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Application Note

Sensitive and Repeatable Analysis of Pesticides in QuEChERS Extracts with APGC-MS/MS

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Abstract

Multi-residue pesticide analysis is challenging due to the low limits of detection required in a diverse range of food commodities. As there are currently in excess of 1000 pesticides in use, laboratories are under increasing pressure to broaden the range of pesticides determined in ever shorter turnaround times. Therefore, the analytical methods they employ need to be sensitive and robust in a wide variety of sample matrices.

Typically, this analysis is carried out using a dedicated GC-MS/MS system using an EI source. As shown by Portolēs et al, this source causes extensive fragmentation of some analytes leading to low sensitivity of the molecular ion.

APGC is a “soft” ionization technique which results in an increased abundance of the parent ion and therefore enhancing sensitivity and specificity. An additional advantage is that the APGC source is interchangeable with the ESI source enabling a single MS platform to be used for the analysis of both LC and GC amenable pesticides.

In this application note, we demonstrate that the Waters Xevo TQ-S equipped with an APGC source provides a sensitive and repeatable solution for the analysis of pesticides in QuEChERS extracts of strawberry, pear, and

spinach.

Benefits

- Increased sensitivity and selectivity for GC amenable pesticides
- Analyze LC and GC compounds on a single MS platform
- Fast and easy processing of MS data using TargetLynx

Introduction

Pesticides are widely used in the production of fruit and vegetables across the globe. Their use is widespread and governments, food producers and food retailers have a duty to ensure they are not present in final products for consumption. Most countries have clearly defined regulations governing pesticide residues. Legislation imposes Maximum Residue Limits (MRLs) for pesticides in food products requiring analytical techniques that are sensitive, accurate and robust. Multi-residue pesticide analysis is challenging due to the low limits of detection required in a diverse range of food commodities. As there are currently in excess of 1000 pesticides in use, laboratories are under increasing pressure to broaden the range of pesticides determined in ever shorter turnaround times. Therefore, the analytical methods they employ need to be sensitive and robust in a wide variety of sample matrices. Typically, this analysis is carried out using a dedicated GC-MS/MS system using an EI source. As shown by Portolēs ***et al***,[1] this source causes extensive fragmentation of some analytes leading to low sensitivity of the molecular ion. APGC is a "soft" ionization technique which results in an increased abundance of the parent ion and therefore enhancing sensitivity and specificity. An additional advantage is that the APGC source is interchangeable with the ESI source enabling a single MS platform to be used for the analysis of both LC and GC amenable pesticides.

In this application note, we demonstrate that the Waters Xevo TQ-S equipped with an APGC source provides a sensitive and repeatable solution for the analysis of pesticides in QuEChERS extracts of strawberry, pear, and spinach.

Experimental

GC conditions

GC system:	7890A GC
Column:	DB5-MS 30 m x 0.25 mm x 0.25 µm film
Carrier gas:	He 1.2 mL/min
Temp. gradient:	Initial 70 °C for 0.1 minute, 33 °C/min to 180 hold for 1 min, 7 °C/min to 300 °C, hold 6.52 min
Total run time:	30 min
Injector temp.:	250 °C
Injection type:	Pulsed split/splitless
Pulse time:	1 min
Pulse pressure:	55 psi
Injection volume:	1 µL
Makeup gas:	N_2 at 300 mL/min
Transfer line temp.:	310 °C

MS conditions

MS system:	Xevo TQ-S

Mode:	API +
Corona:	2.0 µA
Cone gas:	200 L/hr
Aux gas:	250 L/hr
Source temp.:	150 °C

GC-MS/MS cone and collision parameters and MRM transitions used for this study are presented in Table 1.

Sample preparation

QuEChERS is a simple sample preparation technique suitable for multi-residue pesticide analysis in a variety of food and agricultural products.

Strawberry, pear, and spinach samples were homogenized using a domestic food blender. The samples were then extracted using the DisQUE QuEChERS (CEN method 15662) protocol to generate blank matrix extract in acetonitrile. A nine-point calibration range from 0 to 50 ng/mL (equivalent to µg/kg) was prepared by adding a mixed pesticide standard in acetonitrile to each matrix. To test the repeatability at low concentration, each matrix was fortified with the pesticide mix at 1 µg/kg. A deuterated internal standard, chrysene -d12, was added to provide a fixed concentration of 2 ng/mL to each vial prior to analysis and this was used as an injection standard to correct for injection volume variation. All standards were analyzed in triplicate and the low level spike in each matrix was analyzed 10 times using the Waters Xevo TQ-S with APGC source. Two MRM transitions were monitored for each pesticide, the most abundant species for quantification and the less abundant species for confirmation. MRMs were developed by injection of the solvent standards using a fast GC method (10 minute gradient). The first step was to identify the precursor ion, which was followed by further injections to identify optimal product ions. The final step was to optimize the cone voltages and collision energies so that sensitive and specific MRMs were added to the instrumental method.

