# Ion Chromatography Mass Spectrometry methods for cationic polar pesticides analysis

John E. Madden, Anna Blank, Charanjit Saini, Yan Liu, Alexandra Zatirakha
Thermo Fisher Scientific, 1228 Titan Way, Sunnyvale, CA, USA, 94085

## Abstract

**The hyphenation of Ion Chromatography to Triple Quadrupole Mass Spectrometry (IC-MS/MS) is increasing in popularity for the analysis of Polar Pesticides. Advances in the Quick Polar Pesticides (QuPPe) method of sample preparation has made this analysis fast, accurate and routine for anionic polar pesticides such as glyphosate, but the development of methods for cationic polar pesticides has fallen behind. Nevertheless, cationic polar pesticides may occur as residues in food, but are often excluded from pesticide monitoring programs due to the difficulty in the determination of these target analytes.**

## Introduction

The EURL-SRM (European Union Reference Laboratory for pesticides requiring Single Residue Methods) lists 39 polar pesticides of interest, from which 23 fall into the category of cationic species based on their ionic properties and molecular structures. This work is aimed to demonstrate capabilities of several cation-exchange columns in the analysis of the listed cationic polar pesticides via ion chromatography.

## Materials and methods

### Sample preparation

Stock solutions (1000 mg/L in water) for all standards for target analytes were prepared by diluting the calculated amount of pure compound in reagent water. The standard solutions at lower concentrations were prepared by serial dilutions with reagent water.

### Instrumentation

All chromatography work was performed using a Thermo Scientific™ Dionex™ ICS-6000$^+$ ion chromatography system. Eluent was electrolytically generated using a Thermo Scientific™ Dionex™ x EGC MSA eluent generator cartridge.

Mass spectrometry was performed using a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer equipped with a heated electrospray ionization (H-ESI) source operating in the positive ion mode. Data acquisition was performed in the selected reaction monitoring (SRM) mode. Spray voltage was set to 2.8 kV, sheath gas to 45 arbitrary units (Arb), auxiliary gas to 2.5 Arb, sweep gas to 2.0 Arb, ion transfer tube temperature set to 350ºC, and vaporizer temperature set to 300ºC. The collision gas pressure was set to 1.5 mTorr and the source fragmentation voltage to 10 V. All SRM transitions (parent, quantifier, and qualifier ions) were individually tuned for each target analyte by injecting the corresponding standard solution (100 µg/L), as shown in Table 4.

**Figure 1. Gradient separation of polar pesticides and 6 common cations on IonPac CS17 with suppressed conductivity detection**

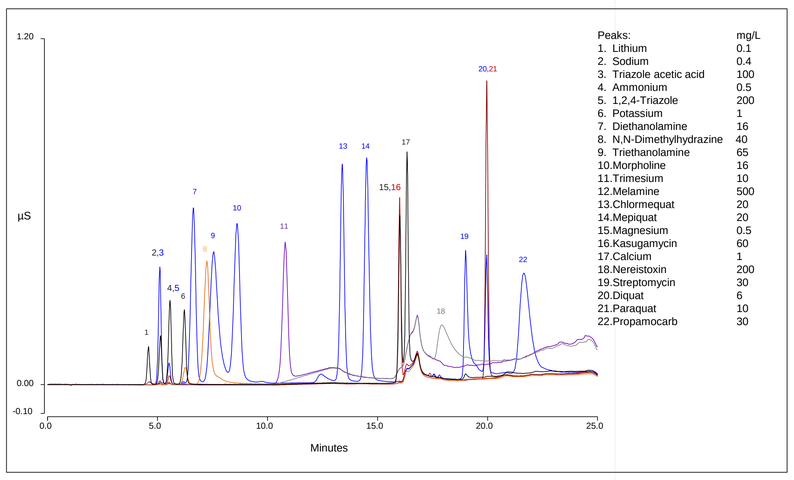


Figure 1: Column : Dionex IonPac CS17 2 x 250 mm. Injection volume: 2 µL. Flow rate: 0.4 mL/min. Eluent: 0 min – 2 mM MSA, 8.5 min – 2 mM MSA, 14.5 min – 10 mM MSA, 20.5 min – 60 mM MSA.

