# Quaternary amine polar pesticides using improved cation-exchange separation technology combined with tandem mass spectrometry detection

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## 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 column, 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 mass spectrometry detection. Similar samples were analyzed using a single quadrupole mass spectrometer in selected ion monitoring (SIM) mode and tandem MS detection using selective reaction monitoring (SRM).

**Results:** Residual contamination of quaternary amines polar pesticides, 0.4 to 1.7 µg/kg, were measured in the oat cereal samples using tandem mass spectrometry in SRM mode. This is well within the MRL of 0.02 to 15 mg/kg. Negligible amounts of quaternary amine polar pesticides were detected in similar samples analyzed by IC-MS with a single quadrupole mass spectrometer in SIM mode. Samples extracted with HCl required a 15 min 1 mM MSA wash to remove the sample matrix from the column. The applications had good recoveries by MS/MS (85-117%) and sensitivity to ~0.1 µg/L (LODs). The sensitivities using a single quadrupole were single digit µg/L.


## 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] Add 10 to 20 mL of methanol/acid to 5 g of ground oats cereal. Extract for 15 min in 80° C shaking hot water bath. Cool and centrifuge. Extract supernatant, filter as needed. Dilute 1:5 with DI water. For quantitative determinations of chlormequat and mepiquat: the recommended acid is 100 mM formic acid; paraquat and diquat: 100 mM HCl.

### Test Method(s)

### Equipment

Thermo Scientific™ Dionex™ ICS-6000 HPIC IC system

Thermo Scientific™ Dionex™ AS-AP autosampler

Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer

Or Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer

### Data analysis

Thermo Scientific™ Chromeleon™ Data Systems (CDS) 7 version 3 software and data management.

**Table 1. IC-MS/MS and IC-MS conditions.**

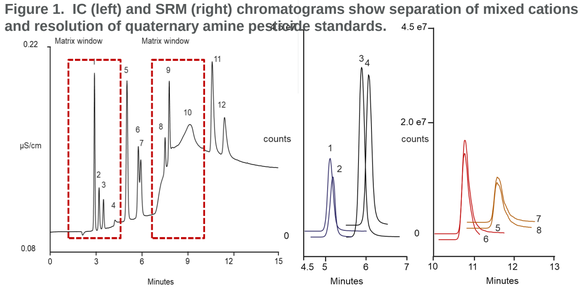
| | | | |
|---|---|---|---|
| Columns: | Thermo Scientific™ Dionex™ IonPac™ CG21-Fast-4µm guard, and Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4µm analytical column, 2 mm i.d. | | |
| MSA Gradient: | 3 mM MSA to 25 mM MSA | | |
| Eluent Source: | Thermo Scientific™ Dionex™ IonPac™ EGC 500 MSA eluent cartridge, 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, Dionex CDRS 600 suppressor, 2 mm, 22 mA, constant current and external water modes at 0.3 mL/min | | |
| 2nd Detection: | Mass spectrometry, HESI-II, SRM mode (Thermo Scientific™ TSQ Altis™ Plus triple quadrupole), or SIM mode (Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer) | | |
| Ion | Precursor (m/z) | Product (m/z) | CE (V) |
| Chlormequat | 126 | 57.9 | 30 |
| Chlormequat-$d_4$ | 122.1 | 62.9 | 30 |
| Mepiquat | 130 | 110 | 30 |
| Mepiquat-$d_{16}$ | 114.1 | 98.1 | 30 |
| Paraquat | 93 | 171 | 19 |
| Paraquat-$d_8$ | 97 | 179 | 19 |
| Diquat | 92 | 88.5 | 19 |
| Diquat-$d_8$ | 96 | 157.1 | 19 |

* ISQ EC mass spectrometer conditions: SIM, same m/z shown in Table 1 precursors except paraquat and diquat. CID: 10 V, method: Scan advanced, Scan time 4-9 min for mepiquat and chlormequat ions, 12-15 min for paraquat and diquat ions. For detailed MS conditions, see Application Notes AN000607, AN001166.[4]

