# MULTIRESIDUE PESTICIDE ANALYSIS IN FRUIT AND VEGETABLE COMMODITIES USING BOTH UPLC AND APGC ON A SINGLE MASS **SPECTROMETER PLATFORM**

Kari Organtini<sup>1</sup>, Eimear McCall<sup>2</sup>, Simon Hird<sup>2</sup>, Gareth Cleland<sup>1</sup> and Narendra Meruva<sup>1</sup>

<sup>1</sup>Waters Corporation, 34 Maple St, Milford, MA 01757 <sup>2</sup>Waters Corporation, Stamford Avenue, Altrincham Road, SK9 4AX Wilmslow UK

# INTRODUCTION

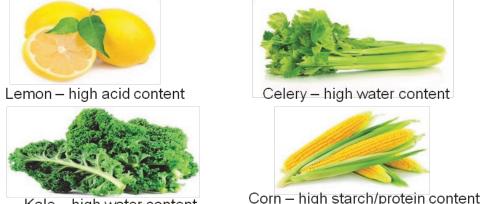
Hundreds of pesticides are commercially available and approved for use on various crops making pesticide residue analysis an important component of ensuring food safety. Maximum Residue Levels (MRLs) are set at the highest level of pesticide that would be expected to be found in that crop when it has been treated in line with good agricultural practice. National authorities control and enforce MRLs by testing samples for pesticide residue levels using analytical surveillance programs. These programs check for compliance with MRLs, assess dietary exposure, and check for use of unauthorized pesticides. The food industry also carries out its own due diligence analyses. With the large amount of pesticides available for use, it is important to be able to rapidly and reliably analyze samples for many pesticides in a single method. Mass spectrometry coupled with both gas (GC) and liquid chromatography (LC) is needed to provide comprehensive analysis of a wide range of pesticide residues with sufficient sensitivity to meet global MRL regulations. The use of Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) sample extraction and clean up has streamlined analytical efficiencies for multi residue analyses.<sup>1</sup> The advantage of ultra performance liquid chromatography (UPLC) coupled with tandem quadrupole mass spectrometry (MS/MS) for multi residue pesticide analysis is widely reported.<sup>2</sup> More recently the use of GC-MS/MS operated at atmospheric pressure (APGC) has been shown to offer significant improvements in performance over electron impact (EI) for challenging pesticides, in terms of selectivity, specificity and speed of analysis.<sup>3, 4</sup> For this analysis, a single workflow for multi residue analysis of pesticides is demonstrated on a variety of fruit and vegetable samples on the same tandem guadrupole MS instrument, with less than 30 minutes needed to switch between chromatographic inlets. The performance of the method is highlighted in terms of sensitivity, repeatability, and linearity for both LC and GC in compliance with the SANTE guidelines (11945/2015) for pesticide analysis.<sup>5</sup>



Figure 1. Xevo TQ-S micro with the Universal Source allowing for UPLC and APGC analysis on the same mass spectrometer.

**Commodities Studied** 

Four different classes of food commodities were studied:



Kale – high water content

#### Pesticide Residue Classes

Each analysis method (APGC and UPLC) contained approximately 200 pesticide residues from various classes.

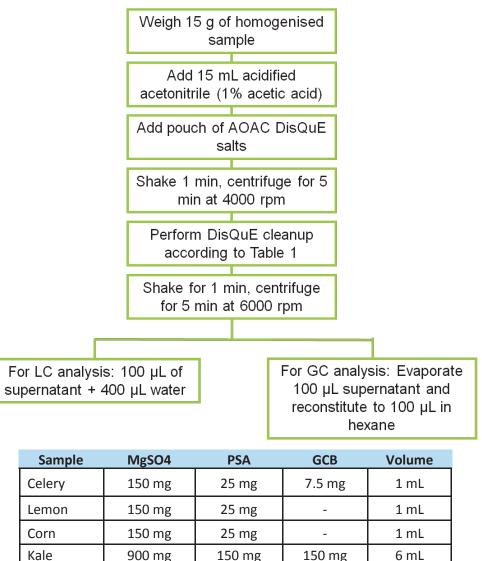
The classes of pesticides studied in the UPLC analysis include:

- Organophosphorus
- Carbamates
- Organonitrogen

The classes of pesticides studied in the APGC analysis include:

- Organophosphorus
- Organochlorine
- Organonitrogen
- Pyrethroids
- Herbicide Methyl Esters

Sample Extraction and Cleanup



#### 900 mg 150 mg 150 mg 6 mL

Table 1. dSPE cleanup conditions used for each sample matrix.

# **METHODS**

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

Method Generation and Management using QuanPedia MS Methods, TargetLynx Processing Methods, and UPLC Methods were generated and maintained using QuanPedia databases.

Water THE SCIENCE OF WHAT'S Options		elect 'Ri	un S	Samp		E Xevo TQ S micro Hclass Q 🕐	
		Select a task					
		Run Sam	ples				
		Create chron quantitative	matographic, MS a methods for an ar	equisition and nalysis			
		Run Com	pounds IS acquisition meth	ad for calastad			
			to confirm chrom				
		Update Ti Update an a based on ob	imes nalysis with new re served changes	etention times			
	Waters		_ ↓		FE Xevo TQ	S micro Hclass	
		<u> </u>				100000 F 29/	
1.61.62	Select Analysis	Sele	ct N	1etho	od		
	Select an analysis from the list below. The analysis includes MS acquisition, chron						
	Analysis						
	Analysis Japanese Positive List Method 2	Information 52 pesticides using positive/negative polarity switching	g 2 MRMs per compound				
	LC Multiresidue Penticide Standards Kit (204)	LC Multi-exolus Pestis de Standards KR (204) Pert number - 186027374 http://www.waters.com/waters.jpartDetail/itm/partNux	mbers18607574				
						2	
	Marine Bottowins	20 tipophilic marine biotoxins including CA, PTX, YTX, Waters application note 7200039380v Mtg.//www.adders.com/websatets/cms/bioes/2	A2A and cyclic imine group				
	Melanine	http://www.waters.com/webassets/cms/Verary/docs/7 Malarrine and cyanuric acid, 2 MRMs per compound 1 Jane Note 72022/08/am		landardii			
	Mycotoxine		lumonisins ZAL ZAN, ZEN I	and HT2 and T2 2 MRMs for con	ferration		
	Mycotoxine 2	14 mycchowine 2 MMMs per confirmation. Young et al., NACKW 2015 poster www.waters.com (poster Library number - PSTR134857					
	Contra a la			200 - 130		Next Canad	
gennen beligt sinsspraisbelaut proje	Andra periode and a limit of annual						
Life View Options Tandars Funds are an area area area area area area are	lans Help					pest, autodwell_one min window.mdb - TarpetLynx XS Met	x. 🗆 🔅 🞫
10 2 alter 2 baterien erferfent (2.20) efterfent (3.1) ##	an 👹 Parentes 📓 Conceptions 📲 Sanakari, ann 👹	Sanas Stanton II Shadaanka	File Edit	Update View Compound	- Help 😍 🐳 🔟 📖		
Type	Information.		Tere Compound 61 Chlorar	List *	💁 🏊 🕾 🕾 🕾 🛸 🛸	Value	
Miller of 2 mass pairs. Time 6 40 t Miller of 2 mass pairs. Time 2 82 5 Miller of 2 mass pairs. Time 4 22 t Miller of 2 mass pairs. Time 7 40 t	is 3.82 ED+ (Floresand) to 5.22 ED+ (Enrethodin)		62 Chlorbr 63 Chlorfe	omuron nvinphos	Compound Properties Compound Name	Carbaryl	1
<ul> <li>Mill of 2 mass pairs, Time T 84 to Mill of 2 mass pairs, Time 5 81 to</li> </ul>	le 8.84, 8.9 × (Solaron) Is 7.89, 8.9 × (Fluorradhuron)	_	64: Chlorft 65: Chlorid	azuron	CAS Number Compound Type	Insecticide	
