QUANTITATIVE ANALYSIS OF PESTICIDES IN QuEChERS EXTRACTS USING UPLC- AND APGC- MS/MS

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INTRODUCTION

Pesticides are widely used in the production of fruit and vegetables across the globe. Governments, food producers and food retailers have a duty to ensure they are not present in final products for consumption. Most countries have regulations governing pesticide residues in food. For pesticides in food products, legislation imposes Maximum Residue Limits (MRLs) which lead to the requirement for analytical techniques that are sensitive, selective and reproducible. 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. The renowned QuEChERS extraction method has been pivotal in this approach, however different chromatographic techniques are typically required for the efficient detection of the multitude of pesticide residues; either by gas chromatography or liquid chromatography, typically coupled with tandem mass spectrometer systems.

Typically, GC analysis is carried out using a dedicated GC-MS/MS system with an EI source. As shown by Portoles et al, [1] EI causes extensive fragmentation of some pesticides leading to poor sensitivity and selectivity, as demonstrated in Figure 1. APGC is a soft ionisation technique which generates high relative and absolute abundance molecular ions resulting in highly sensitive and selective MRM transitions. Furthermore, the APGC source is interchangeable with the LC electrospray source enabling a single MS instrument to be used for the analysis of both LC and GC amenable pesticides [Figure 2]. In this study, we demonstrate sensitive, accurate and repeatable results for the analysis of pesticides in QuEChERS extracts of a selection of commodities below the regulatory limits.

METHODS

A variety of commodities (strawberry, pear and spinach) were extracted by QuEChERS (CEN method 15662 DisQuE #186004831) protocol to generate a nine point calibration range from 0 to 50 µg/kg and replicates at 1 µg/kg (to measure repeatability) A deuterated internal standard, chrysene-d_{12}, was added to give a fixed concentration of 2 ng/mL to each vial prior to analysis and was used as an injection standard to correct for injection volume variation. All standards were analysed in triplicate and the low level spike in each matrix was analysed ten times using the Waters® Xevo TQ-S with the APGC source using the conditions described below.

GC Conditions

Condition	Details
Column	DB5-MS 30 m x 0.25 mm x 0.25 µm film
Carrier gas	Helium 1.2mL/min
Temp. Gradient	Initial 70 °C for 0.1 minute, 33 °C/min to 180 hold for 1 minute, 7 °C/min to 300 °C, hold 6.52 minutes. Total run time 30 minutes.
Injector temperature	250 °C
Injection type	Pulsed split/splitless
Pulse time	1 minute
Pulse pressure	55 psi
Injection volume	1 µl
Make up gas flow	Nitrogen 300 mL/min
Transfer temperature	310 °C

MS Conditions

Parameter	Details
MS system	XEVO TQ-S
Mode	API Positive
Corona	2.0 µA
Cone gas	200 L/hr
Aux gas	250 L/hr
Source temperature	150 °C

Table 1. **Summary of the 20 pesticides analysed, MRM conditions and method performance results**

Compound	Retention time (min)	MRM	Cone voltage (V)	Collision Energy (eV)	Limit of detection (ng/mL)	Correlation Coefficient (R^2)
Aldrin	13.4	363>159	30	20	0.5	0.992
		363>215		20		
Azinphos-Ethyl	14.2	289>261	20	10	0.05	0.99
		289>233		10		
Azinphos-Methyl	20	261>125	20	20	0.5	0.99
		261>167		10		
Buprofezin	15.9	306>106	30	20	0.05	0.99
		306>203		10		
Chlorfenvinphos	14.3	359>170	30	30	0.05	0.994
		359>205		20		
Chlorpyriphos	13.2	350>198	20	20	0.1	0.995
		350>294		10		
Chlorpyriphos-Methyl	12.1	322>125	40	30	0.05	0.99
		322>212		30		
Dichlorvos	6.3	221>145	10	10	0.01	0.99
		221>127		20		
Dicrotophos	9.6	238>112	40	10	0.05	0.99
		238>193		10		
Dieldrin	16	379>325	20	10	0.1	0.995
		379>261		20		
Endosulfan I	15.3	405>323	10	30	0.1	0.99
		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	0.99
		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	0.99
		385>125		30		
Fenarimol	20.7	331>139	40	30	0.1	0.997
		331>268		20		
Heptachlor Epox B	17.7	387>217	20	30	0.1	0.99
		387>252		10		
Mevinphos	7.5	225>127	30	10	0.05	0.99
		225>193		10		
Phenthoate	14.4	321>135	9	20	0.05	0.99
		321>163		12		
Phosphamidon	12	300>127	40	20	0.1	0.993
		300>227		10		

