Determination of cationic polar pesticides in cereals using improved cation-exchange separation technology combined with suppressed conductivity and tandem mass spectrometry

Terri T. Christison, Sachin P. Patil, John E. Madden, Jeff Rohrer, and Scott Pritchett
Thermo Fisher Scientific,1214 Oakmead Parkway, Sunnyvale, CA, USA 94085

Abstract

Purpose: Demonstrate the determination of quaternary amine polar pesticides (mepiquat, chlormequat, paraquat, and diquat) in oat cereal extracts using cation-exchange chromatography and tandem mass spectrometry.

Methods: Four quaternary amine polar pesticide residues were extracted from oat cereals using version 12 of the European Research Laboratory (EURL) Quick Polar Pesticide extraction (QuPPE) method. The analytes were separated on a Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4µm, designed to resolve quaternary amine pesticides and the matrix ions within 15 min plus a 15 min, 1 mM MSA wash to remove the sample matrix from the column (total run time of 34 min). The quaternary amine polar pesticide analytes were determined and quantitated by Tandem MS detection using selective reaction monitoring (SRM).

Results: The oat cereal samples had 0.4 to 1.7 µg/kg residual contamination from quaternary amines polar pesticides, well within the MRL of 0.02 to 15 mg/kg. Samples extracted with HCl required a 15 min 1 mM MSA wash to remove the sample matrix from the column. The application had good recoveries by MS/MS (85-117%) and sensitivity to ~0.1 µg/L (LODs).

Introduction

Polar pesticides are applied as desiccants just before harvest to ensure early and fast drying and to avoid mold contamination. However, this practice results in a higher risk of “pesticide” contamination to the food supply. Due to their ionic and charged nature, ion chromatography separations are more suitable than traditional separation methods. Anionic polar pesticides have been previously demonstrated by IC-MS[1], but cationic polar pesticides are more challenging due to their similar chemical structures and strong interaction with cation-exchange columns. Extraction, separation, and sensitive detection methods are needed to quantify residual polar pesticide contamination in food, including the challenging oat cereals.

Materials and methods

Sample Preparation

Ground oatmeal and ground toasted oat cereal were extracted according to the EURY-FV version 12 extraction method[2] (Figure 1). For quantitative determinations of chlormequat and mepiquat: the recommended acid is 100 mM formic acid; paraquat and diquat: 100 mM HCl.

Figure 1. EURY-FV QuPPE version 12 extraction method recommended for quantitation of quaternary amine pesticides in cereals

Add 10-20 mL of 1:1 methanol/100 mM acid to 5 g of ground oats cereal

Shake 1 min. Extract 15 min in 80 °C shaking hot water bath

Cool 1 min at room temperature and 120 min at -20 ° C

Centrifuge for 15 min at 20 °C, 16,000 x g

Extract supernatant (top). Filter 0.45 µm.
Dilute 1:5 with DI water

Analyze filtered, diluted supernatant by IC-MS/MS

Equipment

Thermo Scientific™ Dionex™ ICS-6000 HPIC IC system

Thermo Scientific™ Dionex™ AS-AP autosampler

Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer

Software

Thermo Scientific™ Chromeleon™ Data Systems (CDS) 7 version 3

IC-MS Conditions

Figure 2. IC-MS/MS flow diagram

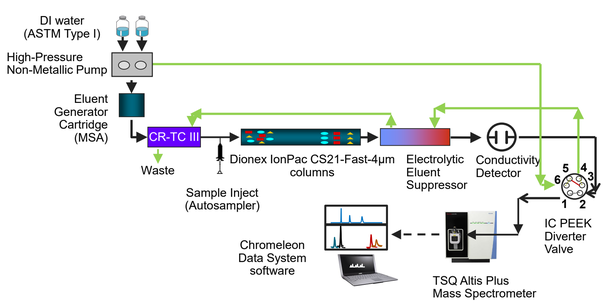


Table 1. IC-MS/MS conditions.

