

Enhancing MRM Experiments in GC-MS/MS Using APGC

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Abstract

In this application note, APGC with Xevo TQ-S and ACQUITY UPLC for multi-residue GC-MS/MS analysis of 25 pesticides in food has been described. APGC is a technique that uses a soft ionization process which can provide abundant molecular ions that can be used as precursor ions in MRM experiments for multi-residue GC-MS/MS analysis.

Benefits

- Increased sensitivity and selectivity for GC amenable pesticides.
- Able to analyze both GC and LC compounds on one MS platform.
- Routine system that is suitable for all food testing laboratories.

Introduction

Gas and liquid chromatography (GC and LC) coupled to mass spectrometry (MS) are the techniques of choice in pesticide residue analysis (PRA) for a wide variety of sample matrices.

GC-MS is commonly applied for non-polar, volatile, and thermally stable compounds and ionization in GC-MS normally occurs under vacuum conditions using either electron ionization (EI) or chemical ionization (CI).

EI is typically employed in the wide majority of GC-MS applications, and is a robust and highly reproducible technique. It is well known that this ionization process produces extensive fragmentation of some molecules, leading in many cases to the absence of the molecular ion $[M]^{\bullet+}$ in EI spectra. CI induces softer ionization and this can lead to better selectivity and sensitivity for some analytes, as well as fewer matrix interferences. But CI ionization is only applicable to specific chemical classes¹⁻³ and sensitivity is limited.

Many recent methods have been reported in PRA based on EI-GC tandem quadrupole mass spectrometry, due to its better performance for quantitative multi-residue analysis compared to EI-GC-single quadrupole mass spectrometry. The selection of adequate precursor and product ions enhance selectivity and sensitivity, minimizing or even eliminating matrix interferences. In this way, very low detection limits can be achieved.

However, the extensive fragmentation due to the high energy transferred to the molecules during the ionization process produces little or no molecular ions for many pesticides, as for example organochlorine (OC) pesticides, organophosphorus (OP), pyrethroids, and chloroacetanilides.⁴⁻⁶ Also, compounds belonging to the same chemical family can show similar EI spectra; so the use of common ions/transitions can complicate the identification and quantification processes, especially if analytes are co-eluting. When the molecular ion is absent or has very low abundance, it is necessary to select a (abundant) fragment ion as precursor. In addition to the loss of sensitivity, the specificity of the method can be also affected.

Atmospheric pressure ionization in GC-MS was first introduced by Horning *et al.*⁷ and the technique offers attractive analytical capabilities in GC-MS analysis. APGC is a technique that operates at atmospheric pressure and uses a soft ionization process which can provide abundant molecular ions that in most cases to be used as precursor ions in MRM experiments for multi-residue GC-MS/MS analysis.

Waters Xevo TQ-S tandem quadrupole mass spectrometer is a highly sensitive and robust instrument that is compatible with both the ACQUITY UPLC System and APGC.

The aim of the project⁸ was to evaluate the potential of APGC-Xevo TQ-S for the quantitative analysis of pesticides in foodstuffs: 25 pesticides with varying chemical properties have been selected and the potential advantages of APGC were evaluated in comparison with EI ionization.

Experimental

Sample preparation

Different fruits and vegetable (apple, orange, tomato and carrot) fortified extracts were used and prepared using the QuEChERS extraction approach (AOAC Official method)⁹

GC conditions

GC system:	7890A
Injector:	Splitless mode
Injection:	1 μ L at 280 °C
Column:	DB-5MS (J&W Scientific, USA) 30 m I.D. 0.25 mm df 0.25 μ m
Column temp.:	70 °C (1 min), 25 °C/min to 150 °C 10 °C/min to 300 °C (3 min)
Transfer line temp.:	310 °C
Carrier gas flow:	2 mL/min (Helium)
Auxiliary gas:	250 L/h (Nitrogen)
Make-up gas:	320 mL/min (Nitrogen)

MS conditions

MS system:	Xevo TQ-S
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APCI corona pin:	1.8 μ A
Cone voltage:	25 V
Source offset:	50 V
Cone gas:	170 L/h (Nitrogen)
Data management:	MassLynx Software v4.1, with Targetlynx Application Manager