RESULTS AND DISCUSSION

Analysis of 20 GC amenable pesticides, difficult to analyse in EI due to excessive fragmentation, was performed using positive ion MRM mode. By varying source conditions either charge exchange or protonation can be selected for an APGC analysis. For the analysis of pesticides, protonation provides more efficient ionisation than charge exchange. Therefore, a vial of water was added to the source to promote protonation. The MRM transitions with optimised cone voltages and collision energies are shown in **Table 1**. Two transitions were monitored for each pesticide to increase method specificity. The high intensity of the precursor/molecular ion generated by APGC makes it possible to use specific and sensitive MRM transitions. In contrast, many pesticide MRM transitions used with EI MS/MS use lower *m/z*, less specific fragment ion as the precursor. The inherent specificity provided by use of the molecular ion as the precursor in an MRM transition over the use of a fragment ion results in more confident detection of lower levels of analytes even in these complex matrices prepared with a simplified, generic sample preparation technique.

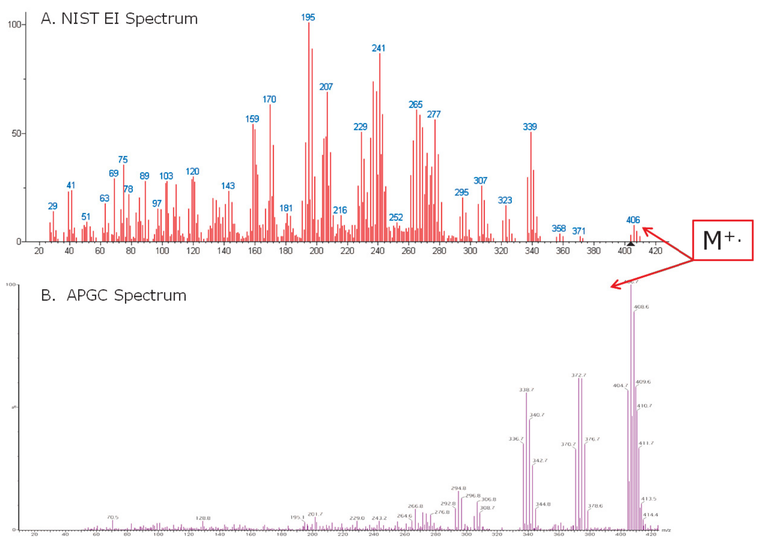

Figure 1. **GC-MSMS spectra for endosulfan by A. EI and B. APCI (APGC)**

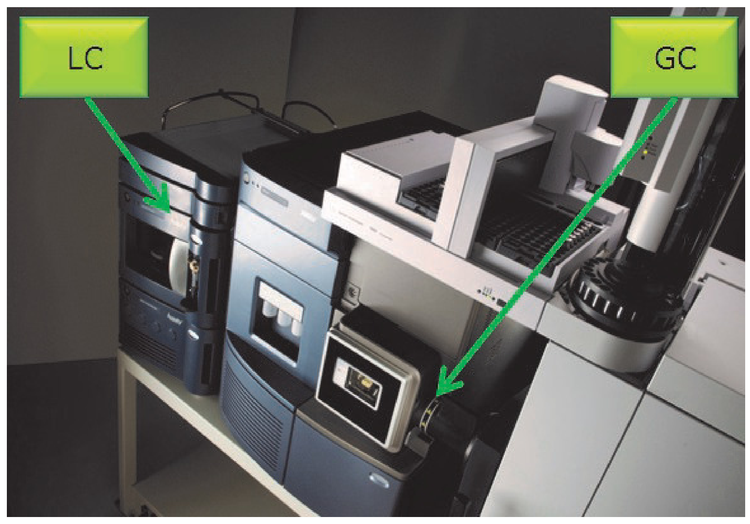

Figure 2. **UPLC and APGC on Xevo TQ-S**

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 ten replicate injections made. The concentration of each pesticide was calculated using matrix matched calibration curves. **Figure 3** and **Table 2** show the mean calculated recoveries and concentrations for each pesticide in all three samples matrices.