## Results

### Evaluation of off-the-shelf columns

In order to reduce the number of columns necessary for evaluation of cationic polar pesticides, existing cation exchange columns were reviewed with the following requirements.

- **Weak cation exchanger**. Many of the target analytes are divalent or polyvalent. The high affinity of strong cation exchangers for divalent analytes makes them unsuitable.
- **Low to very low hydrophobicity**. Many of the target analytes are polar or have high hydrophobicity. Thus, the column needs to have low or very low hydrophobicity.
- **Good for simple and complex amines**. Most of the target analytes are amines or diamines. Columns that are targeted towards complex amines are preferred.
- **Compatible with IC-MS**. The target application is IC-MS/MS. Therefore, the column needs to have low bleed in order to ensure longevity of the ESI probe.

Table 1 shows available off-the-shelf weak cation exchange columns. The IonPac CS17 column was found to meet all requirements and was the most suitable column for further evaluation.

**Table 1. Summary of weak cation-exchange columns characteristics and separation capabilities**

| Column | Capacity (µEq.) | Hydro-phobicity | Common Cations | Simple Amines[1] | Complex Amines[2] | IC-MS compatible |
|---|---|---|---|---|---|---|
| IonPac CS12A | 700 | Med/Low | Excellent | Good | Poor | Yes |
| IonPac CS14 | 325 | Med/High | Good | Good | Good | Yes |
| IonPac CS15 | 700 | Med/Low | Excellent | Good | Poor | Yes |
| IonPac CS16 | 3,000 | Medium | Excellent | Excellent | Poor | Yes |
| IonPac CS17 | 363 | Very Low | Poor | Good | Excellent | Yes |
| IonPac CS18 | 290 | Medium | Good | Excellent | Good | Yes |
| IonPac CS19 | 600 | Medium | Poor | Good | Excellent | No |
| IonPac CS20 | 750 | Medium | Poor | Excellent | Good | Yes |

[1]Hydrophobic amines such as methylamines and polar amines such as alkanolamines
[2]Polyvalent amines such as alkyldiamines and moderately hydrophobic amines such as biogenic amines

### Evaluation of target analytes

The EURL lists 39 target polar pesticides of interest, 28 of which can potentially have positive charge in acidic pH and are therefore good candidates for separation using a cation exchanger. All selected pesticides were run one-by-one on an IonPac CS17 column (2 x 250 mm) analytical column; the results are summarized in Table 2.

### IonPac CS17 method optimization

A mixture of 11 pesticides were separated using gradient elution on the IonPac CS17column. Eluent flow rate, column temperature and eluent gradient were optimized as shown in Figure 2 and Table 3. Using the optimized method 16 polar pesticide along with 6 common cations were analyzed as shown in Figure 1. To test the response using IC-MS/MS the gradient was modified slightly to make the run-time faster but otherwise left unchanged. 13 cationic polar pesticides were evaluated using IC-MS/MS, see Figure 3.

