Application note | 000607

Mass spectrometry

Determination of cationic polar pesticides in cereals using ion chromatography and tandem mass spectroscopy

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Keywords

Dionex IonPac CS21-Fast-4µm column, RFIC, Reagent-Free IC, IC-MS/MS, TSQ Altis Plus triple quadrupole mass spectrometer, quats, diquat, paraquat, chlormequat, mepiquat, ionic pesticides, cationic pesticides

Goal

Demonstrate determinations of quaternary cationic pesticides in oat cereal samples by ion chromatography coupled with tandem mass spectrometry

Introduction

Pesticide contamination in food as a potential health risk is a growing public concern, resulting in increased interest and attention by health researchers and regulatory agencies.[1] Due to those concerns, much of the agricultural industry has largely replaced the traditional, toxic, solvent-based pesticides with the widely used, mostly less toxic, ionic pesticides. Collectively grouped into the category of “Ionic and Highly Polar Pesticides” are herbicides, fungicides, defoliants, and desiccants. For traditional solvent-based pesticides, the regulatory test methods are typically gas and liquid chromatography combined with mass spectrometry. However, polar pesticides, which are ionic and non-volatile, are much more suitable for methods using ion chromatography (IC) separations combined with mass spectrometry (MS) detection. Consequently, determinations of anionic polar pesticide residues, such as glyphosate, glufosinate, and their degradation products, such as AMPA (aminomethyl)phosphonic acid), have been successfully demonstrated in beer, fruit, cereals, vegetables, and

drinking water by the Food Environmental Research Association (FERA, LTD)[2,3] and others[4-15] using IC with MS (tandem MS, high resolution accurate mass (HRAM) MS, or single quadrupole MS). Compared to the anionic highly polar pesticides, determinations of cationic polar pesticide residues have proven more challenging due to their similar chemical structures.[11,16] Important cationic pesticides, such as the highly toxic paraquat and diquat, could not be chromatographically resolved on the previously available cation-exchange columns. Because diquat and paraquat have very similar chemical structures and differ in their *m/z* for molecular and fragment ions by less than 2 a.m.u., they could only be resolved by the HRAM MS.[17] To chromatographically resolve them, a new stationary phase was developed to assure accurate paraquat and diquat reporting.[18,19] Separate quantitation of paraquat and diquat is driven by the differences in their individual toxicity levels (LD50) and often different legal status (one may be permitted while another may be prohibited) in different jurisdictions. This application note demonstrates the advantage of the new, designed-for-purpose Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4µm ion exchange column paired with a triple quadrupole mass detector rather than HRAM MS due to the chromatographic separation of paraquat and diquat.

Earlier, multi-residue extraction methods were developed for polar pesticides. In 2018, the European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables (EURL-FV) developed the Quick Polar Pesticide Extraction (QuPPE) method. Recently the QuPPE method was modified to improve recoveries for mepiquat and chlormequat by using formic acid with methanol at elevated temperature, 80 °C.[20] Hydrochloric acid with methanol at 80 °C was used to improve paraquat and diquat recoveries.[21]

Four cationic pesticides with similar quaternary amine chemical structures—mepiquat (1,1-dimethylpiperidinium chloride), chlormequat (2-chloroethyl(trimethyl)azanium), paraquat (1-methyl-4-(1-methylpyridin-1-ium-4-yl) pyridin-1-ium), and diquat (1,1'-ethylene-2,2'-dipyridylium)—were selected to evaluate the new cation-exchange column optimized for quaternary amines, the Dionex IonPac CS21-Fast-4µm column. These compounds have similar chemical structures and are registered and reregistered (approved) herbicides in the United States.[22] Paraquat, a restricted use pesticide, is widely used for commercial applications. To protect personnel, field workers, and adjoining lands, new labeling is required, manual sprayers are banned, aerial spraying is restricted when it may reach adjoining crops, and personnel must wait longer periods before safely entering a sprayed field.[23] In many countries, including those in the European Union (EU), paraquat and diquat are not registered (approved) pesticides.[24,25] In contrast, the EU has approved plant growth regulators containing chlormequat and mepiquat as the active ingredients, particularly for conventional wheat and oats cultivation. The EU's Maximum Residue Levels (MRLs) for these pesticides in fruits, vegetables, flowers, herbs, and cereals, range typically from 0.01 to 0.05 mg/kg.[26] However, EU MRLs[26] (mg/kg) for oat cereals are much higher, due to the expected presence of these pesticides and the challenge of obtaining accurate values from these complex samples. The EU MRL for paraquat was the exception, remaining at 0.02 mg/kg. The MRLs for the US, EU, and Canada are summarized in Table 1.

Table 1. MRLs of four pesticides

	mg/kg		
	US[28]	EU[26]	Canada[27]
Chlormequat*	40	15	40
Mepiquat*	Not found	3	0.02
Paraquat	--	0.02	0.1
Diquat	0.02	2	0.02

*Expressed as the salt

This IC-MS/MS application demonstrates the advantages of combining the optimum cation-exchange column, suppressed conductivity detection, and tandem mass spectrometry detection. The Dionex IonPac CS21-Fast-4µm cation-exchange column was designed to chromatographically resolve the four quaternary amine pesticides and the matrix ions within 15 min. The electrolytic suppressor neutralizes the eluent for sensitive conductivity detection and for compatibility with MS. Tandem MS detection using selective reaction monitoring (SRM) scans only for the ions of interest, eliminating the sample matrix and increasing sensitivity and selectivity.

In this application note, mepiquat, chlormequat, paraquat, and diquat cationic polar pesticides were determined by cation-exchange chromatography using an electrolytically generated methanesulfonic acid gradient at 0.3 mL/min and 40 °C on a 2 × 150 mm, Dionex IonPac CS21 cation-exchange column. As the quaternary amines eluted from the column, they were detected by suppressed conductivity, heated to ionized gas by heated electrospray ionization (HESI-II), and detected by tandem mass spectrometry in SRM mode by the Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer. The method was applied to modified QuPPE extractions of ground oat cereal samples and was found to be accurate and sensitive.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system*
  - Single Pump SP module, isocratic configuration (P/N 22181-60003) or Dual Pump DP module, isocratic configuration (P/N 22181-60009)
  - Eluent Generator EG module (P/N 22181-60019)
  - Detector Chromatography DC module with one 6-port injection valve (P/N 22181-600570) and Automation Manager with one 6-port valve (P/N 075952)
  - CD Conductivity Detector (P/N 079829)
- Thermo Scientific™ Dionex™ Auxiliary Pump AXP-MS (P/N 063973). This pump is to dispense DI water for the suppressor regenerant. If using a Dionex ICS-6000 DP module, the second pump, if not otherwise needed, can supply suppressor regenerant to the AXP-MS.
- Thermo Scientific™ Dionex™ AS-AP Autosampler with temperature control option (P/N 074926)
  - 100 µL syringe (P/N 074305)
  - 1 to 3 8-position sample trays for 10 mL vials (P/N 069877) typically used for standards
  - 1 to 3 19-position sample trays for 10 mL vials (P/N 074938)
- Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer with HESI-II probe (P/N TSQ03-10002)

*Thermo Scientific™ Dionex™ Integrion HPIC system, RFIC model (P/N 22153-60305) with Integrion CD Conductivity Detector (P/N 22153-62034), extra 6-port valve (22153-62027), and Auxiliary Pump AXP-MS (P/N 063973) can be used for this application.

Table 2 lists the consumable products needed for the IC-MS/MS system.

