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APPLICATION NOTE 72908

Determination of cationic polar pesticides in homogenized fruit and vegetable samples using IC-HRAM MS

Authors

Terri Christison, John E. Madden, and Jeff Rohrer

Thermo Fisher Scientific, Sunnyvale, CA, USA

Keywords

IonPac CS17 column, RFIC, Reagent-Free IC, Integrion, accurate mass spectrometry, multiresidue, IC-MS

Goal

Demonstrate the determination of six cationic polar pesticides in food samples using ion chromatography with the Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer

Introduction

Food safety and perceived health risks from residual agricultural chemicals are an ongoing public concern and a subject of increasing regulatory scrutiny. One such group are known as polar pesticides. These include ionic emergent and desiccant herbicides, fungicides, growth-regulating chemicals, and the resultant metabolites of those compounds. Although pesticide and pesticide residues are commonly determined by GC-MS or HPLC-MS methods, polar pesticides are best determined by ion chromatography mass spectrometry (IC-MS) methods because of their ionic and non-volatile nature. Food Environmental Research Association (FERA LTD) and others have successfully demonstrated determinations of anionic pesticide residues (glyphosate, glufosinate, fosetyl, and metabolites) in beer, fruit, and vegetables using IC-MS/MS and IC-high-resolution accurate mass (HRAM) MS.[1-10] Determinations of cationic polar pesticides were also previously demonstrated using IC-MS/MS, however paraquat and diquat were problematic without HRAM-MS.[11] These applications demonstrate the advantages of using IC coupled with MS over other separation methods to separate ionic compounds.

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Additionally, multiresidue sample preparation methods are needed. Ideally the method must efficiently extract the ions of interest in various fruit and vegetable samples while minimizing the extraction of the sample matrix that can overload columns and cause ion suppression.

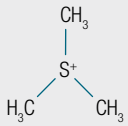
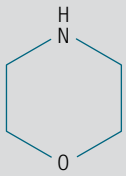
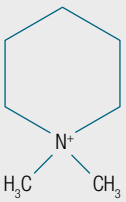
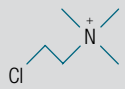
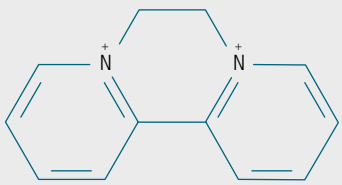
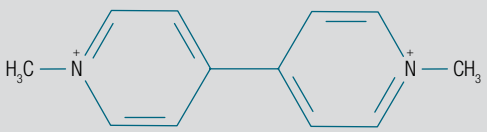
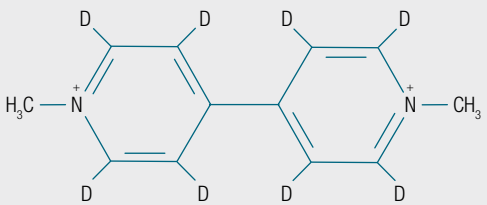
Recently the European Union Research Laboratories for Residues of Pesticides in Food and Vegetables (EURL-FV) developed a multiresidue sample preparation method for polar pesticides, aptly named Quick Polar Pesticides (QuPPe).[12] This method has been successfully demonstrated for anionic polar pesticides across a variety of foods.[2-10] However, the multiresidue sample preparation method has not been demonstrated for cationic polar pesticides.

Cationic pesticides in food can also pose significant health risks and therefore are of regulatory interest. Six chemically similar cations, mostly tertiary and quaternary amine compounds, were selected for this focused application: chlormequat, diquat, mepiquat, paraquat, morpholine, and trimethylsulfonium (Table 1). Although the residues of these compounds are classified and regulated under polar pesticides or polar pesticide residues, they have different agricultural applications. Morpholine is a carrier for waxing fruit used to improve the fruit's aesthetics and provide a moisture barrier to retard spoilage.[13-16] Chlormequat and mepiquat, growth regulators, are used in conjunction or separately to inhibit gibberellic acid, thereby retarding the upward growth of the plant and resulting in increased yields of grain and fruit.[17,18] Although chlormequat and mepiquat are used broadly, they are most commonly applied to cereal grain crops and cotton crops, respectively.[14-16]

