

A Validated Method for the Analysis of 142 Pesticide Residues Using Atmospheric Pressure GC Coupled with Tandem Quadrupole Mass Spectrometry

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APPLICATION BENEFITS

- Routine quantification of 142 pesticide residues in QuEChERS extracts of fruit and vegetables using an ionization mode that provides enhanced sensitivity
- Analysis of LC and GC compounds on a single MS platform
- Fast and easy processing of data using TargetLynx™ Application Manager

WATERS SOLUTIONS

DisQuE™ QuEChERS, AOAC Method
Sample Preparation Kit, Pouches

Atmospheric Pressure Gas
Chromatography (APGC)

Xevo® TQ-S

TargetLynx Application Manager

KEY WORDS

Pesticides, QuEChERS extracts,
Atmospheric Pressure Gas
Chromatography, MS/MS

INTRODUCTION

Pesticides are widely used in agricultural activity across the globe and residues of these pesticides in food products destined for human consumption can be a major food safety risk. Pesticide residues are high on the list of consumer concerns and consequently laboratories are tasked to screen samples for as many pesticides as possible in a single analysis within an appropriate timescale. Most countries have clearly defined regulations governing pesticide residues. Legislation imposes Maximum Residue Limits (MRLs) for pesticide residues in food commodities requiring analytical techniques that are sensitive, accurate, and robust. Multi-residue analysis is challenging due to the low limits of detection required to achieve MRL compliance for a diverse range of pesticides in a wide range of food commodities. There are currently in excess of 1000 pesticides known to be in use, and laboratories are under increasing pressure to increase the scope of the analytical methods for routine monitoring purposes.

Various technologies are used to meet this challenge, the most common being Liquid Chromatography (LC) and Gas Chromatography (GC) coupled to tandem quadrupole mass spectrometry. Implementation of these techniques allows the laboratory to cover a range of compounds with varying chemistries as required by legislation. In GC/MS/MS the traditional ionization mode used is Electron Impact (EI). This is a relatively “hard” ionization method and results in a high degree of analyte fragmentation, which compromises the selectivity and sensitivity of the MS/MS measurement. Atmospheric Pressure Gas Chromatography (APGC) is a “soft” ionization technique resulting in less fragmentation and subsequently increasing the sensitivity and selectivity of MS/MS methods.¹ The APGC source is readily interchangeable with the electrospray (ESI) source enabling a single platform to be used for the analysis of both GC- and LC-amenable pesticides.

In this application note we describe the development and validation of a multi-class method for the routine determination of 142 pesticide residues in various fruit and vegetable matrices. A more detailed description of the method and of the results achieved can be found in the referenced paper.²

EXPERIMENTAL**GC conditions**

GC system:	7890A GC
Column:	DB5-MS 30 m x 0.25 mm x 0.25 µm film
Carrier gas:	He, 2 mL/min
Temp gradient:	Initial 70 °C for 1 min 15 °C/min to 150 °C, 10 °C/min to 300 °C, hold 3 min
Total run time:	30 min
Injector temp.:	280 °C
Injection type:	Pulsed splitless
Pulse time:	1 min
Pulse pressure:	240 kPa
Injection volume:	1 µL
Make-up gas:	N ₂ at 300 mL/min
Transfer line temp.:	310 °C

MS conditions

MS system:	Xevo TQ-S
Mode:	API +
Corona :	1.8 µA
Cone gas:	170 L/Hr
Aux gas:	250 L/Hr
Source temp.:	150 °C

A vial of water was added to the source to promote protonation. Data were processed using TargetLynx Application Manager. TargetLynx, an option with Waters MassLynx® Software that quickly generates results from acquired LC/MS and GC/MS data, permitting accurate quantification and review of results, including evaluation of data quality and analyte confirmation.