RT (min)	Compounds	Cone voltage (V)	MRMs	Collision energy (eV)	RT (min)	Compounds	Cone voltage (V)	MRMs	Collision energy (eV)
4.70	Dichlorvos	10	Q 221 > 145	10	11.56	Oxychlordane	10	Q 421 > 151	20
			q1 221 > 113	30				q1 421 > 115	20
			q2 221 > 127	20				q2 421 > 285	30
5.97	Mevinphos	30	Q 225 > 127	10	11.56	Heptachlor epox B	20	Q 387 > 217	30
			q1 225 > 113	30				q1 387 > 251	20
			q2 225 > 193	10				q2 387 > 252	10
6.96	Molinate	20	Q 188 > 126	10	12.23	Endosulfan I	10	Q 405 > 323	10
			q1 188 > 98	20				q1 405 > 205	20
			q2 188 > 160	10				q2 405 > 217	30
8.00	Dicrotophos	40	Q 238 > 112	10	12.72	Buprofezin	30	Q 306 > 106	20
			q1 238 > 127	20				q1 306 > 203	10
			q2 238 > 193	10				q2 306 > 250	10
8.24	Monocrotophos	20	Q 224 > 127	10	12.73	Dieldrin	20	Q 379 > 325	10
			q1 224 > 113	30				q1 379 > 147	20
			q2 224 > 193	10				q2 379 > 261	20
8.95	Terbufos	10	Q 187 > 131	10	13.10	Endrin	30	Q 379 > 343	10
			q1 187 > 97	20				q1 379 > 243	20
			q2 187 > 113	20				q2 379 > 244	20
9.80	Phosphamidon	40	Q 300 > 127	20	13.36	Ethion	10	Q 385 > 125	20
			q1 300 > 174	10				q1 385 > 97	10
			q2 300 > 227	10				q2 385 > 143	30
9.76	Endosulfan ether	30	Q 341 > 217	30	14.01	Endosulfan sulfate	10	Q 323 > 217	30
			q1 341 > 170	30				q1 323 > 252	20
			q2 341 > 205	20				q2 323 > 287	10
9.94	Chlorpyrifos methyl	40	Q 322 > 125	30	15.63	Azinphos methyl	20	Q 261 > 125	20
			q1 322 > 212	30				q1 261 > 167	10
			q2 322 > 290	20				q2 261 > 183	10
10.77	Chlorpyrifos	20	Q 350 > 198	20	15.66	Pyriproxyfen	10	Q 322 > 185	20
			q1 350 > 294	10				q1 322 > 129	30
			q2 350 > 322	10				q2 322 > 227	10
10.85	Aldrin	30	Q 363 > 159	20	16.04	Fenarimol	40	Q 331 > 268	20
			q1 363 > 215	20				q1 331 > 139	30
			q2 363 > 327	10				q2 331 > 259	20
11.39	Isodrin	30	Q 363 > 159	20	16.17	Azinphos ethyl	20	Q 289 > 137	20
			q1 363 > 215	20				q1 289 > 233	10
			q2 363 > 327	10				q2 289 > 261	10
11.56	Chlorfenvinphos	30	Q 359 > 170	30					
			q1 359 > 99	10					
			q2 359 > 205	20					

Table 1. Retention times and APGC MRM transitions for the compounds.

Results and Discussion

In order to evaluate the capabilities of APGC, 25 pesticides were selected on the basis of their mass spectral behavior in the EI source and those that are commonly analyzed using EI-GC-MS/ MS in order to meet food safety regulatory guidelines.

MRM selectivity and specificity

There are many examples of pesticides known to be analytically problematic when using GC-MS/MS methods under EI conditions, due to EI being a highly energetic ionization mechanism. An example of this can be seen in Figure 1, where we show a spectral comparison for EI and APGC.