Trimethylsulfonium, diquat, and paraquat function as herbicides and desiccants.[14-16] Trimethylsulfonium (TMS) is of particular interest because it is a common counter ion to the herbicide and grain desiccant, glyphosate. There is an increased public concern around use of glyphosate and therefore TMS because glyphosate along with all other desiccants is applied just prior to the harvest and therefore is more likely to be retained as residues.[16,19,20] Due to the tolerance of genetically modified organisms (GMOs) to glyphosate, higher concentrations of glyphosate can be applied, thereby potentially increasing the exposure level, and therefore exposure risk.[19]

Diquat and paraquat are applied on the leaves as a defoliant, desiccant, and herbicide. They pose a human health risk as Parkinson's disease has been linked to exposures to diquat and paraquat.[14-16, 21] Diquat is used more often in lakes and rivers to minimize aquatic weeds; whereas, paraquat is applied in no-till farming as a contact herbicide for grass and weeds. Additionally, paraquat blocks ferrodoxin thereby inhibiting photosynthesis and generating reactive oxygen species (ROS). Due to its toxicity, paraquat is restricted to commercial applications.

Table 1. Chemical structures of cationic pesticides

Cationic pesticide	Structure*
Trimethyl-sulfonium (TMS)	
Morpholine	
Mepiquat	
Chlormequat	
Diquat	
Paraquat	
ISTD d8-Paraquat	

*ChemSpider[22]

Here we demonstrate direct determinations of quaternary amine pesticides and morpholine in homogenized fruit and vegetable samples using cation-exchange chromatography with serial detection by suppressed conductivity and mass spectrometry. These techniques are demonstrated by HRAM-MS in full scan and Parallel Reaction Monitoring (PRM) data dependent MS2 (ddMS2).

This method was modified to achieve a fast 10 min analysis time using HRAM-MS and applied to the group of six cationic pesticides. Mepiquat, trimethylsulfonium, morpholine, and chlormequat exhibited good chromatographic resolution with Rs > 2. In contrast, diquat and paraquat with carbon isotopic masses within 2 *m/z* coeluted but were easily resolved in ddMS2 PRM mode by HRAM-MS. The six pesticides had good accurate mass, meeting the SANTE mass accuracy requirements of <5 ppm.[23] The homogenized food samples did not contain the native cationic pesticides of interest. Sensitivities were measured in the single digit µg/L or less range by spiking pesticides to the samples. Good accuracy was found, with recoveries of spiked in reagents in the standards and the samples within 80 to 120%.

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC™ High Pressure system with reagent-free ion chromatography (RFIC) capabilities, including:
  - Eluent generation capabilities
  - Column oven temperature control
  - Detector-Suppressor compartment temperature control
  - Tablet control
  - Consumable Device tracking
- CD Conductivity Detector
- Thermo Scientific™ Dionex™ AS-AP™ Autosampler with cooling option, 1 mL sample syringe
- Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap HRAM-MS with HESI II probe
- IC-MS Interface
  - IC-MS Installation Kit, P/N 22153-62049
  - Thermo Scientific™ Dionex™ AXP-MS Auxiliary pump (pump solvent for desolvation), P/N 060684
  - Dionex AXP Auxiliary pump (pump water for the suppressor regenerant), P/N 063973
  - Dionex Integrion Auxiliary 6-port valve option, P/N 22153-62027 used as an IC diverter valve

Table 2 lists the consumable products recommended for the Dionex Integrion HPIC system with RFIC capabilities configured for suppressed conductivity and mass spectrometry detections.

