The Multi-residue Analysis of Trace Levels of Pesticides in Wine using a LC-MS/MS

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ABSTRACT

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

Methods: Various wines 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 triple quadrupole LC-MS/MS and comprehensive data handling software. A compound data base of over 700 compounds with optimized SRMs and liquid chromatography conditions was created, along with a screening method for over 550 residues and quantitative methods with over 250 residues.

Results: Results demonstrate that the methods are fit-for-purpose for both quantitative and broad spectrum pesticide residue screening that can be easily implemented in food safety testing labs. Calibrations with matrix matched standards (MMS) and matrix extracted spikes (MES) were performed and analyzed according to the EU SANTE guidelines, yielding acceptable results for the key figures of merit: Limit of Detection (LODs), Limit of Quantitation (LOQs), calibration range/linearity, and recovery.

INTRODUCTION

Food pesticide residue laboratories face significant analytical challenges. Growing target compound lists, large numbers of samples, wide varieties of matrices, and decreasing limits of detection are pressuring labs to become more efficient than ever before. In addition, customers often require more information on contaminants that are not on any target lists that may be a threat. Increasing food safety concerns and growing agricultural trade has resulted in more stringent pesticide regulations globally. To comply with such regulatory standards, quantitative and screening methods for large numbers of pesticide residues are becoming more common in the routine food safety laboratory. Tandem quadrupole mass spectrometry offers a highly sensitive and selective detection in complex matrices. This poster describes a method for analysis of multi-class pesticides in wine using liquid chromatography coupled with a triple quadrupole mass spectrometer.

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.

Add 10 g of wine to a 50 mL polypropylene centrifuge tube

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Add 10 mL of acetonitrile

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Add 100 µL of internal standard and 50 µL of control standard

↓

Cap and shake vigorously for 1 minute

↓

Add salt pouch: 4000mg $MgSO_4$, 1000mg NaCl, 500mg $Na_2Citrate$, 1000mg $Na_3Citrate$

↓

Shake for approximately 1 minute

↓

Centrifuge at 4000 rpm for 5 minutes

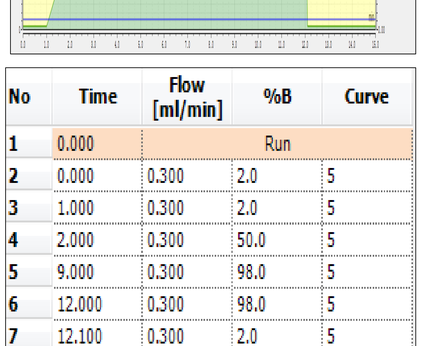
↓

Filter at least 1.5 mL of supernatant through a 0.45 µm filter

↓

Inject 1 µL for analysis

Figure 1: Extraction procedures for wine and 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.



No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	0.300	2.0	5
3	1.000	0.300	2.0	5
4	2.000	0.300	50.0	5
5	9.000	0.300	98.0	5
6	12.000	0.300	98.0	5
7	12.100	0.300	2.0	5
8	New Row			
9	15.000	Stop Run		

Negative Voltage	2500 V
Positive Voltage	3500 V
Sheath Gas	30 units
Auxiliary Gas	6 units
Sweep Gas	1 unit
Ion Transfer Tube Temperature	290 C
Vaporizer Temperature	350 C

Injection Volume	1 µL
Column Temperature	25 C
Analytical Column	Thermo Scientific™ Accucore™ aQ, 100 x 2.1 mm, 2.6 µm
Run Time	15 min
Mobile Phase A	Water with 0.1% Formic Acid+ 5mM Ammonium formate
Mobile Phase B	Methanol with 0.1% Formic Acid+ 5mM Ammonium formate

Figure 2: LC gradient, mobile phase, column, and API source conditions for the Thermo Scientific™ Vanquish™ Flex Binary UHPLC pump with TSQ Quantis™ Triple Quadrupole Mass Spectrometer.