Table 2. Consumables list

Product name	Description	P/N
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kit	Dionex IC Viper fitting assembly kit for the Dionex ICS-6000 HPIC system with CD Detector	302965
Thermo Scientific™ Dionex™ EGC 500 MSA Eluent Generator cartridge	Cation eluent generator cartridge for HPIC high-pressure systems	075779
Thermo Scientific™ Dionex™ CR-CTC™ III Electrolytic trap column	Continuously regenerated cation trap column required for determining quaternary amine analytes, as in this application note. The cable is standard for the Dionex Integrion HPIC and ICS-6000 HPIC instruments.*	104-60001
Thermo Scientific™ Dionex™ ICS-6000 EG Eluent Generator kit	Degasser installed after Dionex CR-CTC III trap column and before the injection valve, used with eluent generation	075522
Thermo Scientific™ Dionex™ CDRS™ 600 suppressor, 2 mm	Suppressor for 2 mm cation columns	088670 or 088670CMD
Thermo Scientific™ Dionex™ IonPac™ CG21-Fast-4µm Guard Column	Cation guard column, 2 × 30 mm	303349
Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4µm Analytical Column	Cation analytical column, 2 × 150 mm	303348
Thermo Scientific™ Dionex™ AS-AP Autosampler vial kit options	10 mL polystyrene, package of 100 caps and septa	055058
Thermo Scientific™ Dionex™ IC PEEK Viper™ Sample Loop	10 µL PEEK Viper Sample Loop	302895
Thermo Scientific™ Extended Mass Reference Solution (EMRS)	Replacement calibrant solution, 100 mL	HAZMAT0100099
IC-MS Installation Kit	IC-MS installation kit includes tubing, mixing tee	22153-62049
HESI-II needle	Replacement HESI-II needle	OPTON-30139
Centrifuge tubes	50 mL centrifuge tubes for sample preparation	14-432-22
Syringe filters	0.45 µm polypropylene syringe filters	44513-PP

* P/N 22181-98150 for cable adapter to Dionex ICS-5000⁺ HPIC system:

IC-MS conditions

Parameter	Setting		
Columns	Dionex IonPac CG21-Fast-4µm guard (2 × 30 mm) Dionex IonPac CS21-Fast-4µm analytical column (2 × 150 mm)		
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	Dionex EGC 500 MSA eluent cartridge, Dionex CR-CTC III trap column and HP EG degas kit		
Flow rate	0.30 mL/min		
Injection volume	10 µL		
Column temperature	40 °C		
Detection/Suppressor compartment	20 °C		
Detection 1	**Suppressed conductivity, Dionex CDRS 600 suppressor, 2 mm, 22 mA, constant current and external water modes**		
Suppressor regenerant	DI water by auxiliary pump at 0.3 mL/min.		
IC background, noise	<1 µS/cm, <1 nS/cm		
IC-MS system backpressure	~3,000 psi		
IC-MS run time	34 min		
Automation Manager AM-HP1 valve functioning as a diverter valve	*Timing (min)*	*Valve position*	*IC flow path*
	Prerun	DC.AM_HP1.A	Divert IC flow away from MS. DI water for Suppressor Regen flows through diverter valve to MS
	-4.0 Equilibration	--	Same
	0.0 Run	--	Same
	4.0	DC.AM_HP1.B	IC flow to MS. Start MS acquisition
	15 min	DC.AM_HP1.A	Divert away from MS
Detection 2	**TSQ Altis Plus triple quadrupole mass spectrometer, +HESI-II, +2.8 kV, SRM mode**		
Flow (N_2)	Sheath: 45 (arb) Aux: 3 (arb) Sweep: 2 (arb)		
MS temperatures	Vaporizer: 300 °C Ion transfer tube: 350 °C		
Desolvation solution	None		
SRM conditions	Polarity: Positive		
	Cycle time (s): 0.8 (check box)		
	Use calibrated RF lens: (check box)		
	Resolution (FWHM) Q1: 0.7 Q3: 1.2		
	CID gas (mTorr): 1.5		
	Source fragmentation: 10		
	Chromatography Peak width: 6 s Filter (check box)		

SRM Table

	Scan start time (min)	Scan end time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)
Chlormequat	4.5	6.0	122.1	57.9	30
Chlormequat	4.5	6.0	122.1	62.9	30
Chlormequat-d_4	4.5	6.0	126	57.9	30
Mepiquat	5.5	7.0	114.1	58	30
Mepiquat	5.5	7.0	114.1	98.1	30
Mepiquat-d_{16}	5.5	7.0	130	110	30
Paraquat	10	13	93	85	19
Paraquat	10	13	93	171*	19
Paraquat-d_8	10	13	97	179*	19
Diquat	10.8	13.8	92	84.5	19
Diquat	10.8	13.8	92	157.1*	19
Diquat-d_8	10.8	13.8	96	88.5	19

* These *m/z* are the singly charged ions and are used only as confirming ions.

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software 7.3 version (MS driver is included) was used.

Reagents

- Degassed ASTM Type I[29] deionized (DI) water
- Solvents for sample extraction
  - Formic acid, 88%, ACS grade (P/N A118P-100)
  - Hydrochloric acid, 37%, ACS grade (P/N AC423795000)
  - Methanol, Optima™ HPLC grade (P/N A454-1)
- Chlormequat (2-chloroethyl(trimethyl)azanium chloride), 98%, Alfa Aesar™. (MW= 158.066 g/mol, CAS 999-81-5, P/N AAA1563006)
- Chlormequat-1,1,2,2-d_4 chloride, 100 µg/mL, Absolute Standards, Inc. (P/N 96081)
- Diquat dibromide, monohydrate (1,1'-ethylene-2,2'-dipyridylium dibromide), SPEX CertiPrep™ (MW = 362.07 g/mol; CAS 6385-62-2, P/N S1752)
- Diquat-d_8 dibromide (dipyridine-d_8) C/D/N Isotopes Inc., 50 mg (MW = 352.08 g/mol; P/N D-7990)
- Mepiquat chloride (1,1-Dimethylpiperidinium chloride), 98%, Honeywell Fluka™ PESTANAL™ (MW=149.662 g/mol, CAS 24307-26-4, P/N 11-101-3665)
- Mequat-d_{16} chloride, 100 µg/mL, Absolute Standards, Inc. (P/N 96082)
- Paraquat dichloride, tetrahydrate (1-methyl-4-(1-methylpyridin-1-ium-4-yl) pyridin-1-ium;dichloride), SPEX CertiPrep (MW = 257.158 g/mol; CAS 1910-42-5, P/N S2915)
- Paraquat dichloride-d_8 (ring-d_8), 100 µg/mL, Absolute Standards, Inc. (CAS 347841-45-6, P/N 95305)

Standard preparation

Stock and intermediate standards

To prepare 100 mL of 1,000 mg/L individual stock standards, add the solid reagent (Table 3) into a 125 mL HDPE bottle. Add 100 g of ASTM Type I DI water. Swirl to dissolve. Refrigerate at 20 °C.

Table 3. Preparation of 100 mL of 1,000 mg/L individual standards

	MW of hydrated salt (g/mol)	FW of cation	Ratio (hydrated salt)/(cation)	Reagent (hydrated salt), weight (mg)	DI water (g)
Chlormequat chloride	158.066	122.62	1.29	129	100
Mepiquat chloride	149.662	114.21	1.31	131	100
Paraquat dichloride	257.158	186.26	1.38	138	100
Diquat dibromide monohydrate	362.07	202.27	1.96	196	100

Mixed intermediate and working standards

To prepare the mixed 5 mg/L intermediate standard, pipet 500 µL of the individual 1,000 mg/L stock standards into a 125 mL HDPE bottle. Dilute with DI water to 100 g total weight. Swirl to mix. Refrigerate at 20 °C or lower.

Working standards (1, 2, 5, 10, 20, 50, 100 µg/L) were prepared from the 5 mg/L intermediate standard and DI water. 5 µg/L ISTD was added to each 5 mL working standard from the 10 mg/L mixed isotopic spiking standard (prepared below).

ISTD isotopic standards

To prepare 500 mg/L (µg/mL) stock standard of the diquat isotopic standard, 9.2 mg of diquat-d_8 dibromide (dipyridine-d_8) (1.83 salt/cation ratio) was dissolved in 10 mL of DI water and further diluted (2,000 µL in 10 mL) to 100 mg/L working standard.

To prepare a 10 mL of 1 mg/L mixed isotopic spiking standard, pipet:

- 128 µL (1.28 salt/cation ratio) of 100 mg/L chlormequat-d_4 chloride
- 128 µL (1.28 salt/cation ratio) of 100 mg/L mequat-d_{16} chloride
- 100 µL of 100 mg/L diquat-d_8
- 192 µL (1.92 salt/cation ratio) of 100 mg/L paraquat-d_8 dichloride into a 20 mL HDPE bottle. Dilute with DI water to 10 g total weight. Swirl to mix. Refrigerate at 20 °C or lower.

Sample preparation

100 mM formic acid extraction acid

In an exhaust hood, pipet 4.2 mL (5.22 g) of 88% formic acid into a 1 L volumetric flask containing 300 mL of DI water. Swirl to mix. Dilute to the 1 L mark. Mix by inverting the flask several times.