Table 2. Consumables list for the Dionex Integrion HPIC system and sample preparation

Product Name	Description	Part Number
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kits	Dionex IC PEEK Viper fitting assembly kit for the Dionex Integrion system configured for eluent generation and conductivity detection: Includes one each of P/Ns: 088815–088821	088798
Dionex AS-AP Autosampler items (plastic vials required for AMPA determinations)	1000 µL syringe	074307
	Vial Kit, 10 mL Polystyrene with Caps and Blue Septa, 100 each	074228
	Vial Kit, 1.5 mL Polypropylene with Caps and Septa, 100 each	079812
	100 µL sample injection loop	042951
Thermo Scientific™ Dionex™ EGC™ 500 MSA Eluent Generator cartridge	Eluent generator cartridge recommended for this application	075779
Thermo Scientific™ Dionex™ CR-CTC™ 600 Electrolytic trap column	Continuously regenerated trap column used with Dionex EGC MSA 500 cartridge and required for the Integrion IC system	088663
Thermo Scientific™ Dionex™ HP Degasser Module	Degasser installed after Dionex CR-TC trap column and before the Injection Valve. Used with eluent generation. Included with the Dionex Integrion IC, with RFIC capabilites	075522
Thermo Scientific™ Dionex™ CERS™ 500e suppressor	Dionex CERS 500e suppressor is the recommended suppressor for 2 mm i.d. columns	302664
Thermo Scientific™ Dionex™ IonPac™ CG17 column	Cation guard column, 2 × 50 mm	060563
Thermo Scientific™ Dionex™ IonPac CS17 column	Cation separation column, 2 × 250 mm	060561
IC-MS Installation Kit	IC-MS installation kit includes tubing, mixing tee, and Thermo Scientific™ Dionex™ SRD-10 Suppressor Regenerant Detector	22153-62049
0.2 µm syringe filters	Syringe filters used when samples require removal of particulates	09-740-113*
Centrifuge tubes	50 mL Centrifuge tubes	14-432-22*

*Fisher Scientific P/N

Software

Thermo Scientific™ Foundation 3.0 software

Thermo Scientific™ SII for Xcalibur with Thermo Scientific™ Xcalibur™ software or Thermo Scientific™ TraceFinder™ software.

Reagents and standards

Deionized (DI) water, 18 MΩ·cm resistivity (ASTM Type I water)[24], 0.2 µm

Fisher Scientific™ reagents

- Acetonitrile, Optima™ grade for desolvation, P/N A955-1
- Morpholine, ACS grade (MW = 87.122 g/mol; P/N M263-1)
- Alfa Aesar™ Trimethylsulfonium bromide, 98% (MW = 157.069 g/mol; P/N AAV2509814)
- Alfa Aesar 2-Chloroethyl)trimethylammonium chloride (chlormequat), (MW=158.066 g/mol; P/N AAA1563006)

Other reagents and standards

- Sigma-Aldrich™ Paraquat dichloride-(rings-d8) hydrate, (MW = 265.21 g/mol; P/N 50636-5mg)
- SPEX CertiPrep™ Paraquat dichloride, tetrahydrate (MW = 257.158 g/mol; P/N S2915)
- TCI America™, (1,1-Dimethylpiperidinium chloride (Mepiquat), 98%, (MW=149.662 g/mol, P/N AAA1563006)
- Ultra Scientific™, Diquat dibromide, >95% (MW = 344.05 g/mol, P/N US-PST-1410)

Ion chromatography conditions

Columns:	Dionex IonPac CG17 guard (2 × 50 mm) and IonPac CS17 separation (2 × 250 mm)
MSA eluent:	2 mM MSA equilibrate 4 min, 2 to 6.4 mM (0.1–2 min), 6.4 to 30 mM (2–5 min), 30 to 60 mM (5–7 min), 60 mM (7–9 min), 10 mM (9–10 min)
Eluent source:	Dionex EGC 500 MSA eluent cartridge, Dionex CR-CTC 600 trap column and high pressure degas module
Flow rate:	0.40 mL/min
Injection volume:	100 µL
Column temp.:	40 °C
Detection/ suppressor compartment:	15 °C
Detection 1:	Suppressed conductivity, Dionex CERS 500e suppressor, 2 mm, 47 mA, external water mode (delivered by a Dionex AXP-MS pump at 0.7 mL/min)
Conductance background:	<1 µS/cm
Conductance noise:	<1 nS/cm
System backpressure:	~3700 psi

