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應用手冊

Determination of OCs, PCBs, and Synthetic Pyrethroids in Animal Fat

Peter Hancock, Mike Hetmanski, Richard J. Fussell

Waters Corporation, Central Science Laboratory, York

Abstract

The approach described in this application note is the use of tandem quadrupole MS/MS for the quantification

and unequivocal confirmation of OCs, International Council for the Exploration of the Seas (ICES) 7 PCBs and SPs at low µg/kg levels in animal fat without the need for an additional clean-up technique following gel-permeation chromatography (GPC).

Benefits

The Quattro micro GC provides high selectivity toreduce any matrix interferences, high sensitivity to reach the reporting levels required by European Union legislation and quantitative and confirmatory data in a single injection

Introduction

The regulations governing permitted levels of persistent organic pollutants (POPs) in food products are becoming more stringent in response to increased awareness of the hazards they pose to humans. POPs, such as organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs), have been linked to adverse effects such as cancer, damage to the nervous system, reproductive disorders, and disruption of the immune system. They do not readily degrade in the environment, are lipophilic and tend to bioaccumulate as they move through the food chain. Therefore, these can potentially occur at high concentrations in fatty foods, such as meats and fish. Due to concerns about the effects of POPs, an international treaty restricting their use was adopted via the Stockholm Convention in 2004.[1] Currently in the UK, maximum residue limits (MRLs) of OCs and synthetic pyrethroids (SPs) in meat products are between 0.02–1.0 mg/kg on a fat basis.[2] To enforce these regulations, high quality analytical methods with adequate confirmation and limits of quantification must be used.

OCs, PCBs and SPs have all been analysed by conventional gas chromatography (GC) with electron capture detection (ECD). This is a sensitive technique for compounds containing halogens but suffers extensively from matrix interferences and confirmatory evidence cannot be provided as it is only a two dimensional technique with retention time and intensity. To add a third dimension, mass spectrometry (MS) can be used with selected ion monitoring (SIM). Usually three or four selected ions are monitored for each compound but confirmatory evidence is reduced if one or more of the selected ions are affected by matrix interferences.

The likelihood of interference will increase as the amount of sample preparation decreases. To minimize sample preparation and/or the likelihood of interference the selectivity of the detection technique should be increased.

The approach described in this application note is the use of tandem quadrupole MS/MS for the quantification and unequivocal confirmation of OCs, International Council for the Exploration of the Seas (ICES) 7 PCBs and

SPs at low µg/kg levels in animal fat without the need for an additional clean-up technique following gel-permeation chromatography (GPC).

Experimental

Extraction Method

1.25 g melted porcine fat was weighed into a 10 mL volumetric flask. For recovery, the samples were spiked at 0.00625 µg/mL or 0.05 mg/kg. The volume was adjusted with ethyl acetate/cyclohexane (1:1). A 1 mL extract was cleaned-up by GPC to give a final extract with a matrix equivalent of 0.125 g/mL in hexane. The internal standard, δ-HCH, was added at a concentration of 0.050 µg/mL equivalent to 0.4 mg/kg.

GC Method

The samples were injected by splitless injection (4 µL, 250 °C, purge at 30 mL/min after 2.1 min) into a carrier gas of helium at a constant flow rate of 1.0 mL/min delivered from an Agilent 6890 GC with a CTC CombiPal autosampler attached. The column employed was a J & W Scientific DB-17MS 30 m x 0.25 mm i.d., 0.25 µm. The following temperature ramp rate was used: 100 °C (1 min) to 200 °C (6 min) at 20 °C/min, to 280 °C/min (15 min) at 10 °C/min. The total run time was 35 min. The temperature of the interface was held at 260 °C during the chromatographic run.

Figure 1. Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer.

The Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer was used (Figure 1) in electron impact (EI+) mode. The ion source was operated at 180 °C with an electron energy of 70 eV and a trap current of 200 µA. The mode of acquisition was multiple reaction monitoring (MRM) at an argon collision gas pressure of 3.0 x 10^{-3} mBar.

The Quattro micro GC was tuned so that the precursor and product ions were resolved with a peak width at half height of less than 0.7 Da. The list of pesticide residues and the MRM transitions, along with the collision energies and retention times for the method are listed in Table 1.