Individual standards for 720 pesticides were purchased from Ultra Scientific, Inc., and solutions of each were prepared at 1 ppm in acetonitrile or methanol for optimization of MS/MS conditions. Up to five SRMs with collision energies 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-MS/MS. 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 250 pesticides and a screening method of over 550 were easily created from the database and used for method validation.

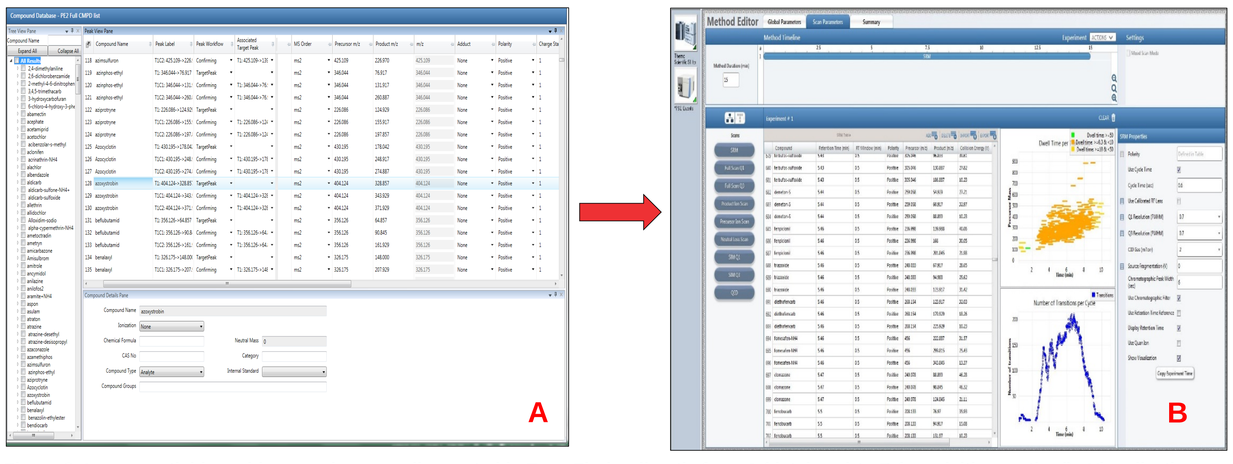
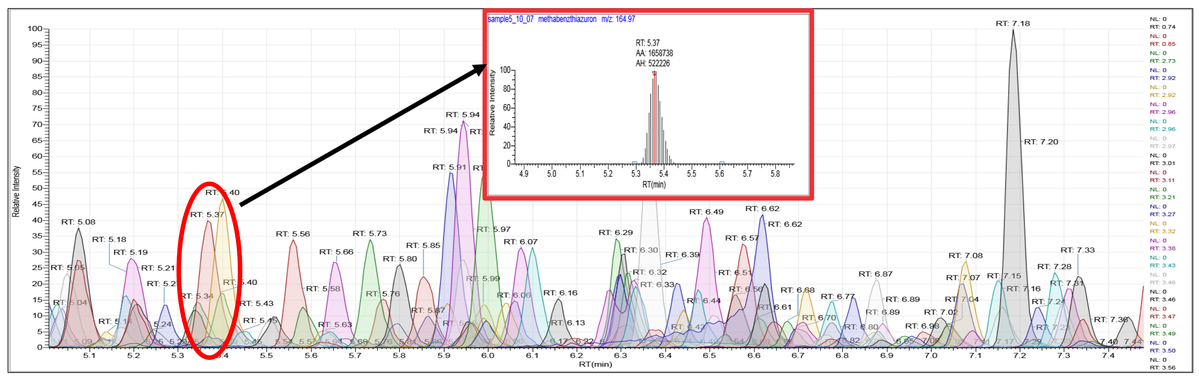