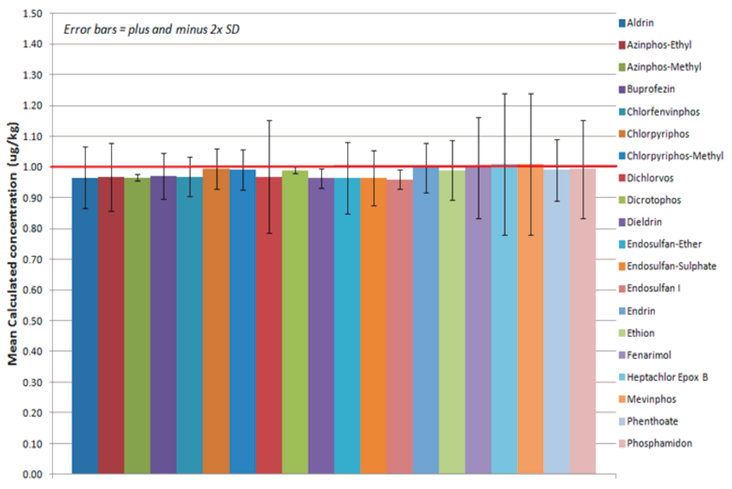

Figure 3. **Pesticides recovery spiked at 1 µg/kg in 3 different food matrices (n=10)**

Table 2. **Mean concentration of each pesticide (n=10) in the three sample matrices**

Pesticide	Spiked samples at 1 µg/kg Mean calculated concentration (n=10)		
	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
Endosulfan I	0.92	0.97	0.98
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	0.048	0.031	0.038
%RSD	5	3	4

Since the sensitivity of this system is well beyond regulatory requirements, a practical application of this performance is to dilute samples, thereby, further reducing matrix effects on chromatography and minimising the amount of material injected on column. The benefit of APGC– XEVO TQ-S is summarized by a collaborator in **Table 3**, with the additional advantage of running UPLC analysis on the same MS/MS.

Table 3. **Comparison of APGC-MS approach versus EI-GC-MS. (*Table courtesy of NofaLab, NL*)**

	APGC	EI
Injection of sample in mg	0.1-0.5 mg	5-50 mg (LOD still higher)
Retention times	Negligible shifts (small scan windows)	Large shifts (large scan windows)
Response	Stable	Large variation and sudden drop
Cleaning the source	Once or twice a year (preventive)	Once or twice a week (when needed)
Replacing the front column/ liner	Every two weeks (preventive)	Once or twice a week (when needed)
Replacing the analytical column	3 to 6 months (when needed)	Once or twice a month (when needed)
Downtime	A week per year (most preventive)	One or two months per year (most trouble shooting)

CONCLUSION

- The method is precise, accurate and reproducible across different sample matrices analysed on different days, exceeds the existing regulations related to pesticide residue analysis.
- Soft ionisation of APGC produces abundant molecular ions for selective and sensitive MRM transitions
- Excellent recoveries (75 to 120 %) and accuracy (< 5 %) was achieved for all analytes in all matrices
- Routine and sensitive multi residues pesticide analysis across different commodities is achievable with QuEChERS using the same workflow for LC-MS/MS on the same system
- Robust system allows for increased sample throughput and system up time for accurate quantitative and confirmatory analysis of GC and LC amenable pesticides on a single MS/MS platform

References

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2. Young, Michael, Tran, Kim Van, Shia, Jeremy C. "Multi-Residue Pesticide Analysis in Ginseng Powder". *Waters application note #720005006EN* (2014)
3. Giroud, Barbara, Antoine Vauchez, Emmanuelle Vulliet, Laure Wiest, and Audrey Bulete. "Trace level determination of pyrethroid and neonicotinoid insecticides in beebread using acetonitrile-based extraction followed by analysis with ultra-high-performance liquid chromatography– tandem mass spectrometry." Journal of Chromatography A 1316 (2013): 53-61

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