Mass spectrometry conditions

IC-HRAM interface makeup solution:	Acetonitrile at 0.23 mL/min
Detection 2:	HRAM-MS, +ESI by HESI II probe
MS scan mode:	Full scan: 50–300 *m/z*, 30 k resolution, AGC 1e6, max IT 100 s
MS source:	Spray: 3.5 kV; S-Lens level, 50
MS N_2 gas flows:	Sheath: 40, Aux: 5; Sweep: 1 arbitrary units
MS temperatures:	Capillary: 425 ºC, Transfer 260 ºC
MS/MS mode:	PRM mode, 30 k resolution, AGC 2e5, max IT 100 ms, fixed first mass: 50.0 *m/z*, NCE 10-140 V Inclusion list
Run time:	10 min

Standard and sample preparation

Standard preparation

Individual 1000 mg/L stock standards were prepared from the reagent and 100 mL of DI water (morpholine: 100 ± 0.1 mg; trimethylsulfonium bromide: 201 ± 0.1 mg; mepiquat chloride: 131 ± 0.1 mg; chlormequat chloride: 129 ± 0.1 mg; diquat dibromide: 182 ± 0.1 mg; paraquat dichloride tetrahydrate: 225 ± 0.1 mg.)

A combined intermediate standard was prepared by diluting the stock standards with DI water. The intermediate standard was diluted sequentially with DI water to create the working standards.

To prepare a 10 mg/L spiking solution (used to spike 10 µL into 10 mL (10 µg/L) of sample or standard) of paraquat-d8 (paraquat-D8 dichloride hydrate, MW 256.21, 99%), first prepare a 10,000 mg/L solution by pipetting 329 µL of DI water into the 5 mg bottle of paraquat-d8. Cap and vortex the 5 mg bottle to mix thoroughly. Dilute an aliquot of the 10,000 mg/L standard, 1000-fold with DI water to 10 mg/L. Store at 6 °C.

Sample preparation

The homogenized food samples were prepared according to a simplified version of the EURL FV Quick Polar Pesticides Extraction (QuPPe)[10] method using methanol in place of acidified methanol. The original QuPPe method has acidified methanol which is more suited to HPLC and not needed for IC analysis methods.[26,27]

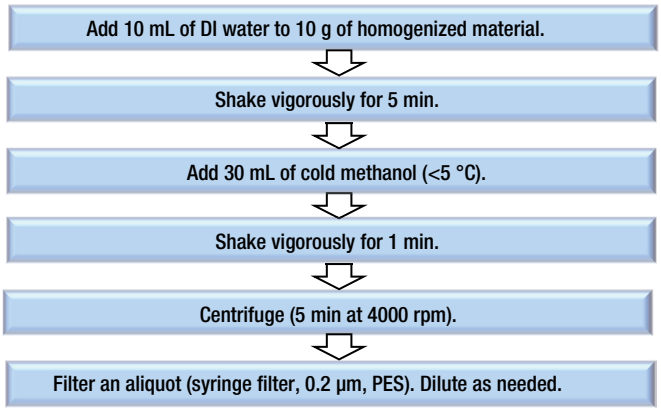


Figure 1. EURL-FV Quick Polar Pesticides extraction (QuPPe) method

Instrument setup and installation

IC system

Install the Dionex Integrion HPIC system with RFIC capabilites, autosampler, and the Dionex SRD 10 Suppressor Regenerant Device according to the flow diagram in Figure 2 and the instructions in Thermo Scientific Technical Note 72611 *Configuring and optimizing an IC-MS system using a compact IC and a single quadrupole mass spectrometer.*[28] Align the Dionex SRD-10 device tubing in the door slots in a manner that prevents crimping of the tubing and resultant backpressure and fatigue to the suppressor. The instructions to test the Dionex SRD-10 device and to reset it after being triggered are in TN72611.