Pesticide	Retention Time	Quantification Transition			Confirmation Transition		
		Precursor Ion	Product Ion	CE	Precursor Ion	Product Ion	CE
Hexachlorobenzene	8.38	284	249	15eV	286	251	15eV
Alpha-HCH	8.78	219	183	5eV	181	145	15eV
Gamma-HCH	10.00	219	183	5eV	181	145	15eV
Beta-HCH	10.75	219	183	5eV	181	145	15eV
Heptachlor	11.12	272	237	13eV	274	239	15eV
PCB 28	11.37	256	186	15eV	258	186	15eV
Delta-HCH, Internal Standard	12.20	219	183	5eV	181	145	15eV
Aldrin	12.53	263	193	25eV	263	191	25eV
PCB 52	12.74	290	220	23eV	292	220	23eV
Oxychlordane	14.26	185	149	5eV	185	121	15eV
Heptachlor epoxide	15.12	183	155	10eV	217	182	15eV
trans-Chlordane	15.42	373	266	15eV	375	266	15eV
PCB 101	15.64	326	256	25eV	328	256	25eV
cis-Chlordane	15.84	373	266	15eV	375	266	15eV
Alpha-Endosulfan	15.96	241	206	12eV	241	170	20eV
pp-DDE	16.68	246	176	21eV	318	248	18eV
Dieldrin	16.87	241	206	12eV	237	143	20eV
PCB 118	17.54	326	256	25eV	328	256	25eV
Endrin	17.82	263	193	30eV	263	191	30eV
PCB 153	17.86	360	290	25eV	362	290	25eV
op-DDT	18.10	235	165	20eV	237	165	20eV
pp-DDD	18.27	235	165	20eV	237	165	20eV
Beta-Endosulfan	18.47	241	206	12eV	241	170	20eV
PCB 138	18.89	360	290	25eV	362	290	25eV
pp-DDT	18.95	235	165	20eV	237	165	20eV
Resmethrin	19.12	171	143	5eV	171	128	15eV
Bifenthrin	19.18	181	166	15eV	181	165	20eV
Endosulfan sulfate	19.57	272	237	11eV	272	235	11eV
PCB 180	20.16	394	324	22eV	396	324	22eV
Lambda-Cyhalothrin	20.57	197	141	10eV	181	152	20eV
Permethrin	22.70	163	127	5eV	163	91	10eV
Cyfluthrin	23.70	163	127	5eV	163	91	10eV
Cypermethrin	24.70	163	127	5eV	163	91	10eV
Flucythrinate	25.00	199	157	9eV	157	107	10eV
Fenvalerate	28.00	167	125	10eV	125	89	15eV
Deltamethrin	31.20	253	93	15eV	181	152	20eV

Table 1. Pesticide residues and MRM method parameters.

The data were acquired using Waters MassLynx Software and processed using the Waters TargetLynx Application Manager.

Results and Discussion

A typical reconstructed ion chromatogram (RIC) for the twenty OCs, seven PCBs and nine SPs is illustrated in Figure 2. The MRM transitions were arranged into thirteen function windows represented by the different colors in Figure 2. These functions can be overlapped slightly to allow for small changes in retention time. Use of function windows allows more time to be spent on each MRM transition, thereby, improving the signal to noise (S/N) ratio for the analytes that gave the lowest response. Dwell times were set so that approximately fifteen data points described each chromatographic peak.