## Results

Figure 1 shows the IC and SRM chromatograms of a mixed standard. MS calibration curves (not shown) were generated by the MS/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 1. IC (left) and SRM (right) chromatograms show separation of mixed cations and resolution of quaternary amine pesticide standards.**

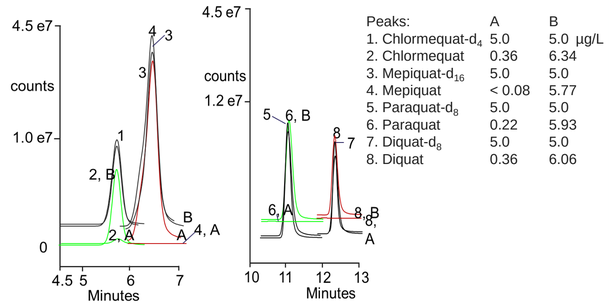



IC Chromatogram. Analyte peaks are well resolved from matrix peaks. Peaks 1. Sodium: 30 µg/L; 2. Ammonium: 10; 3. Potassium: 10; 4. Unknown; 5. Chlormequat: 50; 6. Mepiquat-$d_{16}$: 50; 7. Mepiquat: 50; 8. Magnesium: 10; 9. Calcium: 20; 10. System peak; 11. Paraquat: 50; 12. Diquat: 50

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

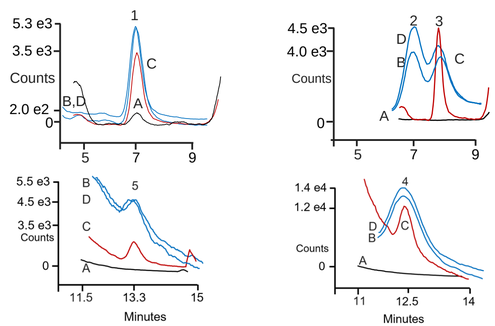
Figure 2 shows the SRM chromatograms of diluted, formic acid-methanol extracted oatmeal sample. Pesticide peaks are well resolved by MS/MS. Figure 3 shows the SIM chromatograms of diluted, HCl-methanol extracted toasted oats.
Tables 2 and 3 summarize the recovery results and calculated results.

**Figure 2. 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 |

**Figure 3. SIM chromatograms of HCl-methanol extracted toasted oats showing: A) quaternary amine pesticides and C) added quaternary amine pesticide standards.**




Samples: A: Sample, B,D: 25 µg/L ISTD, C: Sample A + 25 µg/L

Peaks:

| | A | C | |
|---|---|---|---|
| 1. Chlormequat | 1.2 | 25.5 | µg/L |
| 2. Mepiquat-D16 | -- | -- | |
| 3. Mepiquat | -- | 26.0 | |
| 4. Paraquat | -- | 26.2 | |
| 5. Diquat | -- | 26.4 | |

**Table 2. Recovery results of 5 µg/L of added standard using IC-MS/MS.**

| | 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 |

**Table 3. Recovery results of 25 µg/L of added standard using IC-MS.**

| | Ground Oatmeal | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | <0.43 | 106 | <0.59 | 99.5 | <1.8 | 105 | <3.4 | 100 |
| B | <0.43 | 101 | <0.59 | 104 | <1.8 | 105 | <3.4 | 108 |
| | Ground Toasted Oats | | | | | | | |
| A | 1.0 | 100 | <0.59 | 95.9 | <1.8 | 80.2 | <3.4 | 81.3 |
| B | 1.2 | 97.3 | <0.59 | 104 | <1.8 | 125 | <3.4 | 106 |

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

## Conclusions

- This poster compared two cationic polar pesticide applications: one using IC/MS-MS and the second using IC-MS. The IC-MS/MS method was shown to deliver accurate (86 to 118% recoveries), and sensitive (LODs of < 0.1 µg/L or < 0.5 µg/Kg) determinations of four quaternary amine pesticides (mepiquat, chlormequat, paraquat, and diquat), 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 similarly structured paraquat and diquat ions. More information can be found in AppsLab.com[3]

## 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. Christison, T., Madden, J.E., Rohrer, J. Thermo Scientific technical note TN73990 and application notes AN000607 and AN001166.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