Minimize the length of tubing from the IC to MS by positioning the IC near the MS and by removing any excess red PEEK (0.005 i.d. in, 0.127 i.d. cm) tubing from the Eluent Out port of the suppressor to the MS. For the following consumables to be detected in the Instrument Configuration, connect the cables of the eluent generator cartridge, electrolytic trap column, suppressor, and SRD-10 device into their positions in the IC system.

Configuring the modules in Xcalibur with SII for Xcalibur software

To configure the IC system using SII for Xcalibur software, first close all Xcalibur programs. Open the Configuration program (gear symbol), select the SII for Xcalibur module, select configure, and then add the IC modules. Table 3 shows the summarized instructions. For more detailed instructions, refer to the IC-MS Installation Guide, and operator and product manuals.[29-32] Add these five modules to the IC instrument configuration: Integrion HPIC system (Integrated IC systems), Integrion HPIC Pump Wellness, Dionex AS-AP Autosampler, and AXP auxiliary pump (renamed here in configuration as Pump_ER) and an AXP-MS auxiliary pump (renamed Pump_S).

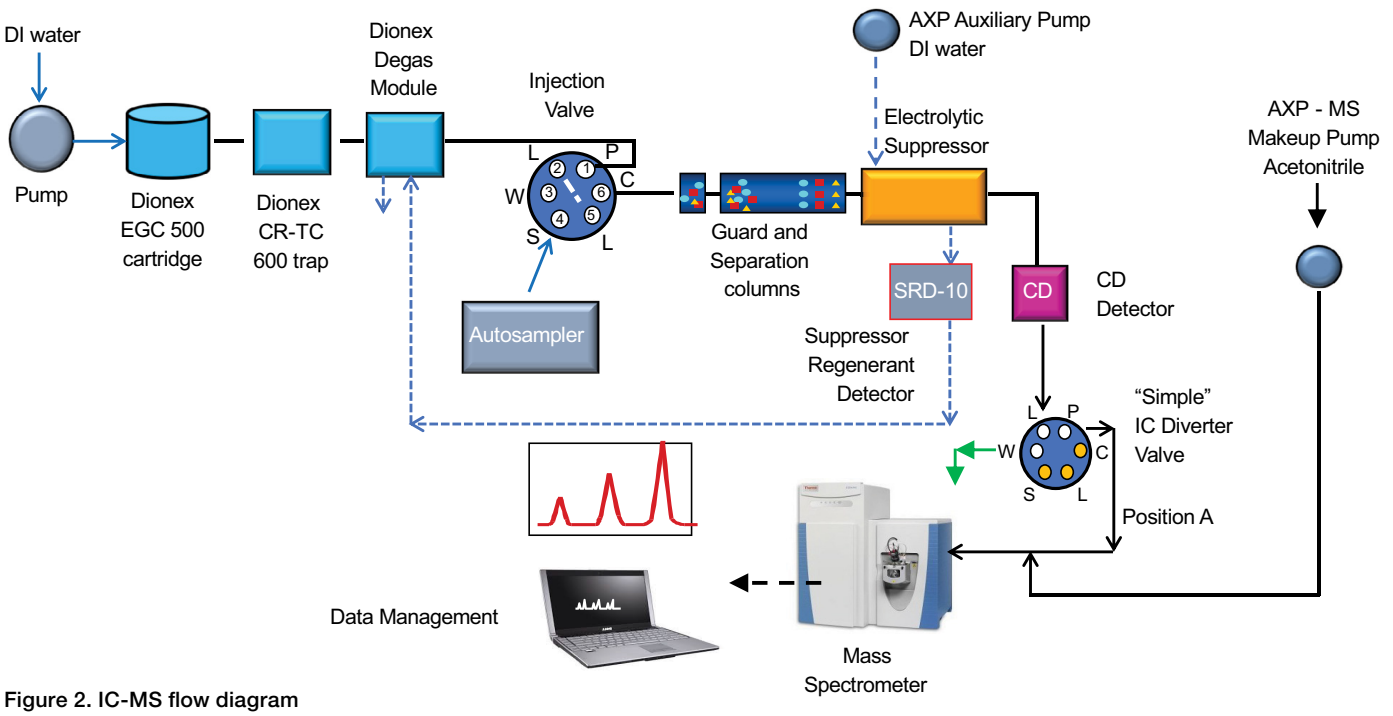


Figure 2. IC-MS flow diagram

Table 3. Summary of system configuration for Dionex Integrion HPIC system with RFIC capabilities in SII for Xcalibur

Tab	Action	Result
Dionex Integrion HPIC module*		
Inject Device	Double left-click on HP_Valve	Opens the device configuration information on the injection valve
	Select Integrion	Changes control of the injection valve from the autosampler to a programmed command by the Integrion IC