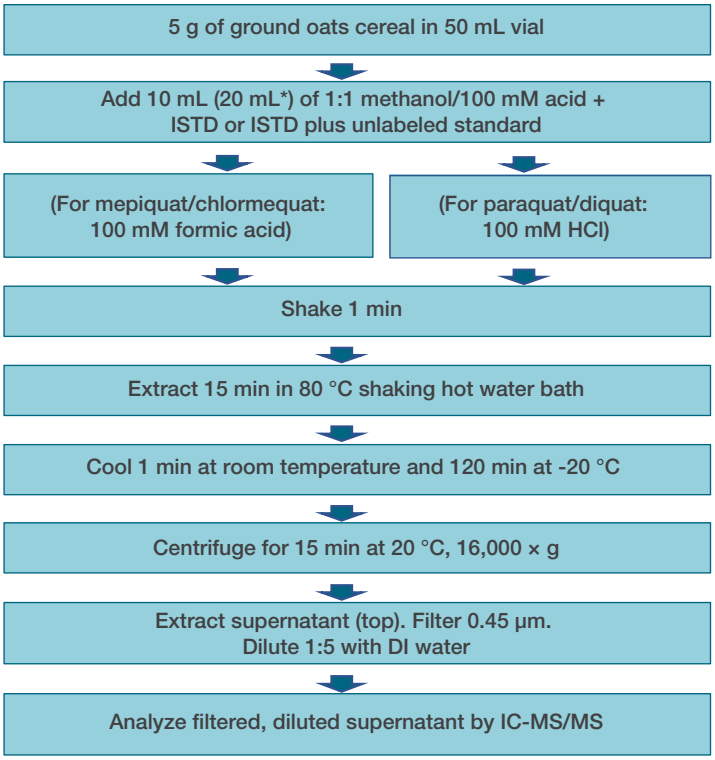
100 mM HCl extraction acid

In an exhaust hood, pipet 8.35 mL (9.85 g) of 37% (concentrated) hydrochloric acid (HCl) into a 1 L volumetric flask containing 300 mL of DI water. Swirl to mix. Dilute to the 1 L mark. Mix by inverting the flask several times.

Samples

Commercial oatmeal and toasted oat cereal samples were evaluated.

The sample extraction follows the EURL-FV Quick Polar Pesticide Extraction method (QuPPE)[21] optimized for the quaternary amines (Figure 1). The oatmeal and toasted oat cereal samples were ground in a food-grade processor. Twelve replicate 5 g ground samples were weighed into 50 mL vials and were extracted by 10 mL each of 1:1 solution of 100 mM acid and methanol (Table 4). The acid-methanol solutions were spiked with 5 µg/L mixed ISTD. Mixed unlabeled standard, 5 µg/L, was added to three replicates of the formic acid-methanol extractions to determine recoveries. Similarly, the 5 µg/L mixed standard was added to three replicates of the HCl-methanol extraction.



*Added 20 mL of methanol/acid to extract toasted oats cereal samples

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

Table 4. Extraction solution recommended by EURL-FV QuPPE method

Compound	100 mM Formic acid	100 mM Hydrochloric acid	Methanol
Chlormequat	5 mL	--	5 mL
Mepiquat	5 mL	--	5 mL
Paraquat	--	5 mL	5 mL
Diquat	--	5 mL	5 mL

As stated in version 12 of the EURL-FV QuPPE method, 100 mM formic acid is sufficient as the acid portion to quantitatively determine chlormequat and mepiquat. However, 100 mM HCl is recommended for paraquat and diquat.

We observed that the 10 mL acid/methanol solution was rapidly absorbed by the ground toasted oat cereal sample to create a slurry. As a result, an additional 10 mL was used to extract the toasted oat cereal (20 mL total).

Instrument setup and installation

Physical and electronic configuration

The Dionex ICS-6000 is a modular, high pressure, Reagent-Free IC™ (RFIC™) dual ion chromatography system. This application runs on one side of the dual system: System 1 (Pump_1, EGC_1,

CR-CTC_1, DC_Valve_1, CD_1). The auxiliary pump (Pump) and DC Automation Manager injection valve (AM_HP1) facilitate the flow to the suppressor regenerant (A mode) and to the MS (B mode) (Figure 2).

In IC-MS applications, a PEEK valve should be used as the diverter valve. In this case, the AM_HP1 valve (in the ICS-6000 DC module) functions as the diverter valve. In the “A” position (divert), the AM_HP1 valve directs the DI water to the MS and the CD effluent to the suppressor Regen In. In the “B” position (analyze), the valve directs the IC effluent to the MS and the regenerant DI water to the suppressor. The timing of the AM_HP1 valve is also intended to divert most of the sample matrix away from the mass spectrometer (see IC-MS conditions).

In IC-MS, a heated electrospray source transfers chromatographically separated ions in solution to ions in a gas stream. Make-up solvent is not needed.

The following instructions assume that your Dionex ICS-6000 HPIC system and the TSQ Altis Plus triple quadrupole mass spectrometer have already been installed by your field service engineer. To set up the IC-MS system, position the Dionex ICS-6000 system near the MS source. Install the power and USB cables and power-up the IC, autosampler, and computer. Add ASTM Type I DI water to the eluent bottles and prime the pumps.

Auxiliary pump, AXP-MS

The auxiliary pump is used to dispense DI water for the suppressor regenerant. Connect the communication cable from the pump to the computer and the power cable. Set the error message action to “abort” if the pressure falls below the lower limit (500 psi). If a serial cable is used for the computer connection, install the serial driver (in the computer) prior to electronic configuration. For reliable operation, prime this pump daily to remove any gas bubbles before starting the sequence.

TSQ Altis Plus mass spectrometer

The TSQ Altis Plus mass spectrometer is a triple quadrupole mass spectrometer. For IC-MS applications, the TSQ Altis Plus system is mass calibrated with the Extended Mass Reference Standard (EMRS) to achieve detection of low mass ions needed for IC.

The installation includes the power connections needed by the mass spectrometer, separate gas lines for the nitrogen and argon gases, a nitrogen source (preferably a nitrogen generator), argon gas tank, a mechanical pump, and turbo vacuum pumps, HESI source, vacuum and exhaust lines, and power, USB, and ethernet communication cords. The mass spectrometer status should be in standby mode after being powered-up and under gas flow and required vacuum. If not, start the gas flow, power-up the mass spectrometer, and start the mechanical and vacuum pumps. After the mass spectrometer achieves vacuum (by mechanical and turbo pumps), set the source conditions to standby mode.

This application is operated solely through the Chromeleon Chromatography Data System (CDS). To electronically configure the IC system, start the Chromeleon Instrument Services program, then start the Instrument Controller program by selecting the *configure instruments link.* Add the ICS-6000 system modules: SP, EG, and DC, and the AS-AP Autosampler, auxiliary pump, and TSQ triple quadrupole mass spectrometer, as described in Table 5. Check and correct the configuration for any errors. Save and close the configuration. To create an “IC-only” instrument method, the TSQ mass spectrometer will need to be temporarily removed from the configuration.