Sample preparation

Fortified orange, carrot, and tomato samples were used to evaluate the linearity, recovery, precision, selectivity, limits of detection (LODs), and limits of quantification (LOQs). Locally purchased apple, lettuce, and courgette (zucchini) samples were additionally analyzed to test the method applicability. Sample preparation was carried out using the DisQuE QuEChERS, AOAC Method Sample Preparation Kit, Pouches, [Part No. 176002922](#), that is designed specifically for the QuEChERS procedure described in the AOAC official method 2007.01.² QuEChERS is a simple sample preparation technique suitable for multi-residue pesticide analysis in a diverse variety of food and agricultural products. Following extraction, 50 µL of the extract (acetonitrile) was transferred into a 2-mL vial and diluted with 300 µL of hexane and 150 µL of acetone. For accurate quantification eight matrix-matched standards were prepared² to cover the range 0.1 to 100 ng/mL (equivalent to 1 to 1000 µg/kg in the sample). These were prepared for each sample matrix as follows: after the cleanup step, 50 µL of the acetonitrile extract obtained from a blank sample were mixed with 250 µL of hexane, 150 µL of acetone, and 50 µL of the pesticide standard solution in hexane at adequate concentration to obtain a calibration range of 0.1 to 100 ng/mL (corresponding to 1 to 1000 µg/kg in sample). All samples were analyzed using the Waters® Xevo TQ-S with the APGC source and a 7890A GC.

RESULTS AND DISCUSSION

The first step in the method development process was the optimization of three MRM's for each target pesticide. Due to the soft ionization characteristics of APGC, the protonated molecule or molecular ion, base peak of the spectrum, could be chosen as the precursor ion in most cases. Examples of APGC mass spectra compared to EI are shown in Figure 1. The goal was to select three sensitive MRM transitions for each compound so that confident identification and quantification of the 142 analytes could be achieved. The high sensitivity achievable using APGC-MS/MS allowed a 10-fold dilution of the QuEChERS extract, thereby strongly reducing the matrix load onto the column.

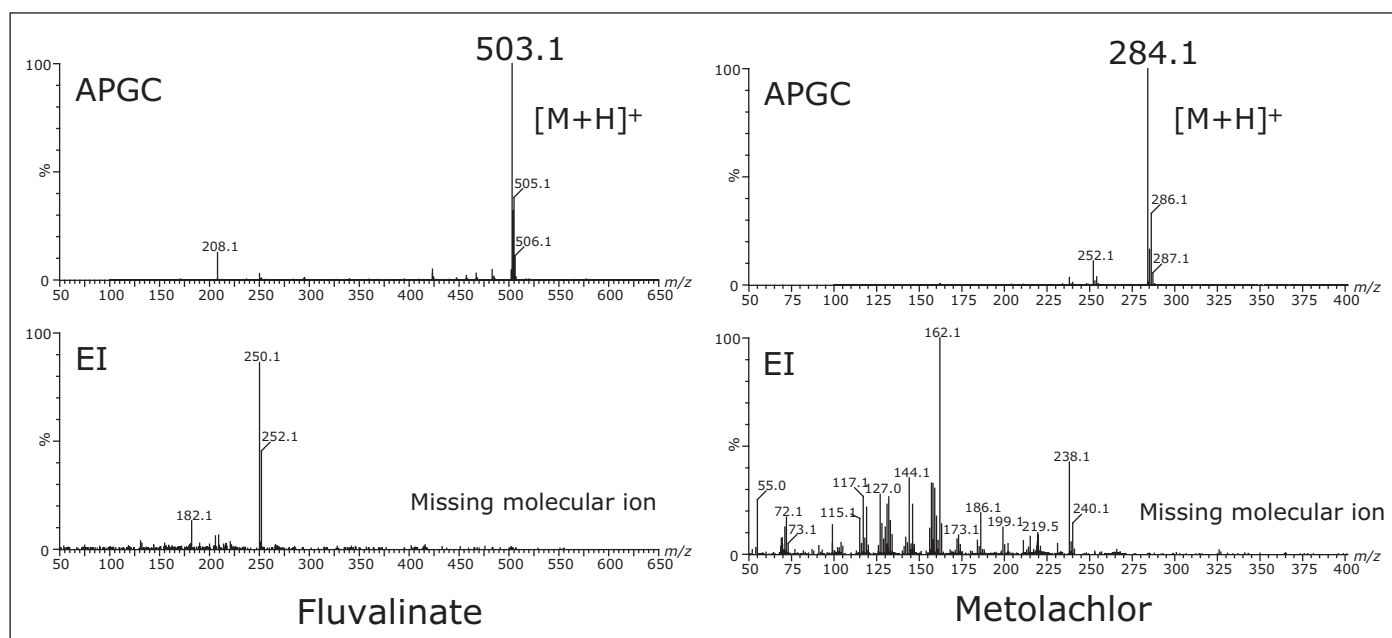


Figure 1. Comparison of spectra generated by APGC (top) and EI (bottom) showing enhanced intensity of the molecular ion in the APGC spectra. EI spectra exhibit extensive fragmentation.