TTL Inputs used to activate the SRD-10 device	Click on the box next to TTL_Input 1	*Activates TTL_Input 1. The other TTL channels are not needed for this application. (Figure 3)*
	Double left-click on TTL_Input 1	*Opens Device Configuration box (Figure 3)*
	Select Pump On	Directs (IC) Pump to turn-off when this channel is activated (If the suppressor regen flow ceases for >5 min) *(Figure 3)*
	Select Inverted edge mode	
Pump Wellness module		
	Select box	Monitors IC pump pressure as a channel
Add Dionex AS-AP Autosampler		
	Options	Select syringe size and vial types in the carousel. Enter sample loop volume.
AXP Auxiliary Pump to dispense DI water through suppressor Regen In channel		
General	Rename Device Name to Pump_ER	Name pump with a unique name. (Pump used for eluent regenerant, Pump_ER)
	Select COM Port from drop down menu	Typically, COM5, COM6, COM7**
Pump Type	Select pump type	AXP, Press OK.
AXP-MS Auxiliary Pump to dispense desolvation solvent to mixing tee		
General	Rename Device Name to Pump_S	Name pump with a unique name. (Pump used for solvent, Pump_S)
	Select COM Port	Should be a different COM channel than the AXP pump
Pump Type	Select pump type	AXP-MS, Press OK.

*The configuration program automatically detects CD detector (Detector), the Dionex eluent generator cartridge (Electrolytics), Dionex CR-TC 600 trap column, and suppressor.
**To identify available COM ports, open the computer's Device Manager program. The AXP pump communication ports will be listed as USB COM ports.