Results and Discussion

The 20 GC amenable pesticides that are difficult to analyze using an EI source due to excessive fragmentation, in strawberry, pear, and spinach was achieved using APGC with the Xevo TQ-S operated in target MRM mode. The MRMs with optimized cone voltages and collision energies are shown in Table 1. Using APGC and by varying the source conditions, analysts can choose to promote either proton transfer or charge transfer as the major ionization process. For the analysis of pesticides the dominant ionization mechanism was $[M+H]^+$. Therefore, a vial of water was added to the source to promote protonation. The high intensity of the parent molecular ion observed in APGC spectra makes it possible to generate specific and sensitive MRM transitions for the target analytes. In contrast, many pesticide MRMs using an EI source rely on using a lower *m/z*, less specific fragment ion as the precursor. These features of APGC ensure that the analyst has confidence in identifying and quantifying pesticides that are detected in fruit and vegetable samples.

Each sample type, including matrix matched standards and replicates was analyzed on three different days. Figure 1 shows a typical calibration curve and residuals plot for endosulphan sulphate generated from the triplicate injection of each matrix-matched calibration standard in strawberry extract. The response was linear from 0.05 to 50 ng/mL with a correlation coefficient R^2 of 0.994. All of the residuals were <15% demonstrating excellent linearity and repeatability of the APGC system. The limits of detection and linearity achieved for all 20 pesticides using the APGC with the Xevo TQ-S System are summarized in Table 1. The limits of detection ranged from 0.01 to 0.5 ng/mL with excellent linearity (R2 >0.99) for all 20 pesticides. This demonstrates that the method can easily achieve the regulatory limits and is applicable to routine quantitative analysis.