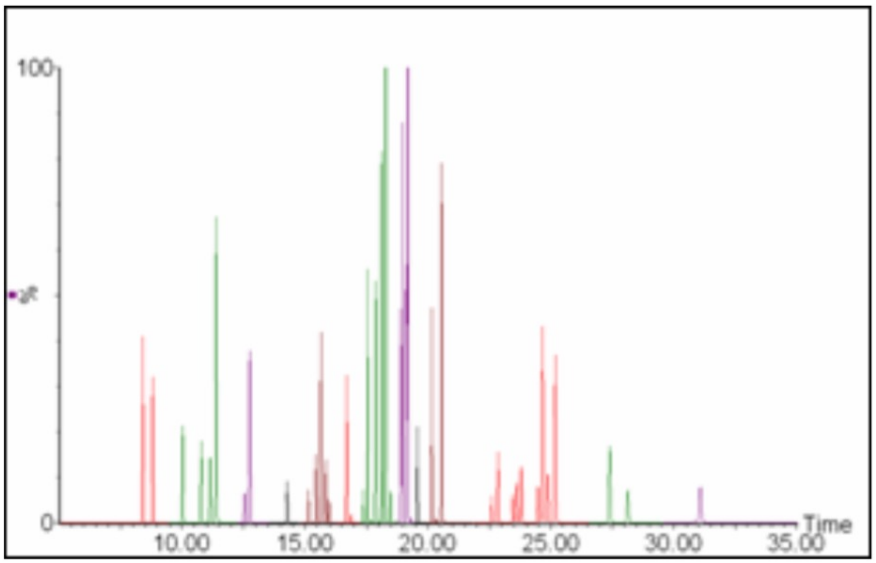


Figure 2. RIC for the OCs, PCBs and SPs.

Comparison of SIM and MRM

Two selected mass chromatograms are illustrated in Figure 3 for hexachlorobenzene (8.52 min) acquired in SIM mode at a concentration in matrix of 0.01 mg/kg. For complete confirmation, three or four selected masses would need to be monitored, so this could impact on the sensitivity shown here. In this example the S/N ratios of both chromatograms would allow for routine integration and quantification of hexachlorobenzene in SIM mode.

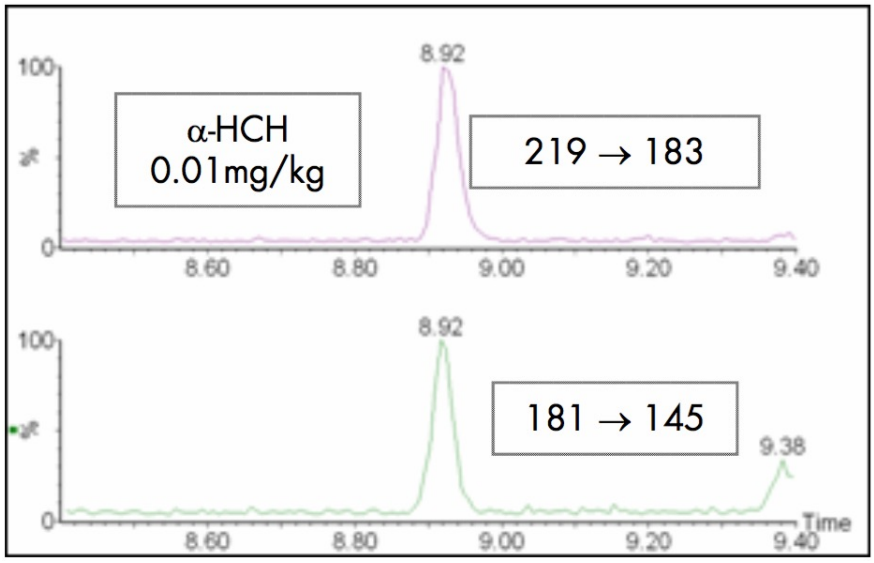


Figure 3. Hexachlorobenzene in SIM mode.

Two selected mass chromatograms are illustrated in Figure 4 for α-HCH (8.92 min) acquired in SIM mode at a concentration in matrix of 0.01 mg/kg. In this example, there is matrix interference (8.83 min) immediately preceding the confirmation peak. For this compound in this matrix, monitoring by SIM is unlikely to allow routine integration and quantification.