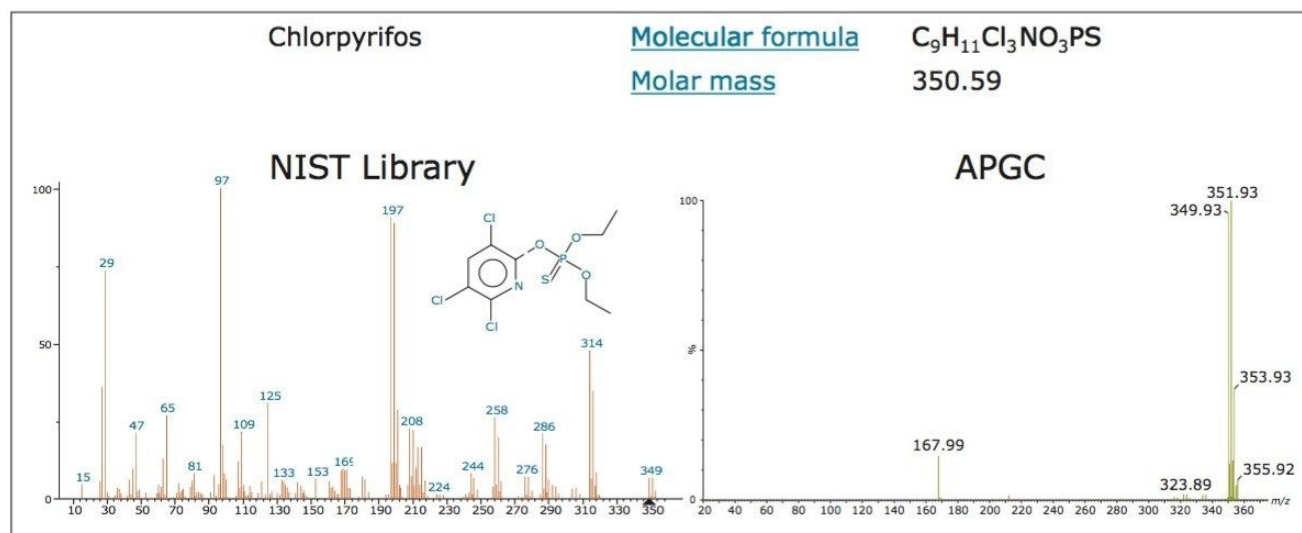


Figure 1. Spectral comparison for chlorpyrifos: EI (left) and APGC (right). The EI spectrum (taken from NIST) is highly fragmented whereas the APGC spectrum shows less fragmentation and provides the $[M+H]^+$ as the base peak.

When a compound undergoes a high degree of fragmentation the resulting MRM is often not specific, and this can affect the selectivity and may lead to a false positive identification.

Figure 2 shows an example of the potential for false positive identification using EI for heptachlor epoxide B and oxychlordane. Pure standards of each compound were analyzed using the typical EI transition for oxychlordane ($235 > 141$, Figure 2a). Due to the structural similarity of the compounds, the heptachlor epoxide B standard was detected at the same retention time with the same transition as oxychlordane. However, using APGC and a more selective transition ($421 > 151$: 421 corresponds to the $[M+H]^+$ ion of oxychlordane in APGC), allowed the determination of oxychlordane without interference from heptachlor epoxide B.

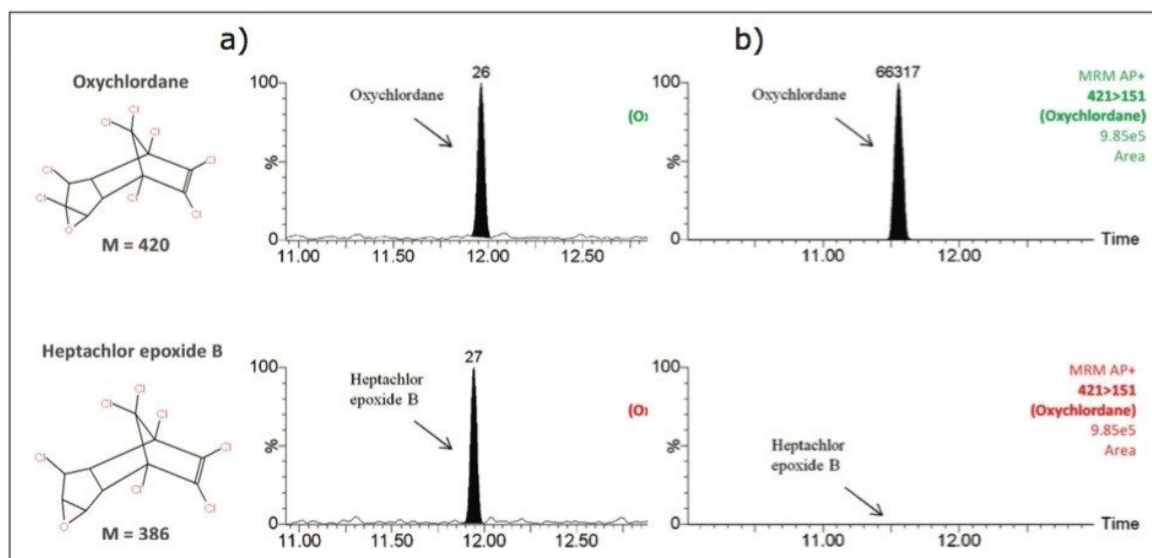


Figure 2. Chromatograms obtained for the selected MRM transitions acquired for heptachlor epoxide B (top), and oxychlordanes (bottom) under (a) EI (100 ppb), and (b) APGC (10 ppb).