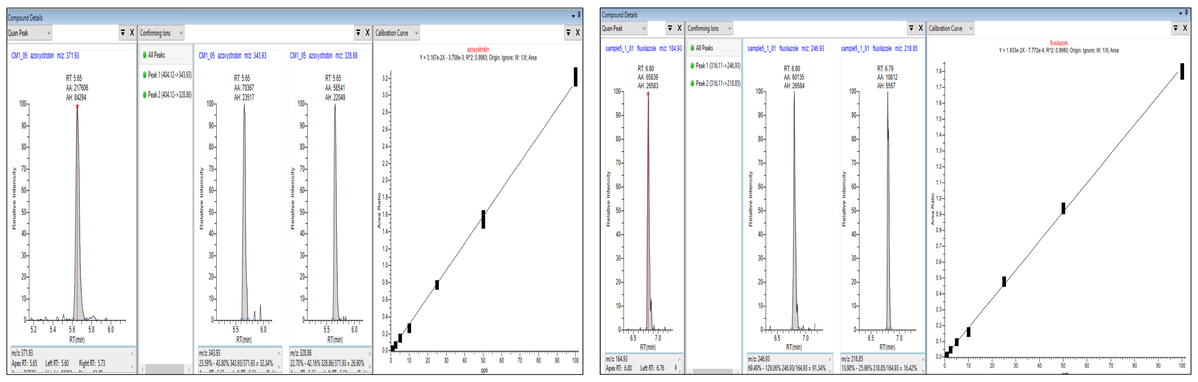
Figure 3: Large pesticide residue panels are easily created in TraceFinder directly from A (database) to B (acquisition method). Visualization tools within the tune program of the TSQ Quantis Triple Quadrupole Mass Spectrometer show SRM density throughout the run, ensuring that enough scans are acquired across each peak with appropriate dwell times for accurate quantitation.

RESULTS

Wine (white and red) samples from various sources were 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 a sufficient number of scans across the target quantitation peak. At least and one or two SRMs can be used for confirmation. Figure 4 shows a 10 ppb MMS for a method containing over 250 pesticides with positive and negative polarity switching occurring throughout the run. Plenty of scans across each peak are present for accurate quantitation.

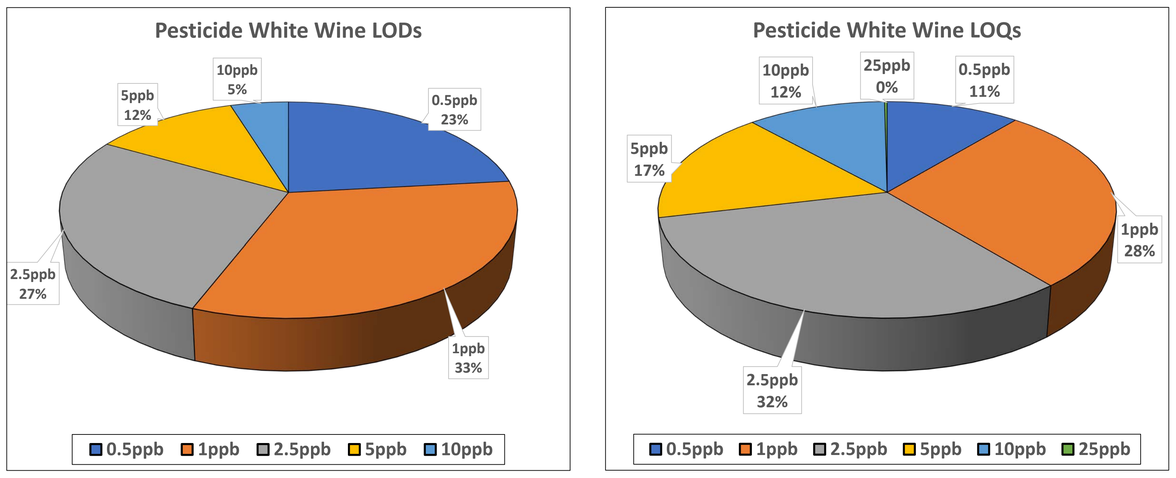


Figures 4: White wine MMS standard at 10 ppb. The peak at 5.37 minutes is Methabenzthiazuron, showing over 15 scans across the quantitation ion used for the analysis. Large pesticide panels of extracted SRMs are easily displayed in TraceFinder Software.