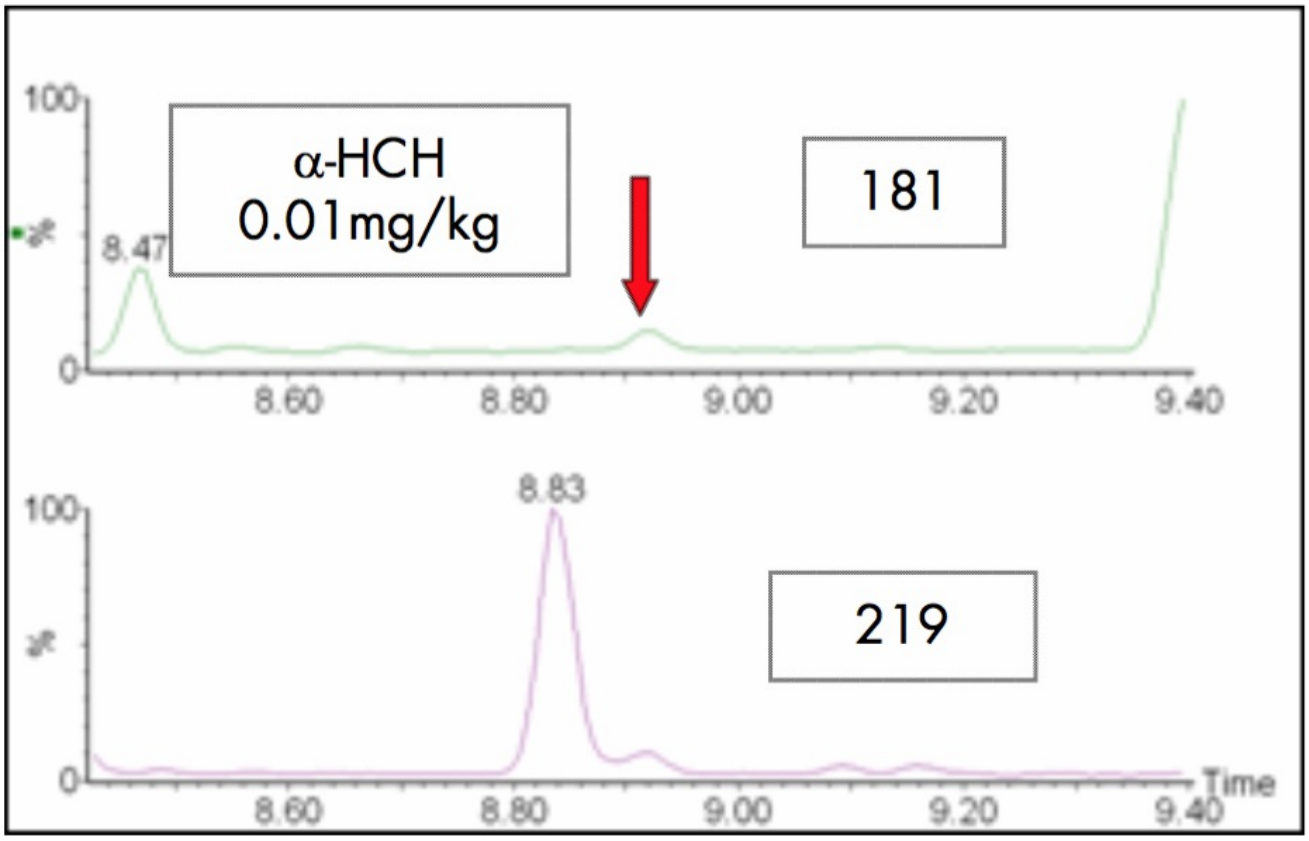


Figure 4. α-HCH in SIM mode.

In contrast, changing to MRM mode the selectivity for α-HCH (8.92 min) is significantly improved at the same concentration in matrix (Figure 5). In this example, routine integration and quantification by MRM could easily be achieved.

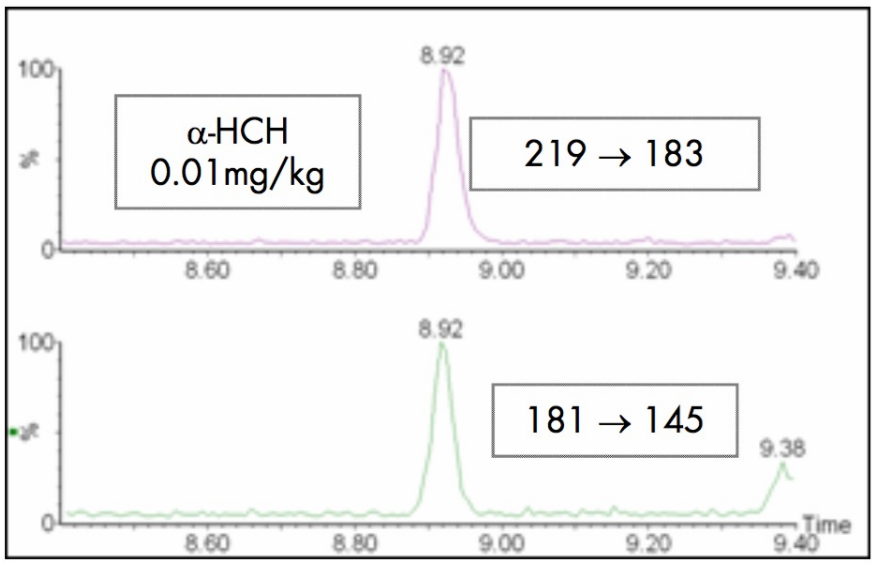


Figure 5. α-HCH in MRM mode.