**Table 2. Summary of suitability of target analytes for cation-exchange IC analysis**

| Analyte | Positive at low pH | Detection Method | Analyte | Positive at low pH | Detection Method |
|---|---|---|---|---|---|
| Ethephon | No | n.a.[1] | Daminozide | Yes | N.S.Cond.[5] |
| HEPA | No | n.a. | Chlormequat | Yes | S.Cond. |
| Glufosinate | No | n.a. | Mepiquat | Yes | S.Cond. |
| N-acetyl-glufosinate | No | n.a. | Difenzoquat | Yes | n.d. |
| MPPA | No | n.a. | Propamocarb | Yes | S.Cond. |
| Glyphosate | No | n.a. | Melamine | Yes | S.Cond. |
| AMPA | Yes | n.d. | Diquat | Yes | S.Cond. |
| Phosphonic acid | No | n.a. | Paraquat | Yes | S.Cond. |
| N-acetyl AMPA | No | n.a. | N,N-Dimethylhydrazine | Yes | S.Cond. |
| Fosetyl-Al | Yes | n.d. | Nereistoxin | Yes | S.Cond. |
| Maleic hydrazide | Yes | UV | Streptomycin | Yes | S.Cond. |
| Perchlorate | No | n.a. | Kasugamycin | Yes | S.Cond. |
| Chlorate | No | n.a. | Morpholine | Yes | S.Cond. |
| Bialaphos | No | n.a. | Diethanolamine | Yes | S.Cond. |
| Cyanuric acid | Yes | UV | Triethanolamine | Yes | S.Cond. |
| Amitrol | Yes | UV | 1,2,4-Triazole | Yes | S.Cond. |
| ETU | Yes | UV | Triazole-alanine | Yes | N.S.Cond. |
| PTU | Yes | n.d. | Triazole-acetic acid | Yes | UV |
| Cyromazine | Yes | UV | Triazole-lactic acid | Yes | UV |
| Trimesium | Yes | S.Cond.[4] | | | |

[1]n.a. – Not applicable
[2]n.d. – Not detected/eluted under any tested condition
[3]UV – Non suppressed UV detection at 204 nm
[4]S.Cond. – Suppressed Conductivity detection
[5]N.S.Cond. – Non-Suppressed Conductivity detection

**Figure 2. Temperature effect on the separation of some polar pesticides on IonPac CS17 column**

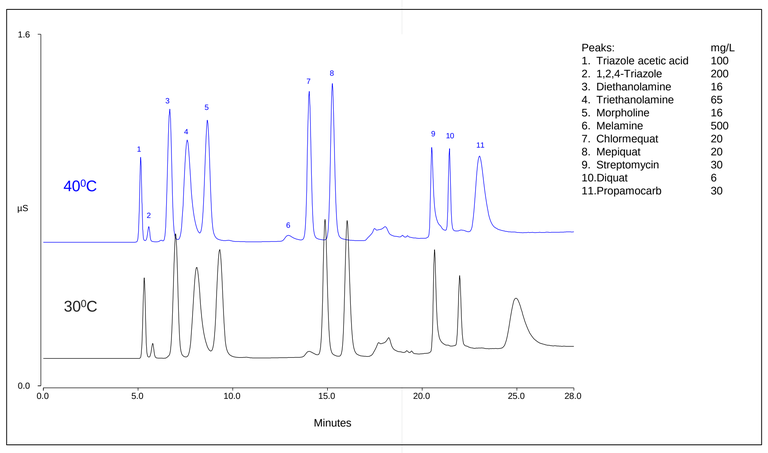


Figure 2: Column: Dionex IonPac CS17 2 x 250 mm. Injection volume: 2 µL. Flow rate: 0.4 mL/min. Eluent: 0 min – 2 mM MSA, 9.3 min – 2mM MSA, 16 min – 10 mM MSA, 22 min – 60 mM MSA.