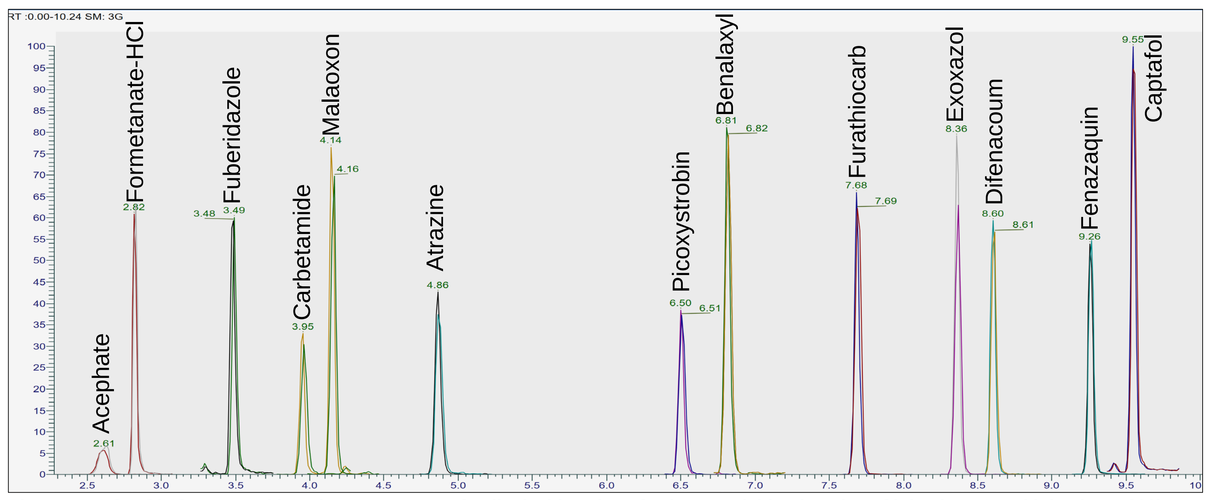
Figures 5: Quantitation ions and confirming ion at 1 ppb in a MES, along with calibration range from 0.5 to 100 ppb in TraceFinder software for azoxystrobin in white wine (left) and Flusilazole (right). The technique allows for confident screening with confirmation well below the MRL concentration.

Figure 5 shows some typical results of calibration curves from 0.5 to 100 ppb. Over 95% of the pesticides studied had calibration curves with r(2) > 0.990. Confirmation ions are displayed in the middle of each panel at 1 ppb for each pesticide, with indicator colors (green) easily visible to show passing ion ratio criteria. The ISVEA laboratory then wanted to expand the list of analytes beyond the original scope, and decided to create a comprehensive screening method using the SRM compound data base. A method of over 550 compounds was developed and optimized to ensure that at least two SRM transitions were detected per compound (one for quantitation and the other for confirmation), and still maintain polarity switching throughout the run. LODs and LOQs were determined as outlined by the SANTE guidelines, with results shown in Figure 6. The method allowed ISVEA to quickly screen samples with confidence at or below the EU MRL for a wide variety of pesticides, giving their customers added confidence in the safety of their products.



Figures 6: LODs and LOQs in white wine obtained following the SANTE guidelines for the screening method of over 550 pesticides at the ISVEA laboratory.

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 7 shows some select pesticides across the retention time range of the method (1-10 minutes), for approximately 300 injections.



Figures 7: LC-MS/MS extracted ion chromatograms of spiked white wine of select pesticides (overlay of injection #1 and injection # 300) demonstrates good robustness of the analytical system and API source.

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 both red and white wines.
- The QuEChERS extraction procedure demonstrated good recovery and precision, with only 1 µL required for injection to meet EU SANTE validation guidance.

REFERENCES

1. EUROPEAN COMMISSION- SANTE/11813/2017, November 2017

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TRADEMARKS/LICENSING