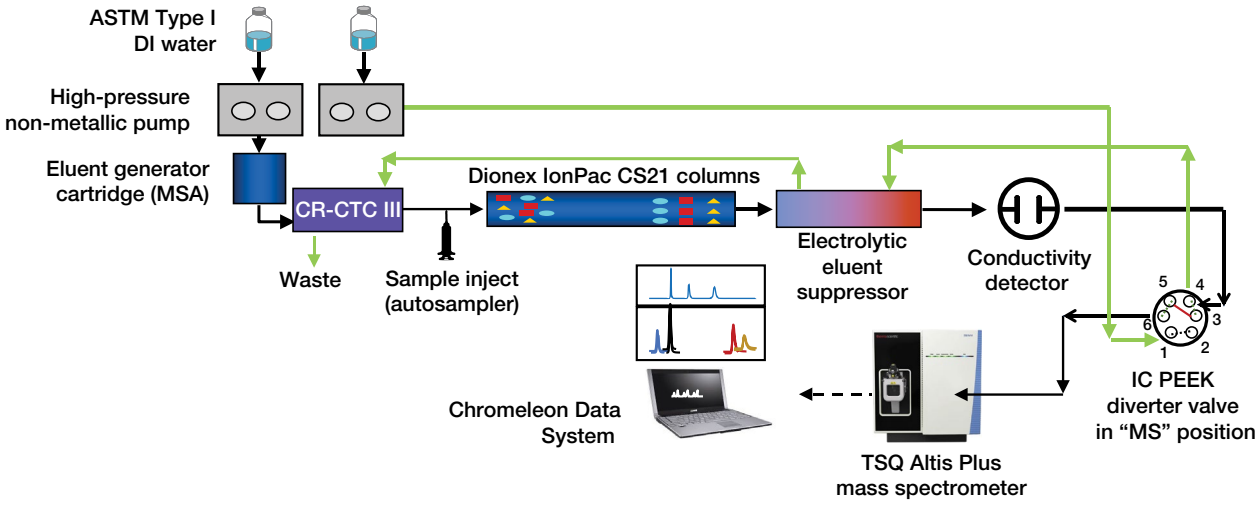


Figure 2. IC-MS/MS flow diagram

Table 5. Electronic configuration parameters

Module	Tab	Action
SP	General	Select Browse. Select the serial number to link module to instrument.
	Device	Link Pump_1 to instrument.
EG	General	Select serial number to link module to instrument.
	Cartridges	Link to instrument. Check EGC_1 and link to Pump_1.
DC	General	Select serial number to link module to instrument, select instrument.
	Detectors	Double click on CD, Link to Pump_1.
	Temperature controls	Check Compartment_TC and Column_TC.
	Suppressors	Double click Suppressor_1, Link to Pump_1.
	High pressure valves	Double click InjectValve_1, Link to Pump_1, select control by autosampler.
AS-AP autosampler	General	Select serial number to link module to instrument.
	Sharing	If this option is present, select instrument.
	Segments / Pump link	Select 10 mL PolyVials for “Red”, “Blue”, and "Green". Leave the pump and TTL links empty.
	Options	Select 1,200 buffer loop, 250 µL syringe, temperature control, and push mode. Enter “10” µL in sample loop.
Auxiliary pump	Add module	Select AXP pump under IC: Dionex modules.
	General	Rename device name to Pump (or another unique name).
	Pump type	Select pump type: AXP-MS.
	Select COM Port	Should be COM port 3 or higher. (If a serial cable is used, the serial driver should be installed prior to configuration.)
	Select “OK”	The correct COM has been selected if no errors are reported in the script.
TSQ mass spectrometer	Add module	Mass spectrometry, mass spectrometer
	General	MSDevice_Sync
		Deselect the hardware inject synchronization. This is not needed for Chromeleon CDS.
		Device name: MSDevice*
		Device type: TSQ Altis*
		Synchronization port: MSDevice_Sync*
TSQ mass spectrometer instrument configuration	System information	Model: Altis
		Serial number: automatically selected*
	Inlet	Diverter valve: select “None”
		Syringe pump: select “Configured”
		Contact closure: Select “High-to-Low Edge”
		CID gas type: Argon*
		Check boxes: Wait for gas to stabilize* Wait for temperature to stabilize* Keep last modified method*
	Ion source	Selected Heated HESI*
		Selected dedicated Heated HESI*
		Box checked: Enable Sweep Gas for NSI source*
	Calibration	Selected EMRS*
	Analog inputs	None selected
	Instrument warnings	Check all boxes*
	Historical data retention	Number of sessions: 1,000*
		Maximum number of years: 1*
		Maximum data storage size (GB): 20*
		Maximum number of records: 200,000*
	Service	Boxes are not checked*
	Save	Select “OK”

* Automatically selected

Plumbing the Dionex ICS-6000 HPIC system

Plumb the Dionex ICS-6000 IC as a standard Reagent-Free IC (RFIC) system using the IC PEEK Viper fittings as indicated on the IC PEEK Viper fittings. In addition, IC PEEK Viper fittings should be used on all liquid connections after the CD detector out, including from the IC to the mass spectrometer, to minimize backpressure on the suppressor that could potentially damage it. The schematics are also illustrated on the inside doors of the Dionex ICS-6000 IC system. Further information can be found in the product manuals of the IC system and the AXP-MS Metering Pump.[30,31] Direct the waste lines to waste containers.

Conditioning electrolytic devices and columns

Temporarily direct the liquid flow away from the mass spectrometer until the IC and IC consumables are fully conditioned. Hydrate and condition the Dionex EGC 500 MSA eluent generator cartridge and Dionex CR-CTC III continuously regenerated trap column according to product manuals or the instructions in the drop-down menu (Chromeleon Console, under Consumables drop-down menu).[32, 33] The Dionex CR-CTC III trap column is required for this application. Long-term use of a Dionex CR-CTC II column or Dionex CR-CTC 500 column for this application will cause column problems.

Condition the columns as described in the Dionex IonPac CS21-Fast-4µm product manual[34] or Consumables Conditioning instructions (Chromeleon Console, under Consumables drop-down menu), using 4 mM MSA, 40 °C at 0.30 mL/min for 30 min or more while directing the effluent to waste. Install the conditioned columns according to Figure 2.

To hydrate the Dionex CDRS 600 suppressor, follow the instructions in the Suppressor Installation Checklist that is included with the suppressor.[35]

Install the suppressor according to Figure 2 and ensure that the backpressure is within the suppressor's specifications. For cation suppressors, optimum results are achieved by minimizing the hydrating and waiting times to those stated in the suppressor installation checklist and immediately installing the suppressor, starting the pump, eluent, and Dionex CR-TC trap column, and powering the suppressor.

System startup, conditioning, and consumables device tracking

To condition the IC system, temporarily direct the IC effluent to waste (instead of to the mass spectrometer) and set the mass spectrometer to standby mode (vaporizer temperature = “150”, ion transfer temperature = 150 °C, and gases = 2 arb). Initially, equilibrate the IC using the Quality Assurance Report (QAR) conditions for the Dionex IonPac CS21-Fast-4µm column.

To create an IC-only instrument program, temporarily remove the mass spectrometer from the configuration. Using the Chromeleon Instrument Method Wizard program, enter the QAR conditions, but do not enter conditions for the mass spectrometer, and turn-off the AXP-MS pump. Create a sequence and start the sequence by approving the consumables in the Consumables Tracking panel. Compare the results against the QAR report. Continue to equilibrate the IC until the total conductivity is <2 µS/cm. Re-connect the liquid connections from the IC to the MS and enter the IC and MS parameters from the Conditions section into the Chromeleon console.

Creating IC-MS methods with emergency shutdown subprograms

Add the TSQ module to the electronic configuration using the commands listed in Table 5, and enter the parameters listed in the Conditions section, including the AM_HP_1 valve timing and the SRM table. Turn on the AXP-MS pump. Set the AXP-MS lower pressure limit to 500 psi. Save the instrument method.

Unexpected failures in the suppressor or the regenerant pump directing water to the suppressor can damage the mass spectrometer. For IC-MS applications, during emergency failures, the diverter valve should be rotated to direct the CD effluent away from the MS. In this configuration, the Dionex ICS-6000 Automation Manager AM_HP1 6-port valve is in the “A” position directing DI water to the MS, and the suppressor is in the recycle mode.

High conductivity emergency trigger

The high conductivity trigger, using the Chromeleon Conditional Trigger function, implements emergency actions when the total conductivity signal exceeds a high level (50 µS/cm) for a set time (180 s). The action commands the AM_HP1 to direct water to the mass spectrometer and puts the suppressor in recycle mode. The high conductivity could be due to an unexpected suppressor failure. (Note: when the trigger conditions are met, the pump will turn off, which turns off the RFIC consumables and suppressor.) These conditions were selected for the application and can be adjusted to lower or higher conductivity or a different elapsed time, depending on the method.

To create an emergency trigger:

1. Open the IC-MS program.
2. Open the Script Editor.
3. Insert a Conditional Trigger on the 0.00 Time, Run line.

Name	"HighConductivity"
Condition	CDet.CD_1_total.signal>=50
TrueTime	180
Delay	5
AllowImmediate	Yes

4. Place the cursor on the End Trigger row and Command column. Select Insert Command.
5. Enter the command to divert IC flow away from the MS while diverting DI water to the MS and to turn-off the Pump_1 motor.

DC.AM_HP1.A	
Pump_1.Motor	off

6. Save the trigger.
7. Save the instrument method.

For more details on creating emergency triggers, see AN73329.[14]

In discussions with the author of TN73390,[19] additional maintenance of the Dionex AS-AP autosampler may be required after six months due to cross-contamination and resulting carryover of diquat and paraquat. It is recommended that the following parts be replaced every six months: AS-AP injection port, transfer line tubing, and buffer loop.