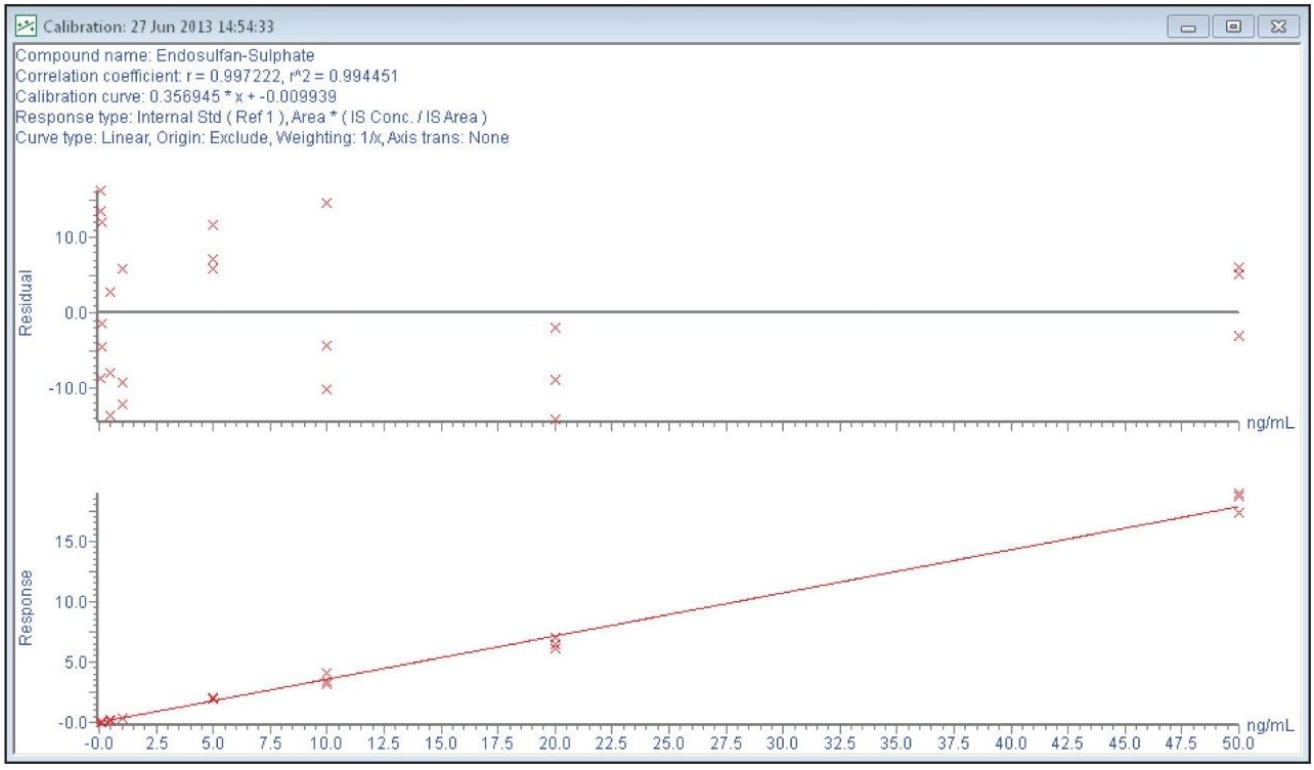

Figure 1. Calibration and residuals plot of the pesticide endosulphan sulphate in strawberry matrix constructed using triplicate injections of each calibration point.

Compound	Retention time (min)	MRM	Cone voltage	Collision energy	Limit of (ng/mL)	Corelation coeffect (R^2)
Aldrin	13.4	**363>159**	30	20	0.50	0.992
		363>215		20		
Azinphos-Ethyl	14.2	**289>261**	20	10	0.05	0.990
		289>233		10		
Azinphos-Methyl	20.0	**261>125**	20	20	0.50	0.990
		261>167		10		
Buprofezin	15.9	**306>106**	30	20	0.05	0.990
		300>203		10		
Chlorfenvinphos	14.3	**359>170**	30	30	0.05	0.994
		359>205		20		
Chlorpyriphos	13.2	**350>198**	20	20	0.10	0.995
		350>294		10		
Chlorpyriphos-Methyl	12.1	**322>125**	40	30	0.05	0.990
		322>212		30		
Dichlorvos	6.3	**221>145**	10	10	0.01	0.990
		221>127		20		
Dicrotophos	9.6	**238>112**	40	10	0.05	0.990
		238>193		10		
Dieldrin	16.0	**379>325**	20	10	0.10	0.995
		379>261		20		
Endosulfan I	15.3	**405>323**	10	30	0.10	0.990
		405>217		10		
Endosulfan-Ether	18.7	**341>205**	30	20	0.01	0.995
		341>217		30		
Endosulfan-Sulphate	17.7	**323>217**	10	30	0.05	0.990
		323>287		10		
Endrin	16.5	**379>243**	30	20	0.05	0.997
		379>343		10		
Ethion	16.8	**385>143**	10	20	0.05	0.990
		385>125		30		
Fenarimol	20.7	**331>139**	40	30	0.10	0.997
		331>268		20		
Heptachlor Epox B	17.7	**387>217**	20	30	0.10	0.990
		387>252		10		
Mevinphos	7.5	**225>127**	30	10	0.05	0.990
		225>193		10		
Phenthoate	14.4	**321>135**	9	20	0.05	0.990
		321>163		12		
Phosphamidon	12.0	**300>127**	40	20	0.10	0.993
		300>227		10		

Table 1. Summary of the 20 pesticides analyzed, MRMs monitored, and quantitative performance results.

To assess the accuracy and precision of the method each sample matrix was spiked at 1 µg/kg (10 times below the blanket MRL of 10 µg/kg) and 10 replicate injections were made. The concentration of each pesticide was calculated using matrix-matched calibration graphs. Figure 2 shows the mean calculated concentrations for each pesticide in all three sample matrices. The accuracy of the method is excellent, with all of the pesticides within 5% of the true concentration. Table 2 shows the mean concentrations for each pesticide in each of the three matrices. The %RSD for all pesticides is also shown to be very good at <5%. This demonstrates that the method is both accurate and reproducible across different sample matrices analyzed on different days.