IC-MS/MS Conditions		
Columns:	Thermo Scientific™ Dionex™ IonPac™ CG21-Fast-4µm guard, and Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4µm analytical column, 2 mm i.d.	
MSA Gradient:	3 mM MSA (-4 to 0 min), 3–6 mM (0.1 to 3.6 min), 6–22 mM (3.6 to 6 min), 22–25 mM (6 to 15 min), 1 mM (15-30 min), 3 mM (30 min)	
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 MSA eluent cartridge, Thermo Scientific™ Dionex™ CR-CTC III trap column and high pressure degas module	
Flow Rate	0.30 mL/min	
Inj. Vol.:	10 µL	
IC Temp.:	Column: 40 °C; Detector-suppressor compartment: 20 °C	
1st Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ CDRS 600 suppressor, 2 mm, 22 mA, constant current and external water modes at 0.3 mL/min.	
Run time:	34 min	
2nd Detection:	TSQ Altis plus triple quadrupole mass spectrometer, HESI-II, SRM mode	
Flow (N_2):	Sheath: 45 (arb), Aux: 3 (arb), Sweep: 2 (arb)	
MS temp.:	Vaporizer: 300 °C, Ion transfer tube: 350 °C	
Make-up solv.:	None	
SRM Cond.:	Polarity:	Positive
	Cycle time (s):	0.8 s
	Resolution (FWHM):	Q1: 0.7; Q3: 1.2
	CID gas (mTorr)	1.5
	Source fragmentation:	10
	Chromatography:	Peak width: 6 s

Table 2. SRM table.

	Precursor (m/z)	Product	
		(m/z)	CE (V)
Chlormequat-d_4	126	57.9	30
Chlormequat	122.1	62.9	30
Mepiquat-d_{16}	130	110	30
Mepiquat	114.1	98.1	30
Paraquat-d_8	97	179	19
Paraquat	93	171	19
Diquat-d_8	96	88.5	19
Diquat	92	157.1	19

Results

Figure 3 shows the IC and SRM chromatograms of a mixed standard. MS calibration curves (not shown) were generated by the MS responses to five standards from 1-100 µg/L and found to be second order, quadratic. The estimated LODs, using 3x S/N *t-test* were 0.07-0.09 µg/L.

Figure 4 shows IC and SRM chromatograms of diluted, formic acid-methanol extracted oatmeal sample. Pesticide peaks are well resolved by MS/MS.
Tables 3 and 4 summarize the recovery results and calculated results.

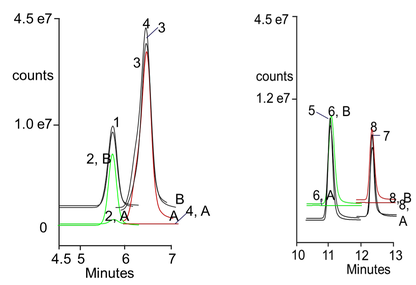
.

Figure 3. IC (left) and SRM (right) chromatograms show separation of mixed cations and resolution of quaternary amine pesticide standards.

IC Chromatogram. Analyte peaks are well resolved from matrix peaks. Peaks
1. Sodium: 30 µg/L; 2. Ammonium: 10; 3. Potassium: 10; 4. Unknown;
5. Chlormequat: 50; 6. Mepiquat-d_{16}: 50;
7. Mepiquat: 50; 8. Magnesium: 10; 9. Calcium: 20; 10. System peak; 11. Paraquat: 50; 12. Diquat: 50

SRM Chromatograms. Mepiquat and mepiquat-d_4 are resolved on this column. Peaks 1. Chlormequat-d_4; 2. Chlormequat; 3. Mepiquat-d_{16}; 4. Mepiquat; 5. Paraquat-d_8;
6. Paraquat; 7. Diquat-d_8; 8. Diquat

Figure 4. SRM chromatograms of formic acid-methanol extracted oatmeal showing: A) quaternary amine pesticides and B) added quaternary amine pesticide standards.



Peaks:	A	B	
1. Chlormequat-d_4	5.0	5.0	µg/L
2. Chlormequat	0.36	6.34	
3. Mepiquat-d_{16}	5.0	5.0	
4. Mepiquat	< 0.08	5.77	
5. Paraquat-d_8	5.0	5.0	
6. Paraquat	0.22	5.93	
7. Diquat-d_8	5.0	5.0	
8. Diquat	0.36	6.06	

Table 3. Recovery results of 5 µg/L of added standard.