Linearity was studied in the range 0.1 to 100 ng/mL using pure solvent standard solutions and injecting in triplicate. The regression coefficients (R^2) were greater than 0.99 for all compounds over the range tested. To ensure accurate quantification, and to account for any enhancement/suppression due to matrix effects, matrix matched calibration standards were used. The LODs obtained for all compounds were low and are summarized in Figure 2. The majority of these ranged between 0.01 and 1 $\mu\text{g}/\text{kg}$ in the three matrices studied with only a few higher than 1 $\mu\text{g}/\text{kg}$. Figure 3 shows four examples, with signal-to-noise (S/N) ratios calculated for the lowest matrix-matched standard in the various matrices.

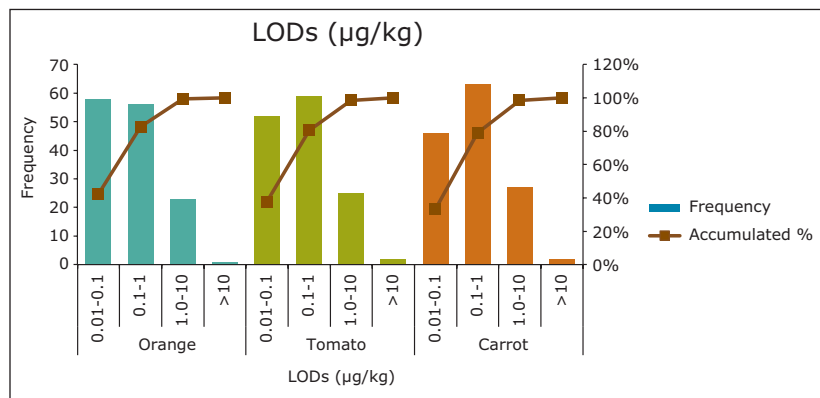


Figure 2. Limits of Detection (LODs) for all 142 pesticides across the three matrices. The bar chart (left axis) shows the number of pesticides with an LOD at a particular concentration range. The line graph (right axis) shows the cumulative percentage of pesticides across the concentration ranges.