**Table 4. Selected reaction monitoring (SRM) transitions**

| Compound | Collision Energy | Transition Type | Parent Ion (*m/z*) | Product Ion (*m/z*) |
|---|---|---|---|---|
| **Chlormequat** | 30 | Quantifier<br>Qualifier | 122.1<br>122.1 | 57.9<br>62.9 |
| **Chlormequat-d4** | 30 | Quantifier | 126.0 | 57.9 |
| **Mepiquat** | 30 | Quantifier<br>Qualifier | 114.1<br>114.1 | 98.1<br>58.0 |
| **Mepiquat-d16** | 30 | Quantifier | 130.0 | 110.0 |
| **Paraquat** | 19 | Quantifier<br>Qualifier | 93.0<br>93.0 | 171.0<br>85.0 |
| **Paraquat-d8** | 19 | Quantifier | 97.0 | 179.0 |
| **Diquat** | 19 | Quantifier<br>Qualifier | 92.0<br>92.0 | 84.5<br>157.1 |
| **Diquat-d8** | 19 | Quantifier | 96.0 | 88.5 |
| **Morpholine** | 23 | Quantifier | 88.0 | 44.0 |
| **Diethanolamine** | 16 | Quantifier | 106.0 | 70.0 |
| **Triethanolamine** | 15 | Quantifier | 150.0 | 132.0 |
| **Trimethylsulfonium** | 16 | Quantifier | 77.0 | 62.0 |
| **Nereistoxin** | 20 | Quantifier | 150.0 | 105.0 |
| **Melamine** | 19 | Quantifier | 127.0 | 85.0 |
| **Cyromazine** | 18 | Quantifier | 167.0 | 125.0 |
| **Propamocarb** | 20 | Quantifier | 189.0 | 102.0 |
| **Streptomycin** | 33 | Quantifier | 600.0 | 263.0 |

**Figure 3. Gradient separation of polar pesticides on IonPac CS17 column with suppressed MS/MS detection**

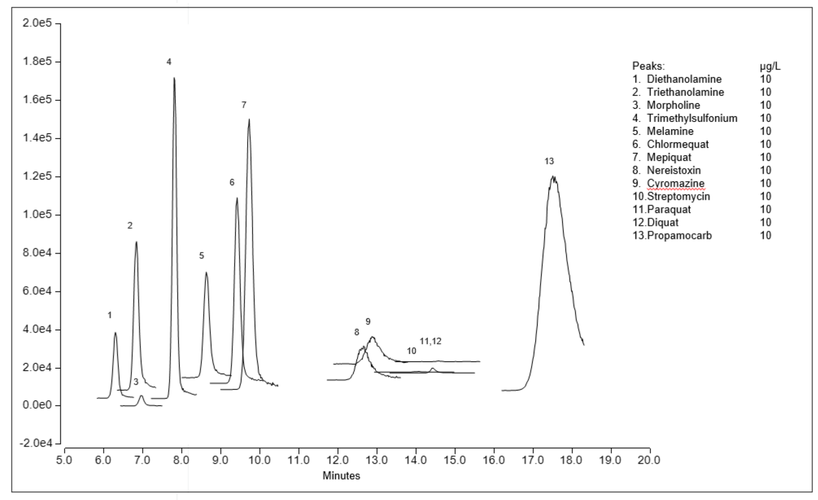


Figure 3: Column : Dionex IonPac CS17 2 x 250 mm. Injection volume: 5 µL. Flow rate: 0.4 mL/min. Eluent: 0 min – 1.0 mM MSA, 4 min – 3.2 mM MSA, 10 min – 15 mM MSA, 14 min – 40 mM MSA. SRM transitions as shown in Table 4.

### IonPac CS21-Fast-4µm method development

The Thermo Scientific™ IonPac™ CS21-Fast-4µm stationary phase column consists of a novel highly hydrophilic carboxylate functionality attached to the surface of a 4 µm macroporous polymeric substrate. This column has unique selectivity allowing the isolation of high levels of matrix ions such sodium, potassium, magnesium and calcium in two narrow windows, see Figure 4, while separating the four quaternary ammonium compounds outside these windows, see Figure 5.