	Chlormequat		Mepiquat		Paraquat		Diquat	
	Found (µg/L)	Rec. (%)	Found (µg/L)	Rec. (%)	Found (µg/L)	Rec. (%)	Found (µg/L)	Rec (%)
	Ground Oatmeal							
A	0.36	117	<0.08	116	0.22	113	0.36	113
B	0.51	98.9	<0.08	118	0.24	95.4	0.37	96.3
	Ground Toasted Oats							
A	<0.09	85.8	<0.08	85.6	0.29	88.2	0.42	94.3
B	<0.09	96.5	<0.08	113	0.24	95.4	0.37	90.8

A: formic acid-methanol extraction.
B: HCl-methanol extraction.

Table 4. Calculated results.

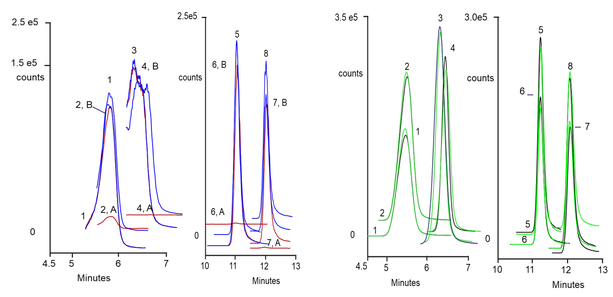
	Chlormequat	Mepiquat	Paraquat	Diquat
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
EU MRLs	15	3.0	0.02	2.0
	Ground Oatmeal			
A	0.00072	< 0.00021	0.00044	0.00071
B	0.00102	< 0.00021	0.00047	0.00074
	Ground Toasted Oats			
A	< 0.00046	< 0.00042	0.00116	0.00168
B	< 0.00046	< 0.00042	0.00094	0.00147

A: formic acid-methanol extraction.
B: HCl-methanol extraction.

Figure 5 shows the matrix effects of HCl-methanol extracted toasted oat cereal. Figure 6 shows that a 15 min wash of 1 mM MSA removes the matrix interference.

Figure 5. Matrix impacting responses: HCl extraction of toasted oats cereal

Figure 6. Added 1 mM MSA wash for 15 min



HCl-methanol extracted toasted oat cereal with 5 µg/L ISTD and unlabeled standard. Peaks 1. Chlormequat-d_4; 2. Chlormequat (4.3,3.3,2.4 µg/L); 3. Mepiquat-d_{16}; 4. Mepiquat (5.7,6.3,6.0); Paraquat-d_8; 6. Paraquat (4.7,3.2,2.5); 7. Diquat-d_8; 8. Diquat (4.6,3.0,1.8)

HCl-methanol extracted of toasted oat cereal with 5 µg/L ISTD and unlabeled standard. Peaks 1. Chlormequat-d_4; 2. Chlormequat (4.3,4.3 µg/L); 3. Mepiquat-d_{16}; 4. Mepiquat (5.7,5.7); Paraquat-d_8; 6. Paraquat (4.7,4.7); 7. Diquat-d_8; 8.Diquat (4.6,4.5)

Conclusions

This application note demonstrated an IC-MS/MS method for accurate (86 to 118% recoveries), and sensitive (LODs of < 0.1 µg/L or < 0.5 µg/kg) determinations of mepiquat, chlormequat, paraquat, and diquat, four quaternary amine pesticides, in oat cereals. These determinations were facilitated by a Dionex IonPac CS21-Fast-4µm column that delivered baseline resolution of cations and quaternary amines, including the similar structured paraquat and diquat ions. More information can be found in Technical Note TN73990 and Application Note AN000607 in AppsLab.com[3,4]

References

1. Kolberg, D.I.S, Mack, D., Anastassiades, M., Hetmanski, M.T., Fussell, R.J., Meijer, T., Mol, H.G. DOI 10.1007/s00216-012-6340-9
2. European Commission, Quick Polar Pesticide method, QuPPe-PO, v.12
3. Madden, J.E., Saini, C., Thermo Scientific TN73990
4. Christison, T., Madden, J.E., Rohrer, J. Thermo Scientific AN000607

Trademarks/licensing

Learn more at thermofisher.com/pesticide-residues-analysis