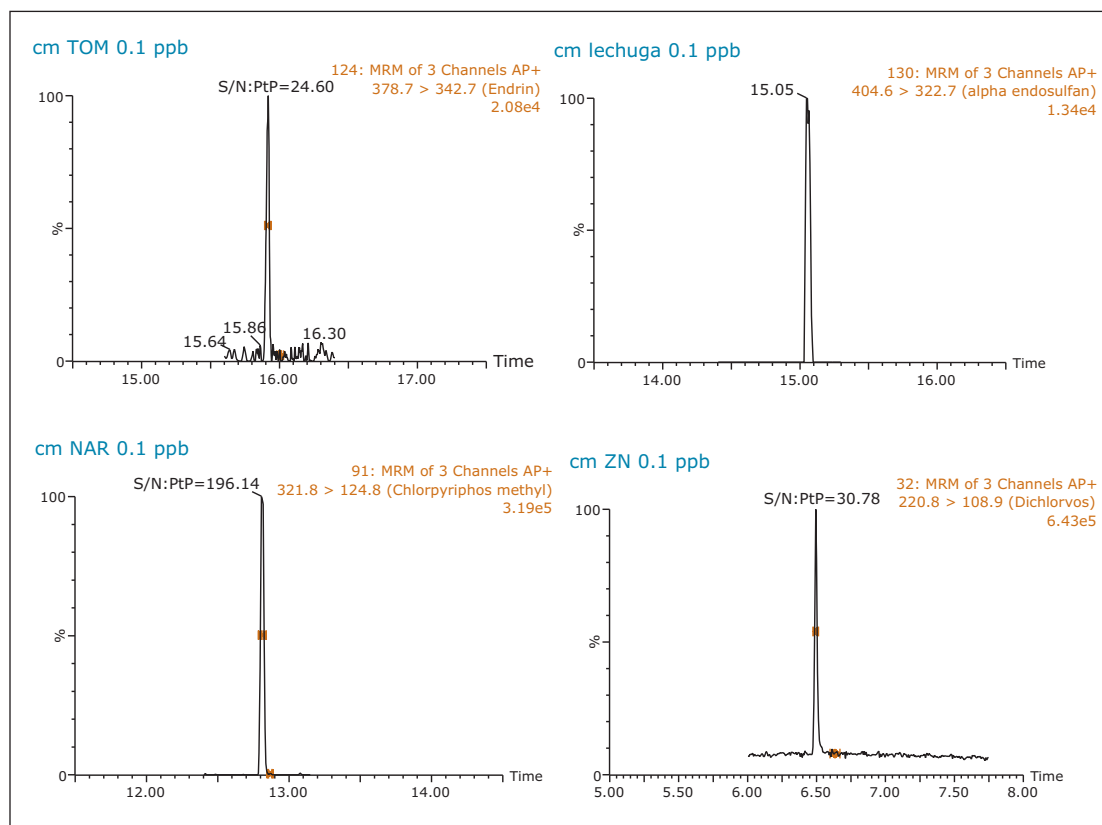


Figure 3. Examples of sensitivity in matrix for four pesticides: endrin, alpha endosulfan, chlorpyrifos methyl, and dichlorvos at 0.1 ppb.

The selectivity of the MRM transitions chosen from the APGC spectra was observed to be excellent as the GC/MS/MS chromatograms did not show interferences for any of the pesticides investigated in this study. An important consideration for the method was to satisfy regulatory criteria regarding ion ratios. For pesticides in the EU the current guideline (SANCO/12571/2013) indicates that the ion ratio in samples should be within 30% of that of the reference value. It was found that in general the ion ratios for the different concentrations of the standards was very consistent, with RSDs <10% in most cases, even when an abundance of the qualifier ion was much lower compared to the quantifier ion.

The developed method was applied to the analysis of real samples with three types of locally purchased orange, tomato, and carrot samples analyzed and expanded to include three types of apple, lettuce, and courgette. A combined total of 43 different pesticides were identified in all of the samples, most at levels well below 0.01 mg/kg and below the EU MRL levels. Figure 4 shows examples of the positive findings in various matrices with confirmatory MRM transitions.

