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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), do not readily degrade in the environment, are lipophilic and tend to bioaccumulate as they move through the food chain. Due to concerns about their effects, an international treaty restricting their use was adopted via the Stockholm Convention in 2004. Currently in the UK, maximum residue limits (MRLs) of OCs and synthetic pyrethroids (SPs) in meat and meat fat are between 0.02 -1.0 mg/kg on a fat basis. To enforce the regulations, high quality analytical methods with adequate confirmation and limits of quantification must be used.

The approach described here is the use of tandem quadrupole MS/MS in multiple reaction monitoring (MRM) mode for the quantification and unequivocal confirmation of OCs, International Council for the Exploration of the Seas (ICES) 7 PCBs and SPs at low mg/kg levels in animal fat. Solvent extracts of porcine fat were cleaned-up using gel-permeation chromatography (GPC) prior to GC-MS/MS. Two MRM transitions were monitored for each of the 36 compounds with a chromatographic runtime of 35 minutes

GC Conditions

GC System	Agilent 6890		
Autosampler	Agilent 7683		
Column	DB-17MS, 30 m x 0.25 mm, 0.25		
Injector	Splitless for 2.1 min, 250 °C		
Injection volume	4 μL		
Flow rate	Constant flow of 1 mL/min heliu		
Oven ramp	0 min	100 °C (hold 1 min)	
	6 min	200 °C @ 20 °C/min	
	20 min	280 °C @ 10 °C/min	

Extraction Method

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

METHODS MS Conditions

MS System Waters Micromass Quattro micro GC Electron impact at 70 eV Ionisation mode 200 µA Trap current 180 °C Source temperature MS/MS Multiple reaction monitoring (MRM) Analyte dependant Collision energy Peak width half height < 0.7Da Resolution Argon at a gas pressure of 3.0×10^{-3} mBar Collision gas





RESULTS AND DISCUSSION

A typical total ion chromatogram (TIC) for the twenty OCs, seven PCBs and nine SPs is illustrated below. The MRM transitions were arranged into thirteen function windows represented by the different colours.



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Two selected mass chromatograms are illustrated below for α -HCH (8.92 min) acquired in selected ion monitoring (SIM) mode at a concentration in matrix of 0.01 mg/kg. 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.



5 µm

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(hold 6 min)

(hold 15 min)

In contrast, changing to MRM mode the selectivity for α -HCH is significantly improved at the same concentration in matrix. Routine integration and quantification by MRM could easily be achieved.



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 recoveries and relative standard deviations (RSDs) of each analyte are listed below. Percentage recoveries of pesticides spiked into fat were generally in the range 72 - 123 % with associated RSD of less than 23 %.

Limits of detection (LOD) and confirmat

	% Recovery		
HCB	99 (23)		
α-HCH	110 (7)		
γ-ΗCΗ	109 (5)		
β-ΗCΗ	109 (16)		
Heptachlor	105 (12)		
Aldrin	110 (5)		
PCB 52	97 (20)		
Oxychlordane	108 (14)		
Heptachlor E	123 (10)		
cis-Chlordane	114 (17)		
PCB 101	95 (21)		
trans-Chlordane	109 (14)		
α -Endosulfan	107 (10)		
pp-DDE	103 (1)		
Dieldrin	107 (9)		
PCB 118	94 (20)		
Endrin	101 (12)		

matched standards, and are illustrated below. All confirmation LOQs were less than 0.02 mg/kg. In the UK, all MRLs for the OCs and SPs are greater than 0.02 mg/kg in meat and meat fat, so this confirmatory method is able to surpass the current legislation.



Using 4 µL splitless injection, matrix-matched calibrations curves were linear over the concentration range 0.0008 - 0.2000 mg/kg, equivalent to 0.1 to 25 ng/mL, with correlation coefficients of greater than 0.9970 obtained for all compounds. δ -HCH was used as the internal standard to correct for any volumetric/injection errors. The data was processed using Waters TargetLynx application manager.

DETERMINATION OF OCS, PCBS AND SYNTHETIC PYRETHROIDS IN ANIMAL FAT

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tion	were	determined	from	the	matrix

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

Two MRM transitions were chosen so that quantification and confirmation could be performed in a single run To illustrate sensitivity, selectivity and the confirmatory ability of tandem quadrupole MS/MS, heptachlor at the 0.01mg/kg concentration level is compared to the matrix blank below. The expected ion ratio from solvent standards is 0.616. For confirmation, any concentration in the extracts must have a ratio between 0.554 and 0.678 (\pm 10 %). At 0.01mg/kg the experimentally derived ion ratio for heptachlor is 0.603 so the presence can be confirmed at this level.



CONCLUSIONS

A method has been described for the determination and confirmation of OCs, PCBs and SPs 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 complex food commodities.