Results and discussion

Column selection

The Dionex IonPac CS21-Fast-4µm column, a high-capacity weak cation-exchanger, was optimized for separation of monovalent and divalent quaternary amine pesticides. The column is functionalized with carboxylic acid groups and composed of DVB macroporous resin beads with 80% cross-linking. As a result, the column has cation-exchange and reversed-phase properties that allow fast elution of the quaternary amines. The column chemistry creates elution windows for the quaternary amines, resolving them from other cations and each other. The monovalent cations elute first (lithium, sodium, ammonium, potassium), then chlormequat and mepiquat, followed by the divalent cations (magnesium and calcium) and paraquat and diquat.

Method qualification

To qualify the method, experiments using mixed cation and quaternary amine standards were evaluated for IC reproducibility linearity range, method detection limits, and the confirming the elution windows for MS detection.

Ion chromatography reproducibility was demonstrated by seven replicate injections of the 5 mg/L QAR standard using the IC-only QAR method (Figure 3). The QAR conditions are designed to verify the four elution windows (monovalent cations, monovalent quaternary amines, divalent cations, divalent quaternary amines). The three analyte peaks (potassium, mepiquat, and magnesium) represent three elution steps: the potassium ion represents the end of the elution window for the monovalent cations, mepiquat represents the end of the elution window for the monovalent quaternary amines, and magnesium represents the beginning of elution of the divalent cations. Paraquat and diquat elute after calcium (not shown). The results show the expected retention times, confirming the elution windows. Table 6 show good reproducibility based on the RSDs of the potassium, mepiquat, and magnesium retention times (0.15, 0.07, 0.09) and peak areas (0.51, 0.27, 0.31).

Columns:	Dionex IonPac CS21-Fast-4µm and guard, 2 mm i.d.
Eluent source:	Dionex EGC 500 MSA, Dionex CR-CTC III trap column, HP EG degas kit
Eluent:	4 mM Methanesulfonic acid (MSA)
Flow rate:	0.30 mL/min
Injection volume:	10 µL
Column temp.:	40 °C
Detection 1:	Suppressed conductivity, 20 °C, Dionex CDRS 600, 2 mm, 22 mA, recycle mode, auxiliary pump = off
Diverter valve:	Divert flow to waste
Detection 2:	TSQ Altis Plus triple quadrupole mass spectrometer on standby.

Peaks:			
	1. Sodium	--	mg/L
	2. Potassium	5.0	
	3. Mepiquat	5.0	
	4. Magnesium	5.0	

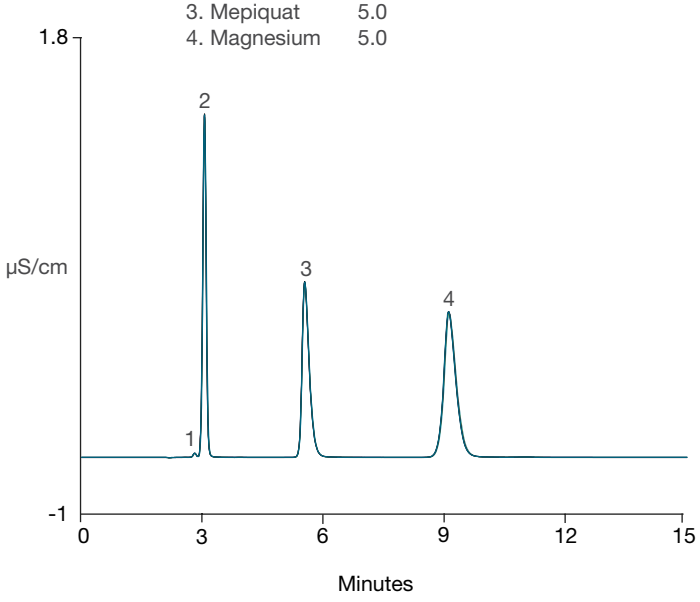


Figure 3. Seven replicates of QAR standard (5 mg/L) using the column qualification conditions

Table 6. IC retention time and peak area reproducibilities of seven replicates

	Suppressed conductivity				Mass spectrometry			
	Retention time (min)	RSD	Peak area (µS*min)	RSD	Retention time (min)	RSD	Peak area (µS*min)	RSD
Chlormequat	5.07	0.19	0.0085	1.52	5.19	0.13	1.90e6	1.02
Mepiquat	5.81	0.08	0.0046	5.03	6.07	0.11	4.85e6	1.14
Paraquat	10.85	0.09	0.0077	5.13	10.93	0.09	9.11e6	6.55
Diquat	11.71	0.10	0.0045	6.27	11.79	0.17	4.97e6	8.66

n = 7

To evaluate the IC-MS elution windows, a mixed standard of cations, quaternary amine pesticides, and internal standards was separated using a gradient from 3 to 25 mM MSA at 0.3 mL/min and 40 °C. Figures 4A and 4B show the IC chromatogram with highlighted matrix windows and the SRM chromatograms of the quaternary amine pesticides.

Columns:	Dionex IonPac CS21-Fast-4µm and guard, 2 mm i.d.
Eluent source:	Dionex EGC 500 MSA, Dionex CR-CTC III trap column, HP EG degas kit
Eluent:	Methanesulfonic acid (MSA) gradient
Gradient:	3 mM (-4 to 0 min), 3-6 mM (0 to 3.6 min), 6-22 mM (3.6 to 6 min), 22-25 mM (6 to 15 min), 3 mM (15 min)
Flow rate:	0.30 mL/min
Injection volume:	10 µL
Column temp.:	40 °C
Detection 1:	Suppressed conductivity, 20 °C, Dionex CDRS 600, 2 mm, 22 mA, external water mode
Auxiliary pump:	0.3 mL/min for DI water regenerant

Peaks:			
1.	Sodium	30	µg/L
2.	Ammonium	10	
3.	Potassium	10	
4.	Unknown	--	
5.	Chlormequat	50	
6.	Mepiquat-d_4	50	
7.	Mepiquat	50	
8.	Magnesium	10	
9.	Calcium	20	
10.	System peak	--	
11.	Paraquat	50	
12.	Diquat	50	

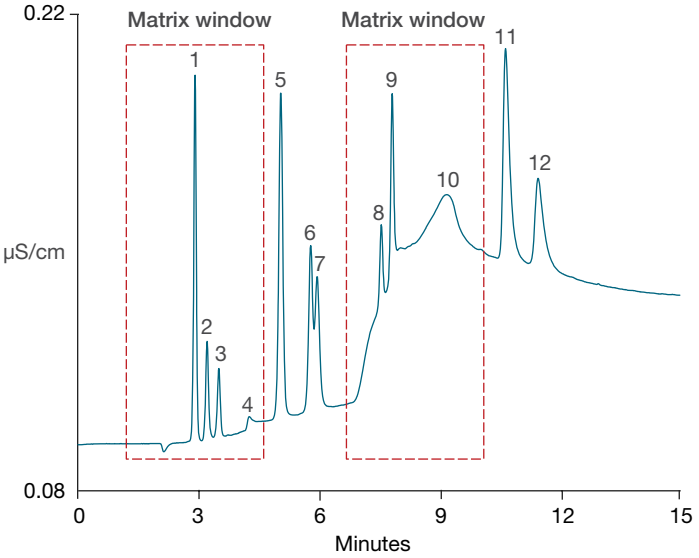


Figure 4A. Mixed cation and quaternary amine pesticide standard showing matrix windows

The SRM chromatograms show the matrix elution windows for the quaternary amine pesticides. Unexpectedly, mepiquat-d_4 and mepiquat exhibit slightly different selectivity on the Dionex IonPac CS21-Fast-4µm column by eluting at slightly different retention times, 5.80 min and 5.96 min by CD detection, 5.89 and 6.06 by MS detection, respectively. The chlormequat, paraquat, and diquat ISTDs eluted as expected, at the same time as the unlabeled standards.