Mittel #2 mass pairs, Time 8 28 5 Mittel #2 mass pairs, Time 2 68 5 Mittel #2 mass pairs, Time 2 68 5 Mittel #2 mass pairs, Time 5 75 5	5 3 99 ES+ (Deams) 5 5 79 ES+ Carbelande:	2	66 Chlorim 67 Chloro	suron ethyl suron	Acquisition Function Number	insecticide 28	
Mill of 2 mass pars, Time 3 75 k Mills of 2 mass pars, Time 4 25 to Mills of 2 mass pars, Time 4 25 to Mills of 2 mass pars, Time 7 04 to	n 475,85+ (Develophen) n 539,85+ (Cartestaten-Sitydron) n 816,85+ (Perincart)		68: Chlorpy 69: Chlorpy	rifos riphos-methyl	Quantification Trace Use absolute mass window?	202 > 145 145	
BHB 427 mess parts. The main 11%           BHB 427 mess parts. The main 11%           BHB 427 mess parts. The main 10%           BHB 427 mess parts. The main 10%	6-958-851 (Promition) 6-953-851 (Promition) 6-953-851 (Promition)		70: Chlorise 71: Chlorise	Muron Iuron	Chromatogram mass window (Da)	0 0000	
Mitter of 2 mans pairs, time 2 58 5 Mitter of 2 mass pairs, Time 3 54 5 Mitter of 2 mass pairs, Time 3 54 5	0 8 10 85+ Esturani 6 4 54 85+ (Changen)		72 Cindor 73 Cinosu	furon	Locate Peak Using Locate Peak Selection	Retention Time Nearest	
Milli of 2 mass pairs, time 4 96 to Milli of 2 mass pairs, time 3 91 to Milli of 2 mass pairs, time 4 54 to	6 4 91 53+ (Triplaner) 6 5 14 53+ (Triplaner)		74: Clethor 75: Clethor	ám I	Predicted Retention Time Retention Time Window (mins) #	7 5270 0 5000	
MRW of 2 mass pairs. Time 8 801 MRW of 2 mass pairs. Time 1 801 MRW of 2 mass pairs. Time 8 01 MRW of 2 mass pairs. Time 8 01 MRW of 2 mass pairs. Time 8 401	e ni eo, c.S.: (Thilbencard) le 8.06, (S.+. Oktobercard) le 9.00, C.S.+ Chetholencard)		76. Clodina 77: Clofent 78: Clomaz	fop-propargyl azine	Relative Retention Time Reference	None	
<ul> <li>Milli of 2 mass pairs. Time 2 75 h</li> <li>Milli of 2 mass pairs. Time 2 15 h</li> </ul>	Is 375. ES+ (Menguran) Is 316. ES+ (Menautoria)	-	78 Clonau 79 Cloquin 80 Clothia	tocet - mexyl	Response Uses Response Type	Area External (absolute - no internal stand	ards)
Mith of 2 mess pairs. Time 5 20 h Mith of 2 mass pairs. Time 5 24 h		-	Ready		-		NUM
	MS Met	hod		Da	ata Pro	cessing	
					Meth	lod	

Figure 2. Xevo TQ-S micro with the Universal Source allowing for UPLC and APGC analysis on the same mass spectrometer.

## **UPLC-MS/MS** Parameters

LC System: ACQUITY UPLC H Class Column: ACQUITY BEH C18 2.1 x 100 mm, 1.7um Mobile Phase A: Water + 10 mM ammonium acetate Mobile Phase B: Methanol + 10 mM ammonium acetate MS System: Xevo TQ-S micro Ionization Mode: ESI+

## **APGC-MS/MS** Parameters

GC System: 7890A Autosampler: CTC PAL RTC

Column: 30 m x 0.25 mm x 0.25 µm Rxi-5MS

MS System: Xevo TQ-S micro

Ionization mode: API+

Ionization mechanism: **Proton transfer** (3 vials of water in source)

For full method details, see Waters application note 720006013en

## Fast Scanning Speeds of Xevo TQ-S micro

Each analysis method contained approximately 200 pesticide residues acquiring at least 2 MRM transitions per compound. The mass spectrometer must be able to rapidly scan through all of the MRM channels to acquire the required data.