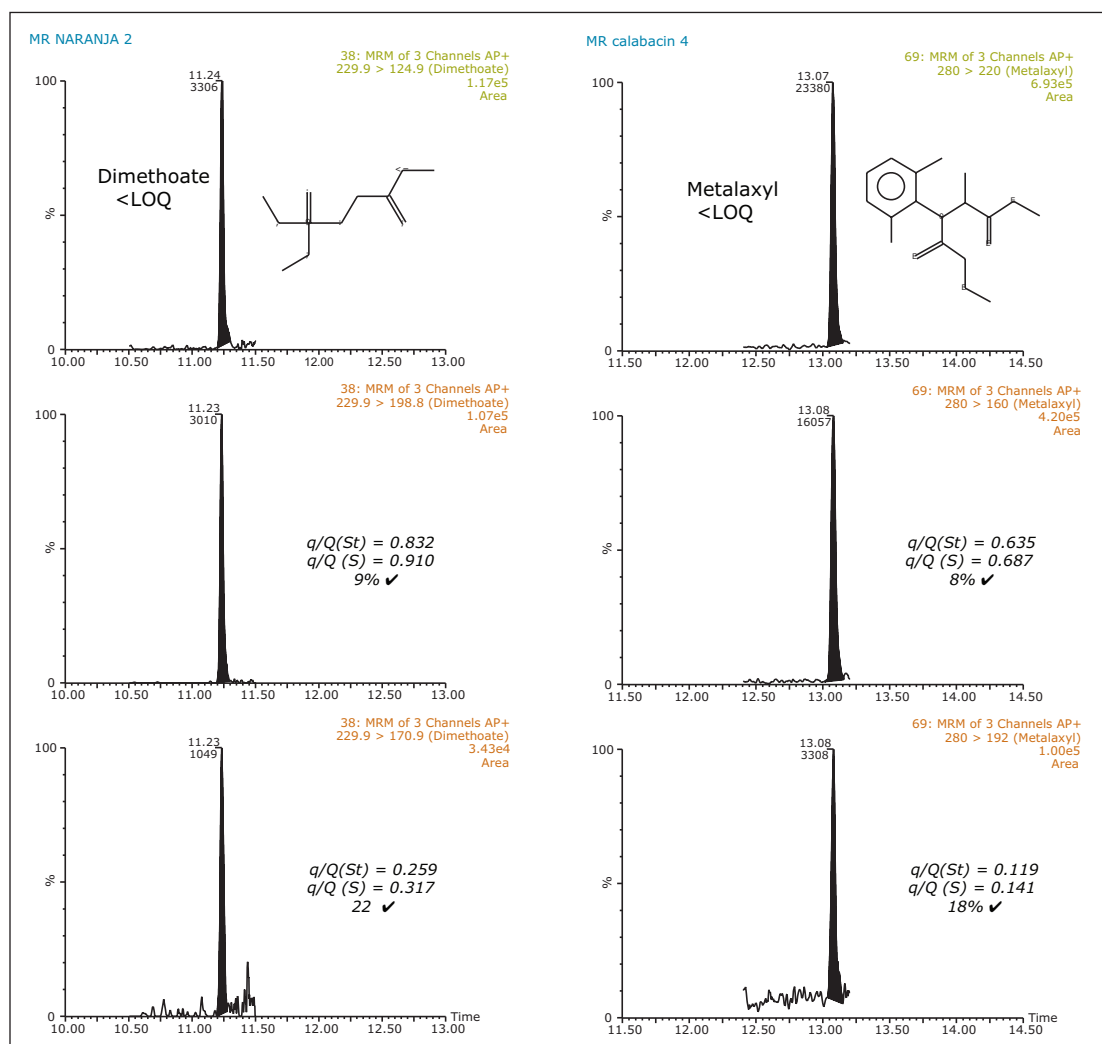


Figure 4. Examples of ion ratios of confirmatory MRM transitions for dimethoate (left) and metalaxyl (right) at low level, below the LOQ. These compounds are challenging with traditional EI methods.

The sensitivity and performance of the Xevo TQ-S with APGC currently exceeds existing regulations related to pesticide residue analysis. This additional sensitivity enables samples to be diluted and therefore reducing matrix interferences and minimizing the amount injected on column. This in turn has major benefits for system cleanliness and reduces instrument maintenance requirements.

CONCLUSIONS

- A method based on QuEChERS extraction/clean up and APGC-MS/MS analysis for the determination of 142 pesticides has been presented.
- Excellent sensitivity and selectivity was achieved by the APGC source and the soft ionization allowed the quasi-molecular ion to be used as the precursor ion.
- LODs generally ranged from 0.01 to 1 µg/kg in orange, tomato, and carrot matrices. This increased sensitivity allowed for the dilution of sample extracts and thereby reducing matrix effects and GC maintenance.
- The validation results demonstrate that the method is applicable to the quantitative routine residue analysis of 142 GC-amenable pesticides.
- This method was successfully applied to the analysis of real samples and 43 different pesticides were identified and confirmed using ion ratios. None of the detected pesticides exceeded their EU MRL.

References

1. TPortolés, L Cherta, J Beltran, A Gledhill, F Hernández. Enhancing MRM Experiments in GC/MS/MS Using APGC. [Waters Application Note No. 720004772en, August, 2013.](#)
2. L Cherta, TPortolés, J Beltran, EPitarch, J G J Mol, F Hernández. Application of Gas Chromatography (Triple Quadrupole) Mass Spectrometry with Atmospheric Pressure Chemical Ionization for the Determination of Multi-Class Pesticides in Fruit and Vegetables. *J Chrom A*. Nov 1; 1314: 224-40 (2013).

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