In summary, some compounds will have acceptable selectivity for quantification and confirmation via SIM mode. However, as the matrix complexity is increased and/or the sample clean-up is reduced,other compounds require the more selective MRM technique for routine quantification and confirmation.

Matrix matched standards were prepared in the concentration range 0.1 to 25 ng/mL. Equivalent mg/kg concentrations were at the 0.0008, 0.0016, 0.0040, 0.0060, 0.0100, 0.0200, 0.0400, 0.0800, and 0.2000 mg/kg. δ-HCH was used as an internal standard to correct for any volumetric errors. The standards were injected in a typical batch analysis. The data was then processed using Waters TargetLynx Application Manager. Correlation coefficients of $r^2 > 0.9970$ were obtained for all compounds in matrix. A representative curve for α-endosulfan with a correlation coefficient of $r^2 = 0.9998$ is illustrated in Figure 6.

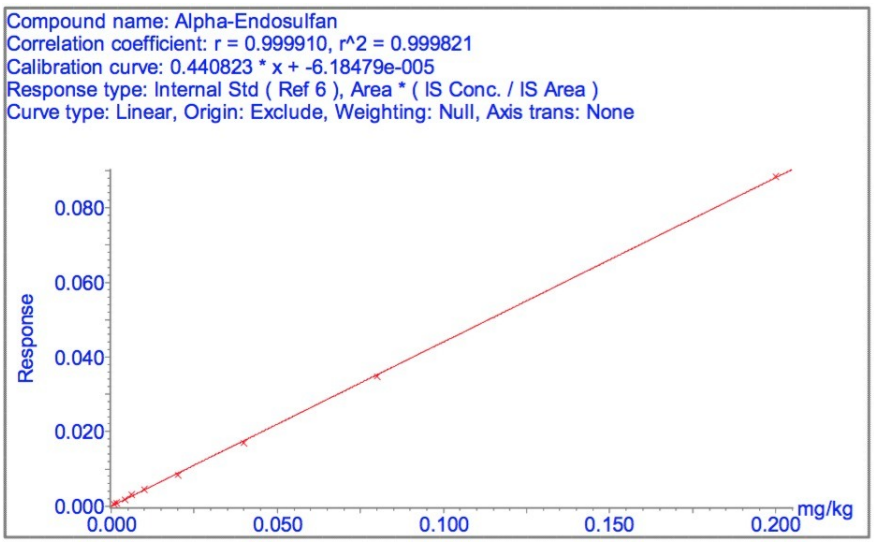


Figure 6. Representative calibration curve for α- endosulfan, 0.0008–0.2000 mg/kg.

To test the extraction method described, four recovery experiments were performed in porcine fat, spiked at 0.05 mg/kg. Each analyte was compared to its calibration curve of matrix-matched standards. The mean recovery and relative standard deviation (RSDs) of each analyte is listed in Table 2.

	% Mean Recovery	% RSD
Hexachlorobenzene	99	23
Alpha-HCH	110	7
Gamma-HCH	109	5
Beta-HCH	109	16
Heptachlor	105	12
Aldrin	110	5
PCB 52	97	20
Oxychlordane	108	14
Heptachlor epoxide	123	10
cis-Chlordane	114	17
PCB 101	95	21
trans-Chlordane	109	14
Alpha-Endosulfan	107	10
pp-DDE	103	1
Dieldrin	107	9
PCB 118	94	20
Endrin	101	12

Table 2. Mean Recovery and %RSD for 0.05 mg/kg recovery samples (n = 4).

	% Mean Recovery	% RSD
PCB 153	98	18
op-DDT	85	10
pp-DDD	116	14
Beta-Endosulfan	91	12
PCB 138	97	17
pp-DDT	72	11
Resmethrin	109	18
Bifenthrin	112	16
Endosulfan sulfate	113	15
PCB 180	93	21
Lambda-Cyhalothrin	111	13
Permethrin	102	10
Cyfluthrin	99	11
Cypermethrin	95	14
Flucythrinate	108	19
Fenvalerate	102	12
Deltamethrin	83	13

Table 2. Mean Recovery and % RSD for 0.05 mg/kg recovery samples (n = 4).