**Figure 4. Separation of the matrix components in a simulated matrix using the Dionex IonPac CS21-Fast-4µm column with suppressed conductivity detection.**

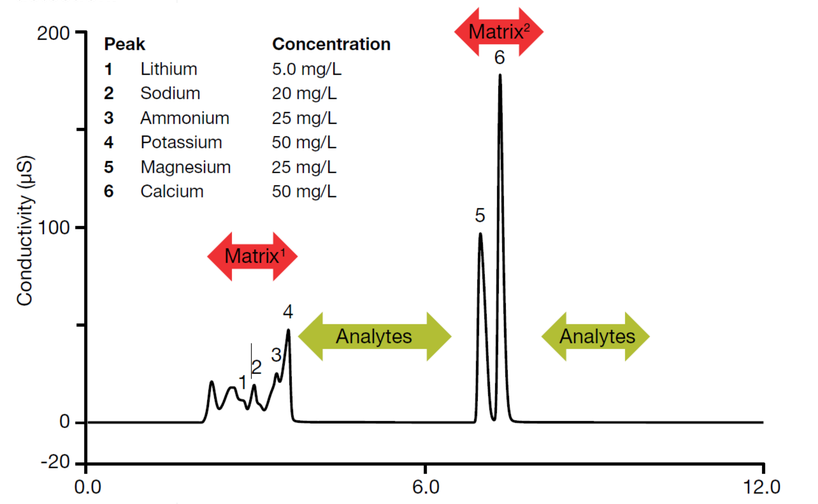


Figure 4: Column : Dionex IonPac CS21-Fast-4µm 2 x 250 mm. Injection volume: 10 µL. Flow rate: 0.3 mL/min. Eluent: 0 min – 3.0 mM MSA, 3.6 min – 3.0 mM MSA, 6.0 min – 22.0 mM MSA, 15 min – 25 mM MSA.

**Figure 5. Separation of the four quaternary ammonium polar pesticides — chlormequat and mepiquat (Analyte Zone 1), and paraquat and diquat (Analyte Zone 2) using the Dionex IonPac CS21-Fast-4µm column with suppressed Mass Selective detection.**

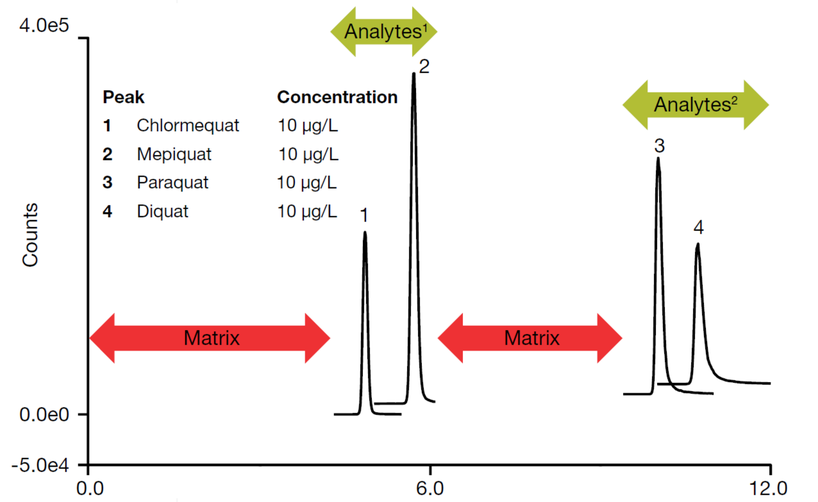


Figure 4: Column : Dionex IonPac CS21-Fast-4µm 2 x 250 mm. Injection volume: 10 µL. Flow rate: 0.3 mL/min. Eluent: 0 min – 3.0 mM MSA, 3.6 min – 3.0 mM MSA, 6.0 min – 22.0 mM MSA, 15 min – 25 mM MSA. SRM transitions as shown in Table 4.

## Conclusions

- The IonPac CS17 column was found to be an excellent column for the screening of cationic polar pesticides. However, its inability to separate paraquat and diquat makes it unsuitable for the quantification of the four quaternary ammonium polar pesticides.
- The IonPac CS21-Fast-4µm column was developed for the specific purpose of separating and thus quantification of the four quaternary ammonium polar pesticides by IC-MS/MS.
- Methods were developed for both the IonPac CS17 column and IonPac CS21-Fast-4µm column.

## Acknowledgements

We would like to thank Christopher Pohl for his invaluable input during the development of the IonPac CS21-Fast-4µm column.