Figure 3. The fast scanning of the Xevo TQ-S micro collects the required data points (minimum 12 points per peak) while maintaining peak quality.

## **Pesticides in Matrix**

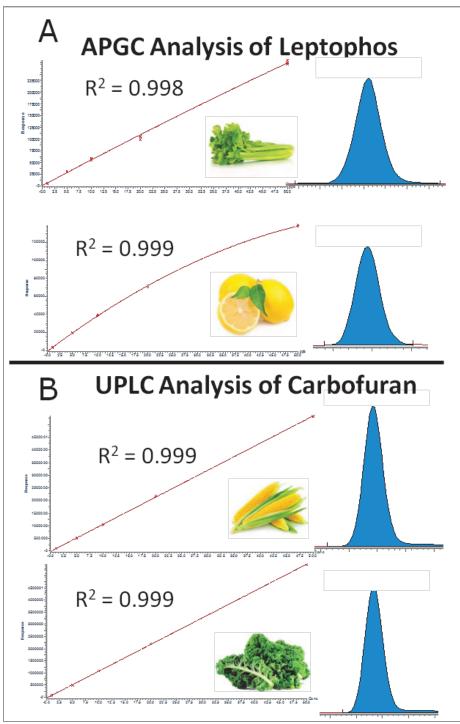
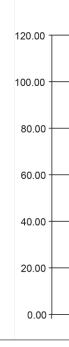
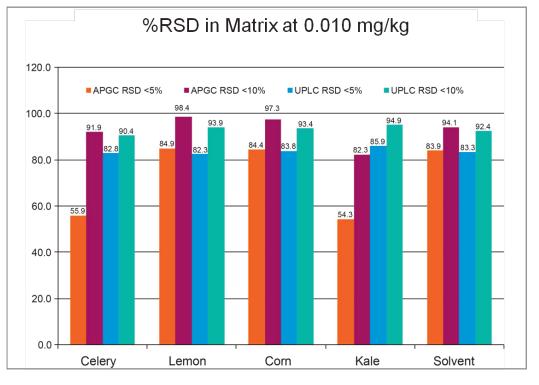


Figure 4. Matrix matched calibration curves and chromatograms for standards at 0.001 mg/kg. (A) APGC analysis of leptophos in celery and lemon, (B) UPLC analysis of carbofuran in corn and kale.

Matrix matched standards were prepared in celery, lemon, corn and kale over a range of 0.001 - 0.050 mg/kg and replicate injections made using the UPLC and APGC methods. The data were fitted with the best fit calibration; for the UPLC data, the response was shown to be linear whereas the APGC response over the range investigated was nonlinear and so was fitted with a quadratic calibration. (Figure 4). This is in accordance with the SANTE/11945/2015 guidelines.<sup>5</sup> A majority of the compounds in both analysis methods had correlation coefficient ( $R^2$ ) values of 0.995 or greater. Residuals from triplicate injections at each calibration point were within ±20%.

Figure 5 demonstrates the percentage of pesticides in each method detected in the spiked matrices at 0.010 mg/kg, but many pesticides could also be detected at 0.001 mg/kg as demonstrated in Figure 4. The precision of the measurements was excellent with more than 90% of the detected pesticides exhibiting RSDs less than 10% (n=3). The exception was the APGC analysis of the kale matrix which had more than 80% of pesticides exhibiting RSDs less than 10% (Figure 6). Ion ratios in matrix were also shown to be within 30% tolerance of the reference values.





tive RSDs.