The mean recoveries were in the range 72 to 123%, with RSDs between 1 and 23%. Inclusion of SPs compromises the clean-up and results in higher concentrations of matrix co-extractives in the final extract. Further optimization of the extraction to improve precision is being carried out. The high response and selectivity provided by tandem quadrupole MS/MS ensured that additional clean-up of the GPC extracts was not necessary for the majority of pesticides.

Limits of detection (LOD) and confirmation (LOQ) were determined from the matrix matched standards, and are illustrated in Figure 7. The LOD was defined as the amount injected that gave a signal equivalent to three times the baseline noise. All the compounds have an LOD of 0.006 mg/kg or less. The LOQ was defined as the response for both the quantification and confirmation transitions sufficient to confirm the identity. As all the UK MRLs are greater than 0.02 mg/kg in animal products, this confirmatory method is able to meet the current legislation requirements.

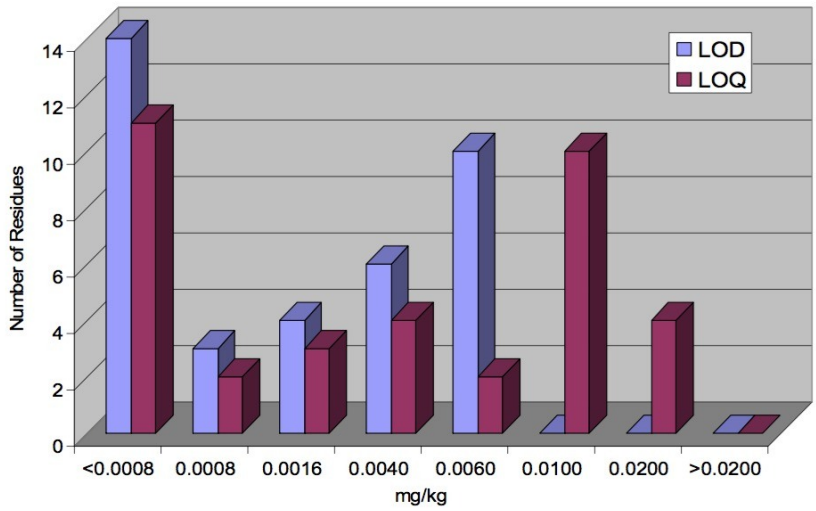
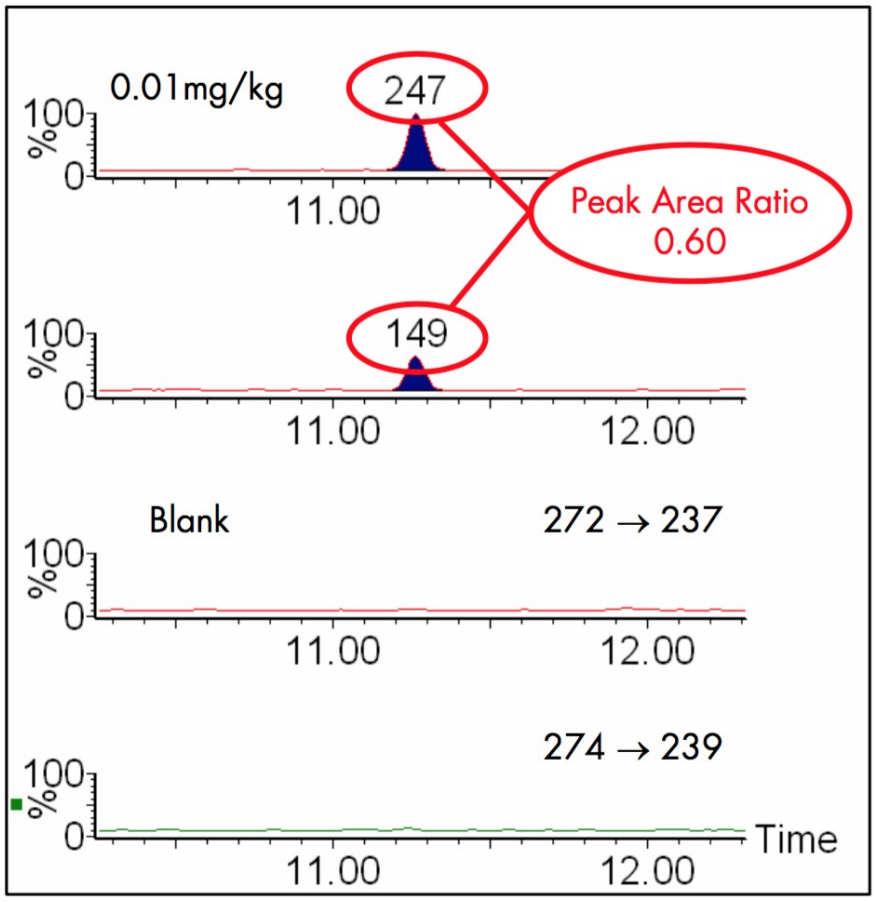