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp.:	Vaporizer: 300 °C; Ion transfer tube: 350 °C
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Standard:	5 µg/L

Peaks:	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)
1. Chlormequat-d_4	126	57.9	30
2. Chlormequat	122.1	57.9	30
3. Mepiquat-d_{16}	130	110	30
4. Mepiquat	114.1	98.1	30
5. Paraquat-d_8	97	179	19
6. Paraquat	93	171	19
7. Diquat-d_8	96	88.5	19
8. Diquat	92	157.1	19

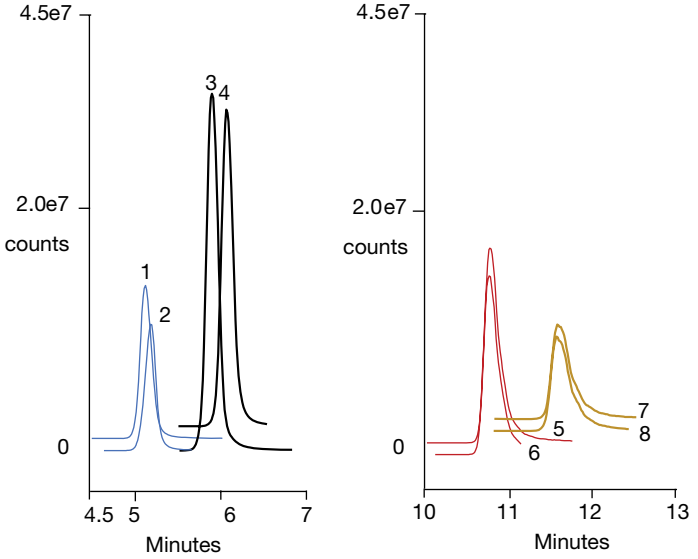


Figure 4B. Mixed cation, quaternary amine pesticide standard, showing SRMs of the pesticides

To evaluate the relationship between concentration and response, calibration curves were prepared using seven calibration standards (1, 2, 5, 10, 20, 50, 100 µg/L) with 5 µg/L ISTD. The results displayed a second-order relationship with excellent fit over the whole range (Table 7). Figure 5 shows the calibration plot for paraquat, which is representative of the other three pesticides. The limit of detection (LOD) was determined by analyzing seven replicates of a 1.0 µg/L calibration standard. The LODs were determined by multiplying the Student's t-test constant (n=7) and the standard deviation (σ).

Table 7. Summary of calibration and limit of detection results

	Calibration			LOD* (µg/L)
	Range (µg/L)	Type	Coefficient of determination (r^2)	
Chlormequat	1–100	Quadratic, offset	0.9999	0.09
Mepiquat	1–100	Quadratic, offset	0.9999	0.08
Paraquat	1–100	Quadratic, offset	0.9999	0.07
Diquat	1–100	Quadratic, offset	0.9999	0.08

*LOD: 1.0 µg/L standard, (n=7), Student's *t*-test x σ (standard deviation)

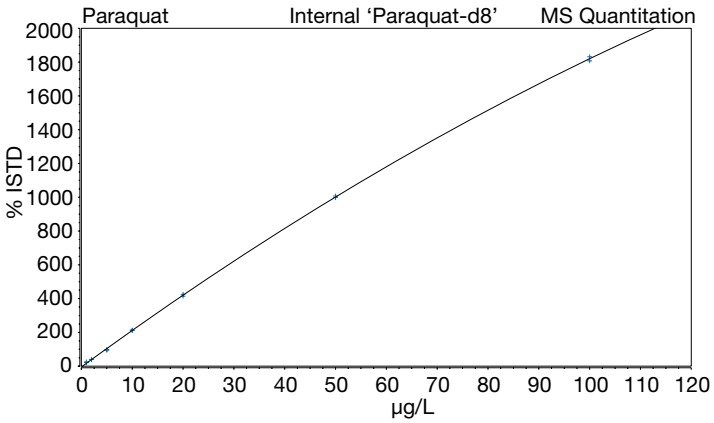


Figure 5. Calibration plot of paraquat

Sample analysis

Oatmeal cereal extractions

The qualified method was applied to acid-methanol extractions of ground samples of oatmeal and toasted oats cereals, and the same samples spiked with standards. The quaternary amine pesticides were extracted in two sets: using formic acid-methanol for chlormequat and mepiquat and HCl-methanol for paraquat and diquat as recommended by EURL-FV QuPPE procedures. Figures 6A and 6B show the IC and SRM chromatograms of formic acid-methanol extractions. The IC chromatograms (Figure 6A) show very large peaks of the sample matrix with small quaternary amine peaks in the baseline. The SRM chromatograms (Figure 6B) show good retention time reproducibility and strong responses of the added standard. Trace concentrations of residual native chlormequat, paraquat, and diquat (0.22–0.36 µg/L) were found in the sample extract.

Figure 7 shows the SRM chromatograms of a ground oatmeal extracted with HCl and methanol. The chlormequat and mepiquat peaks are slightly distorted, but they did not impact quantification.

Columns:	Dionex IonPac CS21-Fast-4µm and guard, 2 mm i.d.
Eluent source:	Dionex EGC 500 MSA, Dionex CR-CTC III trap column, HP EG degas kit
Eluent:	Methanesulfonic acid (MSA) gradient
Gradient:	3 mM (-4 to 0 min), 3-6 mM (0 to 3.6 min), 6-22 mM (3.6 to 6 min), 22-25 mM (6 to 15 min), 3 mM (15 min)
Flow rate:	0.30 mL/min
Injection volume:	10 µL
Column temp.:	40 °C
Detection 1:	Suppressed conductivity, 20 °C, Dionex CDRS 600, 2 mm, 22 mA, external water mode
Auxiliary pump:	0.3 mL/min
Sample prep.:	5 g of ground oatmeal. Modified QuPPE (Figure 1) with 10 mL 50:50 100 mM formic acid/methanol
Sample:	A. Spiked with 5 µg/L ISTD prior to extraction. B: Replicate Sample A spiked with 5 µg/L unlabeled pesticides

Peaks:		B
	2. Chlormequat	5 µg/L
	4. Mepiquat	5
	6. Paraquat	5
	8. Diquat	5

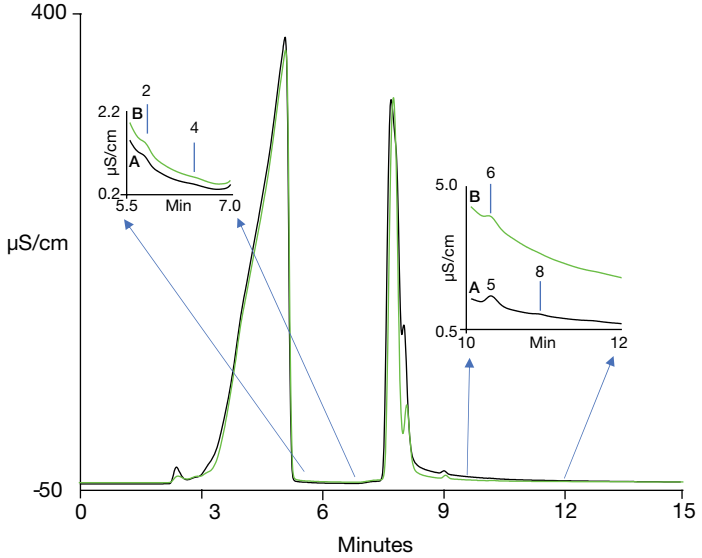


Figure 6A. IC chromatograms. A) Formic acid/methanol extract of ground oatmeal, B) Sample A with added standard

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground oatmeal cereal Modified QuPPE (Figure 1) with 10 mL 50:50 100 mM formic acid and methanol
Sample:	A: Extracted oatmeal sample + 5 µg/L ISTD B: Replicate Sample A + 5 µg/L unlabeled standard

Peaks:	A	B	µg/L
1. Chlormequat-d_4	5.0	5.0	
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	5.0	
6. Paraquat	0.22	5.93	
7. Diquat-d_8	5.0	5.0	
8. Diquat	0.36	6.06	