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

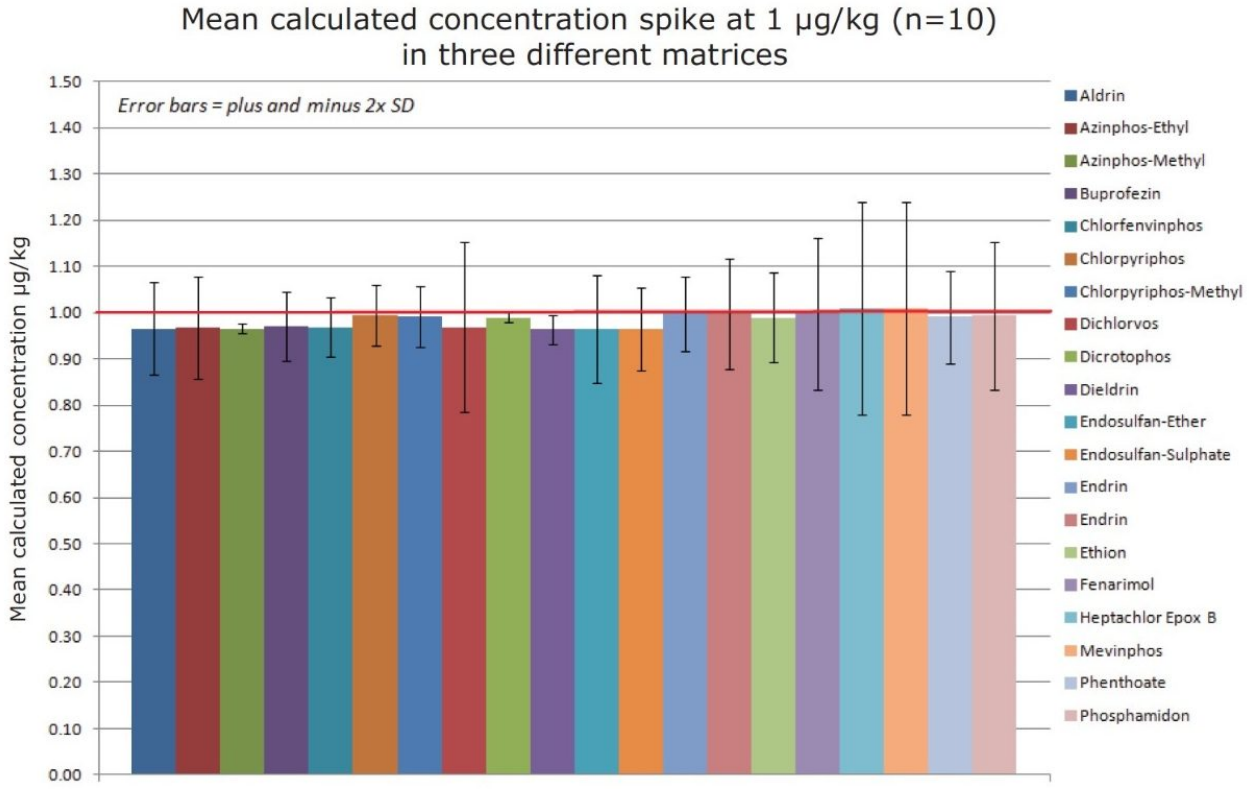

Figure 2. Bar chart showing the mean concentration of pesticides spiked at 1 µg/kg in three different matrices (n=10).

	Spiked samples at 1 µg/kg Mean calculated concentration (n=10)		
Pesticide	Strawberry	Pear	Spinach
Aldrin	0.99	1.01	0.90
Azinphos-Ethyl	1.03	0.94	0.94
Azinphos-Methyl	0.94	0.93	1.02
Buprofezin	0.98	0.97	0.96
Chlorfenvinphos	0.97	1.01	0.93
Chlorpyriphos	1.02	1.01	0.96
Chlorpyriphos-Methyl	1.01	1.01	0.95
Dichlorvos	1.04	1.00	0.87
Dicrotophos	0.99	0.99	1.00
Dieldrin	0.97	1.01	0.99
Endosulfan-Ether	0.99	1.01	0.90
Endosulfan-Sulphate	0.99	0.99	0.91
Endrin	1.02	1.02	0.95
Ethion	0.99	1.05	0.93
Fenarimol	1.00	1.04	0.95
Heptachlor Epox B	1.10	1.00	0.93
Mevinphos	1.14	0.98	0.92
Phenthoate	1.01	1.02	0.93
Phosphamidon	1.04	1.04	0.90
Mean	1.01	1.00	0.94
SD	0.045	0.031	0.038
%RSD	4	3	4

Table 2. The mean concentration of each pesticide (n=10) in the three sample matrices. RSD(%) <5.

Conclusion

- The ability to analyze both LC and GC compounds on a single MS platform is a significant advantage when trying to analyze an increasing number of compounds in a wide variety of sample matrices.

- APGC is a soft ionization technique that produces abundant [M+H]+ ions for the majority of pesticides, which makes it possible to generate selective and sensitive MRM transitions that EI-GC-MS/MS systems fail to achieve routinely.
- APGC in combination with the Xevo TQ-S has been shown to be sensitive, accurate, and reproducible for 20 pesticides that are difficult to analyze by conventional EI-GC-MS/MS.
- APGC offers the performance and versatility required for routine quantitative, multi-residue pesticide analysis in QuEChERS extracts of fruit and vegetables.

References

1. T Portoles, L Cherta, J Beltran, F Hernandez, *J Chromatogr A.* 1260 (2012) 183.

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Atmospheric Pressure Gas Chromatography (APGC) <https://www.waters.com/10100362>

Xevo TQ-S <https://www.waters.com/10160596>

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