Figure 7. Limits of detection and confirmation for all residues in mg/kg.

Two MRM transitions were chosen so that quantification and confirmation could be performed in a single run assuming that the ion ratio between the transitions was consistent between standards and samples. The confirmation criterion chosen was that the ion ratio of the sample was within ±10% of the standard. The ratio was then used to confirm or reject compounds in the extracts.

To illustrate sensitivity, selectivity and confirmatory ability of tandem quadrupole MS/MS, heptachlor (Figure 8), PCB 52 (Figure 9) and bifenthrin (Figure 10) at the 0.01 mg/kg concentration level are compared to the matrix blank.


0.01mg/kg
247
100
%
0
11.00
Peak Area Ratio
0.60
149
100
%
0
11.00
12.00
Blank
272 → 237
100
%
0
11.00
12.00
274 → 239
100
%
0
Time
11.00
12.00

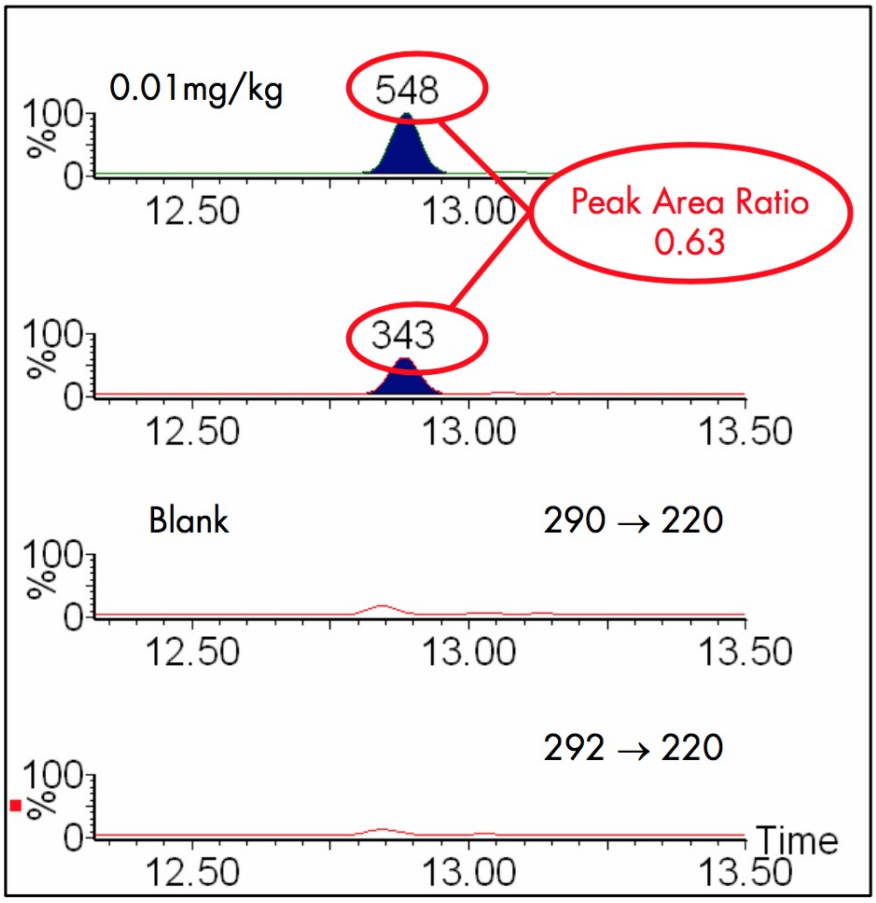


Figure 9. Sensitivity, selectivity and confirmation of PCB 52, 0.01 mg/kg.