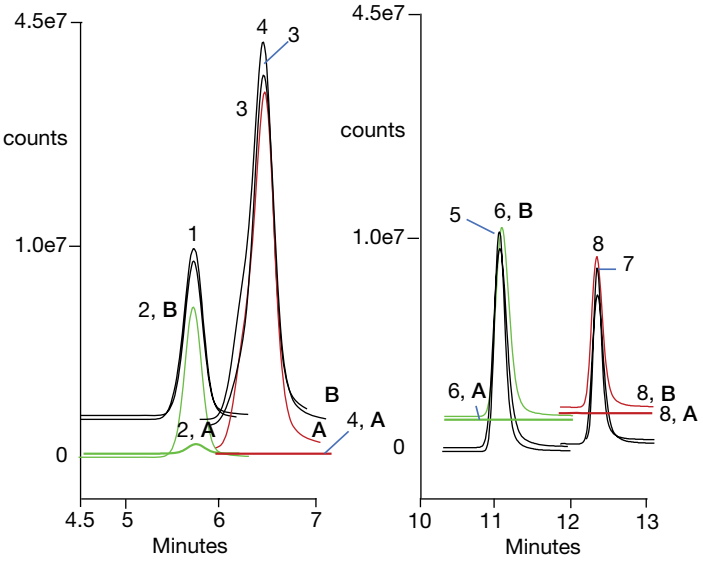


Figure 6B. SRMs of quaternary amines from (A) ground oatmeal, (B) spiked with added standard: formic acid /methanol extract

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground oatmeal cereal Modified QuPPE (Figure 1) with 10 mL 50:50 100 mM hydrochloric acid and methanol
Sample:	A: Extracted oatmeal sample + 5 µg/L ISTD B: Replicate Sample A + 5 µg/L unlabeled standard

Peaks:	A	B	µg/L
1. Chlormequat-d_4			
2. Chlormequat	0.52	5.31	
3. Mepiquat-d_{16}			
4. Mepiquat	<0.08	6.03	
5. Paraquat-d_8			
6. Paraquat	0.22	4.85	
7. Diquat-d_8			
8. Diquat	0.37	5.16	

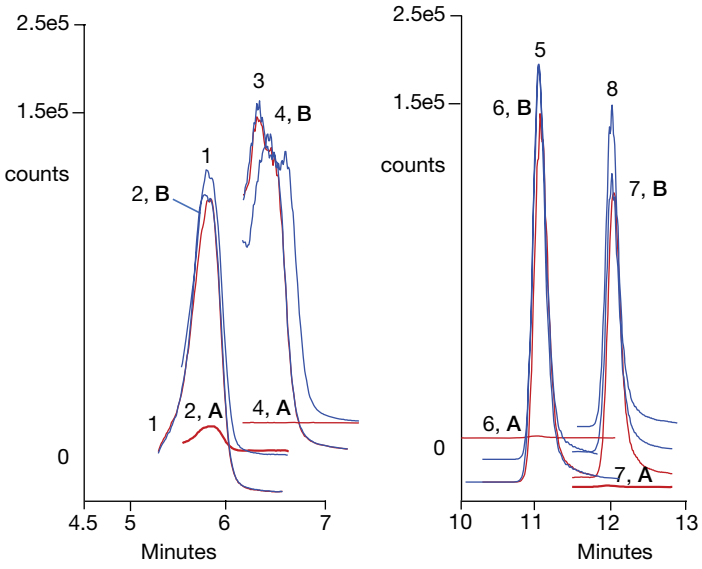


Figure 7. SRMs of quaternary amines from (A) ground oatmeal, (B) spiked with added standard: HCl acid/methanol extract

Toasted oat cereal extractions

Both acid extractions were impacted by the toasted oat cereal sample matrix, as indicated by the SRM retention time shifts (retention time losses ranged from 0.05 to 0.3 min) and ~30% drop in peak areas (relative to the first injection of the triplicate injections). The impact was greatest when analyzing HCl extractions of the toasted oat sample (Figure 8). Additionally, the formic acid extracted samples had distorted chlormequat and mepiquat peaks. These effects were only detected in SRM mode.

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground toasted oat cereal Modified QuPPE (Figure 1) with 20 mL 50:50 100 mM hydrochloric acid and methanol
Sample:	A–C: Extracted toasted oat cereal samples + 5 µg/L ISTD + 5 µg/L unlabeled standard

Peaks:	A	B	C	
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	
5. Paraquat-d_8				
6. Paraquat	4.7	3.2	2.5	
7. Diquat-d_8				
8. Diquat	4.6	3.0	1.8	

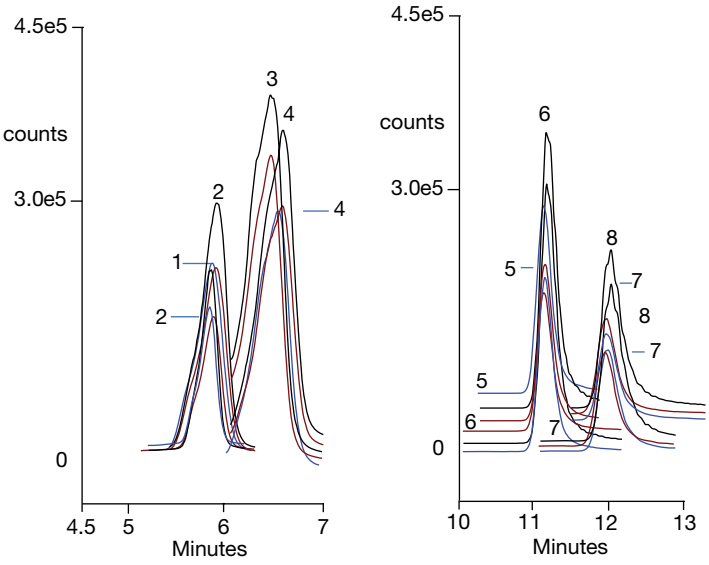


Figure 8. Toasted oats cereal matrix impacting responses, HCl extraction

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground toasted oat cereal Modified QuPPE (Figure 1) with 20 mL 50:50 100 mM formic acid and methanol
Sample:	A–C: Extracted toasted oat cereal samples + 5 µg/L ISTD + 5 µg/L unlabeled standard

Peaks:	A	B	C	
1. Chlormequat-d_4				
2. Chlormequat	4.27	4.34	4.17	µg/L
3. Mepiquat-d_{16}				
4. Mepiquat	4.29	4.30	4.29	
5. Paraquat-d_8				
6. Paraquat	4.66	4.61	4.67	
7. Diquat-d_8				
8. Diquat	5.11	5.13	5.11	

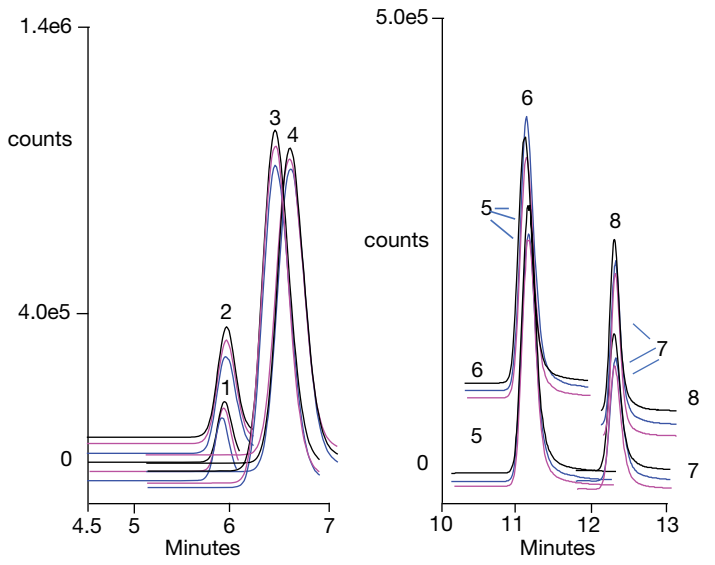


Figure 9. SRM chromatograms of triplicate injections of toasted oat sample extractions by formic acid using 40 min run showing excellent reproducibility. Baseline offset.

To increase the retention time and peak area reproducibilities, experiments were conducted to elute the sample matrix faster by increasing the wash concentration (up to 75 mM MSA) and time (from an extra 5 to 25 min), extending the separation to 40 min, and alternating 15 min sample runs with DI water injections. The wash experiments did not improve the retention time and peak areas reproducibilities. However, the experiments with extended separation time and alternating sample and DI water injections were effective. These experiments suggest that large hydrophobic compounds from the sample matrix, transparent to both detectors, interfered with paraquat and diquat elution. Pretreating the sample extractions with a cartridge designed for removing hydrophobic compounds, the Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge, improved the results. However, paraquat and diquat were not fully recovered from the cartridges and the mepiquat peak was distorted. To improve reproducibility, a 15 min 1 mM MSA step was added to the gradient of the 15 min method. The final method recommended for acid-methanol extractions of oat cereal samples is shown in the Conditions section.