Sensitivity

The Xevo TQ-S is a highly sensitive and robust tandem quadrupole mass spectrometer when coupled to an ACQUITY UPLC System,¹¹⁻¹³ and data from this work (and other work carried out) suggests that this is also the case when using APGC.

Using the MRM method developed the instrumental sensitivity and linearity was evaluated, and linearity was studied by injecting solvent standards in duplicate in the range 0.1 to 100 ppb (corresponding to 0.1 to 100 µg/kg in sample). The majority of compounds showed a linear response from 0.1 to 100 ppb with $r^2 > 0.99$ (data not shown).

Fortified extracts of the compounds in various fruits and vegetables were prepared and Figure 3 illustrates representative examples for ethion and fenarimol in tomato and carrot, respectively. The sensitivity of APGC coupled to the Xevo TQ-S led to an estimation of LOQ (calculated as $10 \times S/N$) between 0.02 and 2 ppb depending on the analyte and matrix under study. Responses for the spiked samples (apple, orange, tomato, and carrot) were compared to those of standards in solvent. No significant matrix effects were observed, although a little enhancement of the response of some analytes in the sample extracts occurred.

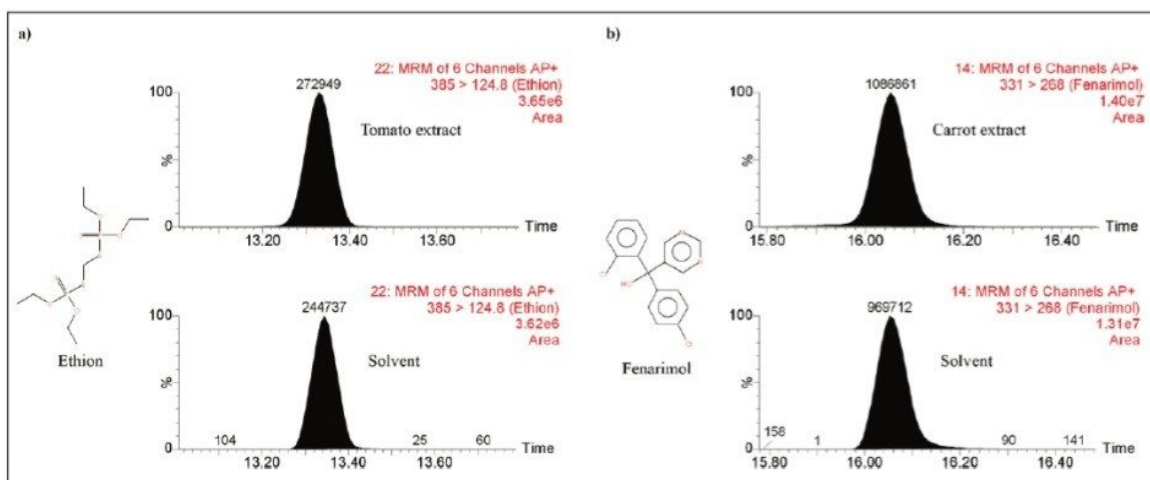


Figure 3. MRM chromatograms for (a) ethion in spiked tomato extract (10 ppb) and in solvent standard (10 ppb), and (b) fenarimol in spiked carrot extract (10 ppb) and in solvent standard (10 ppb),

The sensitivity of the Xevo TQ-S currently exceeds existing regulations related to pesticide residue analysis for both GC and LC-amenable pesticides. The additional sensitivity enables analysts to dilute samples, significantly reducing matrix interferences and minimize the amount injected on-column. This in turn has major benefits for system cleanliness and reduces instrument maintenance requirements.

Conclusion

For many food testing labs, both GC and LC are important techniques that enable a broad range of compounds to be analysed. With the introduction of APGC it is now possible to have access to both types of separation on the Xevo TQ-S.

The Xevo TQ-S used in conjunction with an ACQUITY UPLC has been reported to be a very sensitive and robust tandem quadrupole mass spectrometer, and preliminary work using the same MS system, but with APGC also indicates that high sensitivity can also be achieved for GC-amenable compounds.

As shown by Portoles *et al.*,⁸ pesticides that demonstrate high fragmentation under EI can be easily analyzed

using APGC, where the $[M+H]^+$ became the base peak of the spectrum for the majority of compounds. The soft and reproducible ionization process results in an increase in sensitivity (compared to other GC-MS/MS methods) in the subsequent APGC–Xevo TQ-S method.

The Xevo TQ-S in combination with both the ACQUITY UPLC and APGC makes it an attractive MS for routine food testing laboratories due to its chromatographic versatility, the ability to choose more selective MRMs, and the system sensitivity.

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