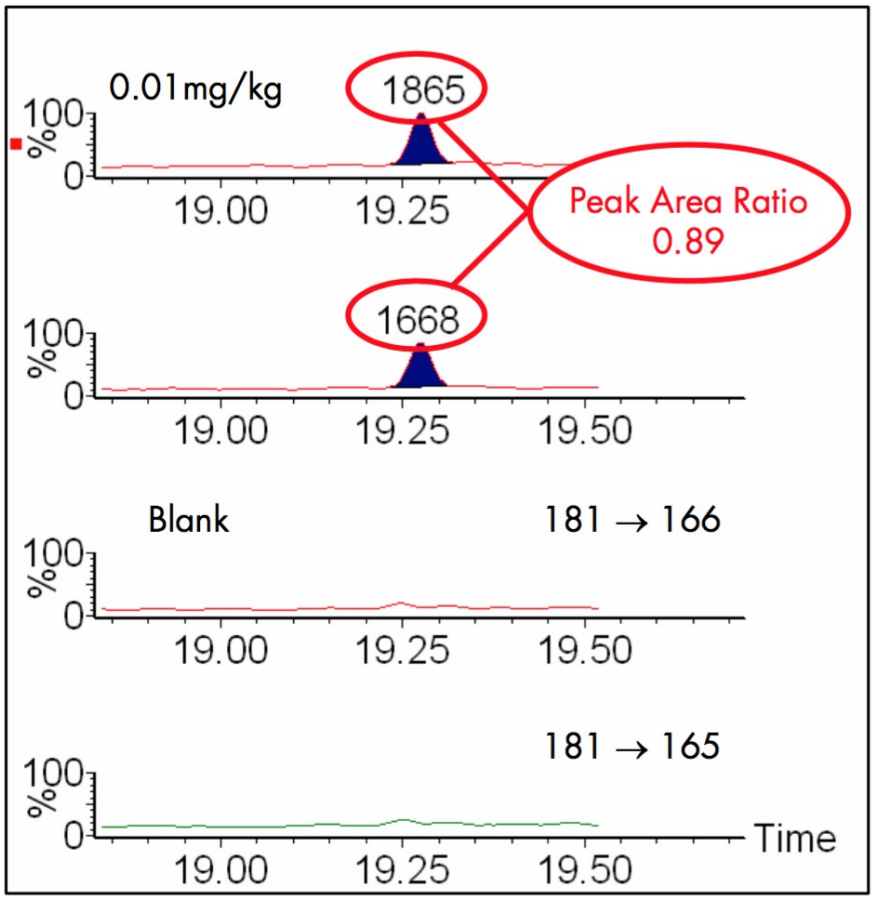


Figure 10. Sensitivity, selectivity and confirmation of bifenthrin, 0.01 mg/kg.

For both heptachlor and PCB 52, the expected ion ratio from the solvent standards is 0.62. For the confirmation criteria to be passed any concentrations in the extracts must have a ratio between 0.56 and 0.68. At 0.01 mg/kg the ion ratios for heptachlor and PCB 52 are 0.60 and 0.63 respectively, so the presence of both can be confirmed at this level.

For bifenthrin, the expected ion ratio from the solvent standards is 0.86. For the confirmation criteria to be passed, any concentrations in the extracts must have a ratio between 0.78 and 0.95. At 0.01 mg/kg the ion ratio is 0.89 so the presence of bifenthrin can be confirmed at this level.

Conclusion

A method has been described for the determination and confirmation of OCs, PCBs, and synthetic pyrethroids in animal fat using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer.

The Quattro micro GC provides high selectivity to reduce any matrix interferences, high sensitivity to reach the reporting levels required by European Union legislation and quantitative and confirmatory data in a single injection.

The tandem quadrupole MS/MS technique should be applicable to a much larger range of analytes in a range of more complex food commodities.

The TargetLynx Application Manager provides advanced quantification with a range of automatic quality control checks.

References

1. http://www.pops.int/
2. Statutory Instrument No. 2591, The Pesticides (Maximum Levels in Crops, Food and Feeding Stuffs) Amendment No. 2 (2003).

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