# **Waters** THE SCIENCE OF WHAT'S POSSIBLE.

# **RESULTS AND DISCUSSION**

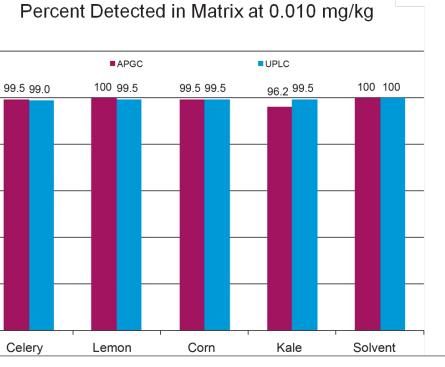


Figure 5. Percentage of pesticides detected in the 0.010 mg/kg standard for each matrix using both APGC and UPLC.

Figure 6. Percentage of compounds detected at 0.010 mg/kg at respec-

#### **RADAR Provides Another Layer of Information**

RADAR allows a full scan to be acquired with MRM transitions and provides extra information in the sample analysis. Figure 7 shows two different matrices spiked with the same concentration of tetrachlorvinphos (red trace). Sample A quantified two times higher than sample B. The full scan data (blue trace) shows a large matrix interference in sample A at the retention time of tetrachlorvinphos which may suggest matrix enhancement as the reason for the difference in qauntitation. This information can help in the development of more extensive sample clean up or change in chromatographic conditions to move the peak of interest away from the matrix interference.

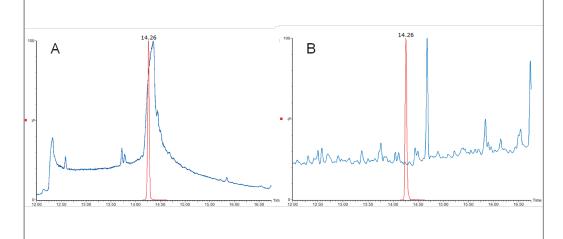


Figure 7. RADAR full scan (blue trace) indicates matrix interference with tetrachlorvinphos MRM (red trace) in sample A, but no interference in sample B.

## CONCLUSIONS

- Complex multi residue pesticide analysis was demonstrated using both UPLC and APGC analysis on the same tandem quadrupole instrument
- Instrument methods were generated and maintained using QuanPedia databases making method generation and maintenance fast and simple.
- The reliable scanning speed of the TQ-S micro produced accurate and precise measurements for large multi residue methods.
- The performance of the analysis complied with the SANTE guidelines for pesticide residue analysis.
- Detection at the default maximum residue limit of 0.010 mg/kg was easily achieved for > 99% of pesticides analyzed with good precision (RSDs < 10%) for most analytes in the food samples.
- The flexibility of the Universal Source architecture provides access to both UPLC-MS/MS and GC-MS/MS on the same instrument, allowing for an increase of laboratory efficiency, while maintaining required sensitivity and repeatedly.

#### References

- 1. D. Shah, E. McCall, G. Cleland. Single LC-MS/MS Method for Confirmation and Quantification of Over 400 Pesticides in a Complex Matrix Without Compromising Data Quality. Waters Application Note 720005559EN. January 2016.
- Kovalczuk T., Jech M., Poustka J., Hajslova J., 2006, UPLC-MS/MS: A novel challenge in multiresidue pesticide analysis in food. Analytica Chimica Acta, 577
- Tienstra M., Portoles T., Hernandez F., Mol J.G.J., 2015, Fast gas chromatographic residue analysis in animal feed using split injection and atmospheric pressure chemical ionisation tandem mass spectrometry, J. Chrom. A., 1422.
- 4. Cherta L., Portoles T., Beltran J., Pitarch E., Mol J.G.J., Hernandez F., 2013, Application of gas chromatography- mass spectrometry with atmospheric pressure chemical ionisation for the determination of multiclass pesticides in fruits and vegetables, 1314.
- 5. European Commission. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. 2015, rev. 0.