurring throughout the run. Plenty of scans across each peak are present for accurate quantitation. Method robustness is key to any laboratory. The screening method showed excellent reproducibility in terms of a) consistent peaks shapes and long column lifetime, with over 1000 injections (and still going strong) and b) consistent peak response over time. Figure 5 shows some select pesticides across the retention time range of the method (1-10 minutes), for approximately 250 injections.

**Figures 4: Chromatogram of over 500 pesticides in 15mins in olive oil MMS spiked at 10 ppb. The peak at 4.32 minutes is Hexazinone showing over 11 scans across the Full Scan quantitation ion used for the analysis. Large pesticide panels of extracted high resolution masses are easily displayed in TraceFinder Software.**



**Figures 5: Robust LC-MS reproducibility of pesticides spiked in olive oil. Selected pesticides (overlay of injection #1 to 250 with extracted mass tolerance of 3ppm).**

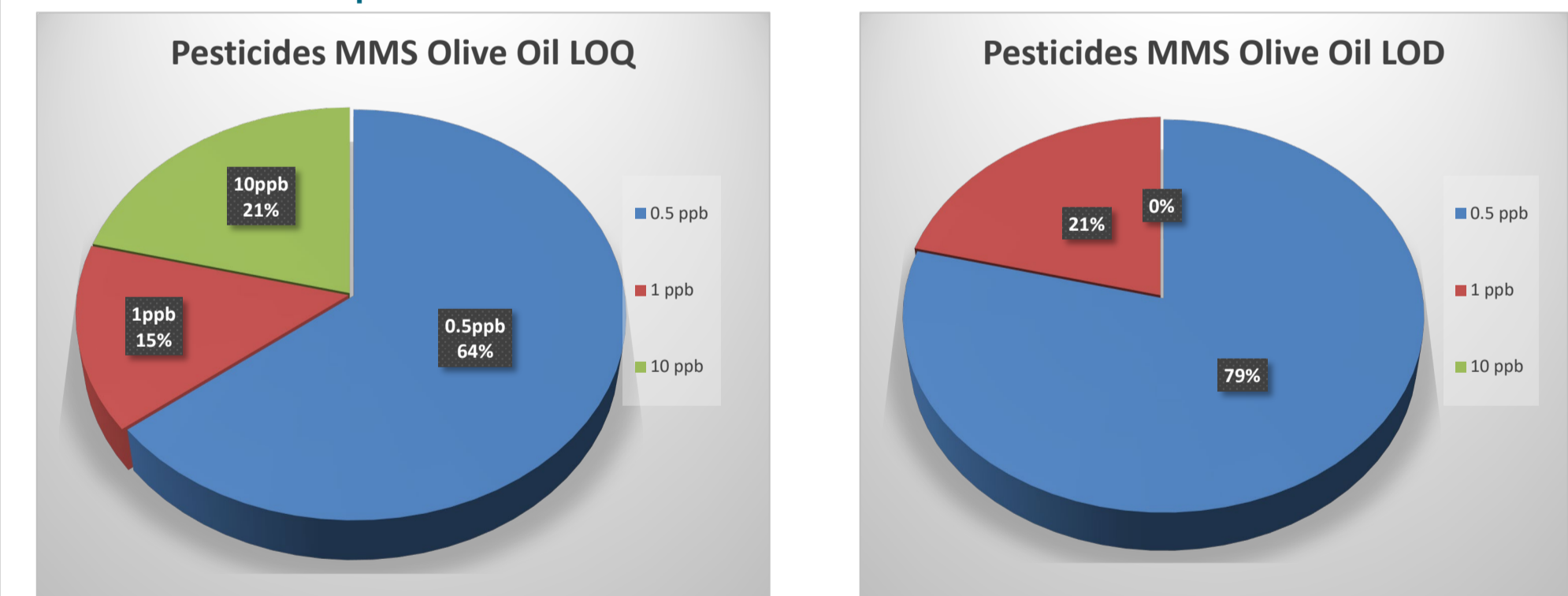


**Figures 6: Quantitation ions and confirming ion in MMS in olive oil, along with calibration range from 0.5 to 100 (500) ppb in TraceFinder software for Ametryn @1ppb (top) and Fenazaquin 0.5ppb (bottom). The technique allows for confident quantitation and screening well below the MRL concentration.**



Figure 6 shows some typical results of calibration curves from 0.5 to 100 (500) ppb. Over 95% of the pesticides studied had calibration curves with  $r^2 > 0.990$ . Confirmation fragment ions are displayed in the middle of each panel at 1 and 0.5 ppb for each pesticide, with indicator colors (green) easily visible to show passing ion ratio criteria. A method of over 500 pesticides was developed and optimized to ensure that at least two fragment ions were detected per compound and still maintain polarity switching throughout the run. LODs and LOQs were determined as outlined by the EU guidelines, with results shown in Figure 7.

**Figures 7: LODs and LOQs in olive oil obtained following the SANTE guidelines for the screening method of over 500 pesticides.**



## CONCLUSIONS

- Large pesticide panels for quantitative analysis and screening at levels below EU MRLs have been shown to provide excellent sensitivity and robustness in a routine laboratory setting for olive oil
- The QuEChERS extraction procedure demonstrated good recovery and precision, with only 1 µL required for injection to meet EU SANTE validation guidance.
- On-going work is required to evaluate the performance of the residues in MES extracts

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSES

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