Figure 9 shows chromatograms of triplicate injections of formic acid-methanol extractions of toasted oat cereal run in series using a 40 min run. Similarly, Figure 10 shows the results of HCl-methanol extractions using sample injections of the 15 min method alternated with DI water injections. Figure 11 shows the results of samples extracted with HCl using a 15 min wash with 1 mM MSA. The three cases show good reproducibility and minimal loss in peak response, <5%.

Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground toasted oat cereal Modified QuPPE (Figure 1) with 20 mL 50:50 100 mM hydrochloric acid and methanol
Sample:	A-C: Extracted toasted oat cereal samples + 5 µg/L ISTD + 5 µg/L unlabeled standard

Peaks:	A	B	C	
1. Chlormequat-d_4				
2. Chlormequat	4.27	4.34	4.17	µg/L
3. Mepiquat-d_{16}				
4. Mepiquat	5.72	5.62	5.65	
5. Paraquat-d_8				
6. Paraquat	4.71	4.78	4.71	
7. Diquat-d_8				
8. Diquat	4.57	4.53	4.54	

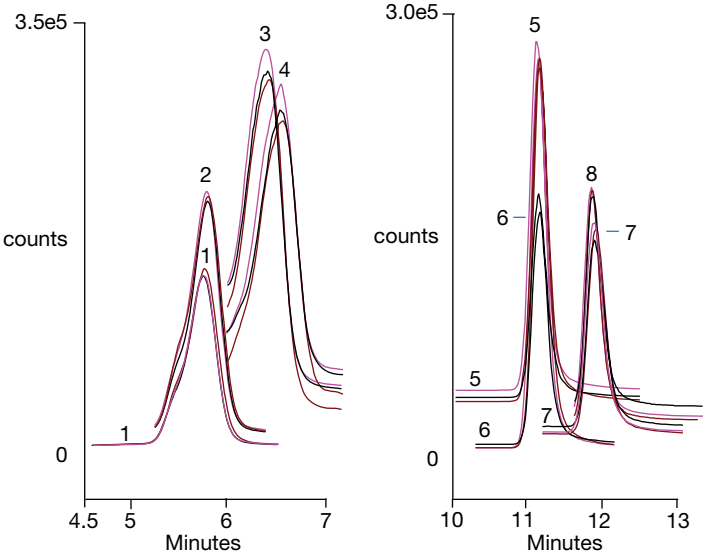


Figure 10. SRM chromatograms of triplicate injections of toasted oat sample extractions by HCl using a 15 min run alternately with 15 min DI water showing excellent reproducibility

Method accuracy was determined by measuring recoveries of 5 µg/L added standard. These results, 86–118% recoveries (Table 8), suggest that the method is accurate. Similar results were obtained for formic acid and HCl extractions, although slightly better recoveries, closer to 100%, were achieved for diquat and paraquat using the HCl extraction method.

MSA gradient:	3 mM (-4 to 0 min), 3-6 mM (0 to 3.6 min), 6-22 mM (3.6 to 6 min), 22-25 mM (6 to 15 min), 1 mM (15 to 30 min), 3 mM (30 min)
Detection 2:	TSQ Altis Plus triple quadrupole, +HESI-II, +2.8 kV, SRM mode
Desolvation:	None
MS N_2 gases (arb):	Sheath: 45; Aux: 3; Sweep: 2
Source temp. (°C):	Vaporizer: 300; Ion transfer tube: 350
SRM Polarity:	Positive
SRM Cycle time (s):	0.8 s
SRM Resolution (FWHM):	Q1: 0.7; Q3: 1.2
SRM CID gas (mTorr):	1.5
SRM Source frag. (V):	10
SRM Chromatog.:	Peak width: 6 s; Filter
Sample preparation:	5 g of ground toasted oat cereal Modified QuPPE (Figure 1) with 20 mL 50:50 100 mM hydrochloric acid and methanol
Sample:	A,B: Extracted toasted oat cereal samples + 5 µg/L ISTD + 5 µg/L unlabeled standard

Peaks:	A	B	
1. Chlormequat-d_4			
2. Chlormequat	4.31	4.28	µg/L
3. Mepiquat-d_{16}			
4. Mepiquat	5.68	5.70	
5. Paraquat-d_8			
6. Paraquat	4.71	4.72	
7. Diquat-d_8			
8. Diquat	4.59	4.52	

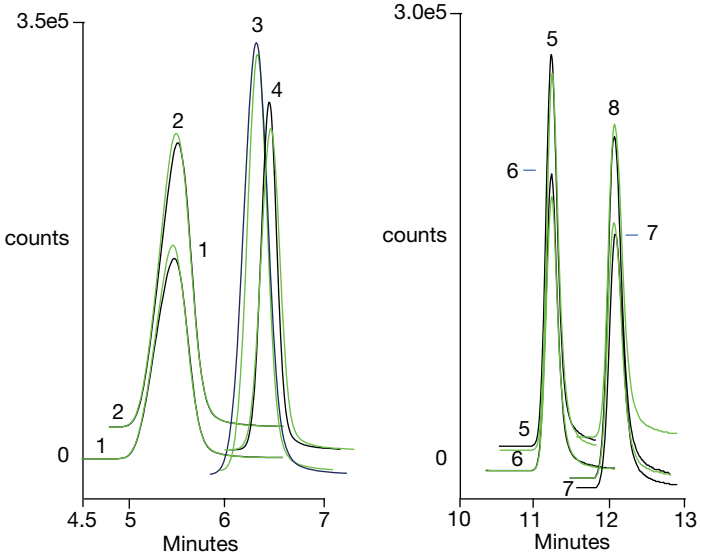


Figure 11. SRM chromatograms of duplicate injections of toasted oat sample extractions by HCl with 1 mM MSA wash showing excellent reproducibility

Table 9 shows the calculated amounts of pesticides found in these cereal samples. The quaternary amine pesticides were either not detected in the oat cereal samples or found at trace concentrations, <2 µg/kg (ppb). The highest concentrations of quaternary amine pesticides were diquat and paraquat, found in the toasted oat cereal at 1.5 to 1.7 µg/kg (0.00147 to 0.00168 mg/kg), well below the EU MRLs of 2 mg/kg (diquat) and 0.02 mg/kg (paraquat).

Table 8. Summary of measured results and recoveries of added standard

	Chlormequat			Mepiquat			Paraquat			Diquat		
	Measured (µg/L)	RSD	Recov (%)	Measured (µg/L)	RSD	Recov. (%)	Measured (µg/L)	RSD	Recov. (%)	Measured (µg/L)	RSD	Recov. (%)
Oatmeal / Formic acid-methanol	0.36 ± 0.02	5.7	117	0.08	--	116	0.22 ± 0.02	9.1	113	0.36 ± 0.02	5.6	113
Oatmeal / HCl-methanol	0.51 ± 0.03	5.9	98.9	<0.08	--	118	0.24 ± 0.02	8.3	95.4	0.37 ± 0.02	5.4	96.3
Toasted oat cereal / Formic acid-methanol	<0.09	--	85.8	<0.08	--	85.6	0.29 ± 0.02	6.9	88.2	0.42 ± 0.03	7.1	94.3
Toasted oat cereal / HCl-methanol	<0.09	--	96.5	<0.08	--	113	0.24 ± 0.03	12.5	95.4	0.37 ± 0.03	8.1	90.8

+ added 5 µg/L

Table 9. Calculated results

	Chlormequat* (mg/kg)	Mepiquat* (mg/kg)	Paraquat (mg/kg)	Diquat (mg/kg)
EU MRLs	15	3	0.02	2
Oatmeal / Formic acid-methanol	0.00072	<0.00021	0.00044	0.00071
Oatmeal / HCl-methanol	0.00102	<0.00021	0.00047	0.00074
Toasted oat cereal / Formic acid-methanol	<0.00046	<0.00042	0.00116	0.00168
Toasted oat cereal / HCl-methanol	<0.00046	<0.00042	0.00094	0.00147

*Total concentration is as the pesticide salt

Conclusion

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 similarly structured paraquat and diquat ions.

Precautions

Carryover from the sample extractions, particularly when extracting the toasted oats sample, impacted the peak responses and retention times. Three solutions were developed: 15 min wash at 1 mM MSA, extending the run time to 40 min per sample, and alternating sample injections with DI water. The 15 min wash at 1 mM MSA condition was incorporated into the Conditions section.

Further information on pesticide applications using IC-MS can be found in the Thermo Scientific Digital Applications library.[37]

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