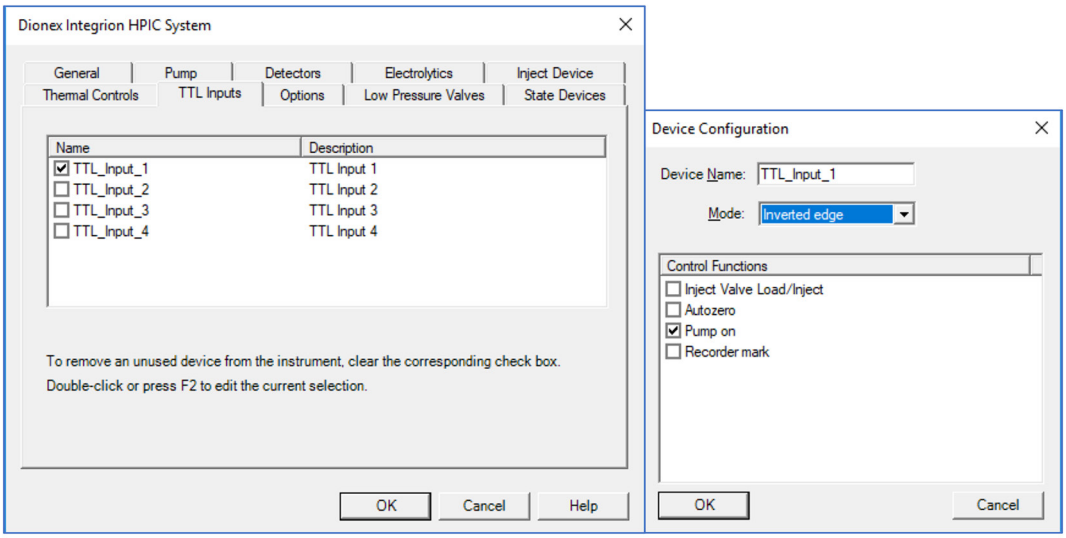


Figure 3. Configuring the Dionex SRD-10 Device

To activate the SRD-10 device in the configuration, select the TTL tab in the Integrion HPIC module, click on TTL_Input_1, and Pump on. (Figure 3). Additional information can be found in the IC-MS Installation guide and the product manual.[29,30] Add the Q Exactive module to the instrument configuration if it is not already added.

Conditioning electrolytic devices and columns

Important: Do not remove consumable tracking tags on the columns and consumable devices. These tags are required for consumables monitoring functionality.

Hydrate and condition the Dionex EGC 500 Eluent Generator Cartridge and Dionex CR-CTC 600 Continuously Regenerated Trap column according to TN72611, product manuals, or the instructions in the drop-down menu (Consumables, Conditioning on the SII for Xcalibur Instrument Console panel after accessing direct control). Condition the columns 30 min according to the instructions found in the same resources.

Hydrate the Dionex CERS 500e suppressor for 10 min according to the startup instructions in the device box and product manual. Temporarily install tubing from the Eluent Out port to the Regen In port. Pump water or eluent (<10 mM) at the application flow rate or lower to the Eluent In port for 10 min with the suppressor off. Allow the suppressor to sit another 20 min without flow to complete the hydration. Install the suppressor according to flow diagram in Figure 2. As a precaution, do not turn on the suppressor until liquid flow is observed flowing out of each stage of the flow path: the Dionex AXP Auxiliary Pump, the suppressor Regen Out port, and the Dionex SRD-10 device.

IC-HRAM interface

A properly functioning electrolytic suppressor is a critical part in the IC-MS interface as it neutralizes the acidic mobile phase thus producing low chemical noise for the IC and the MS to deliver quality results. In all IC-MS applications, it is important to minimize the backpressure exerted on the suppressor to <150 psi.

In addition to excessive red PEEK tubing, the mixing tee and MS probe can also cause backpressure from restricted flow. At the end of each day when the sequence is complete, it is a good practice to flush aqueous-soluble solvent (water or methanol) through the mixing tee and MS probe to minimize salt buildup. It is also a good practice to verify that the flow is not restricted through the mixing tee prior to starting an analysis.

Additional items are recommended for IC-MS applications in event that the suppressor fails to neutralize the eluent:

1. The Dionex SRD-10 device
2. A conditional trigger activating at high conductivity embedded in the instrument method
3. A Virtual Channel to monitor the voltage of an ERS-type suppressor imbedded in the instrument method

Dionex SRD-10 device

The suppressor in a running IC will be unable to neutralize the eluent if suppressor regenerant ceases flowing through the suppressor. The Dionex SRD-10 device monitors air/bubbles in the regenerant flow tubing. It turns off the Dionex Integrion pump (TTL connections and the configuration settings) after measuring an absence of flow for >5 min. It functions independent of the IC, and therefore is independent of the instrument method or sequence.

High conductivity trigger

If the suppressor backpressure is >150 psi, the suppressor will eventually be damaged, resulting in un-neutralized eluent and higher than expected total conductivity. A conditional trigger (high total conductivity (>50 µS/cm) for 180 s) can be programmed in the instrument method to minimize the un-neutralized eluent entering the MS by initiating commands to stop the IC system or rotate the diverter valve to waste. To create a high conductivity trigger, open an instrument method, select the Script Editor, and insert a conditional trigger on the Start Run line (Figure 4).

Enter the commands to turn off the IC pump (System. StopFlow), and the auxiliary pumps (Pump_ER.State and Pump_S.state. Set the state to “off”.) (Figure 5)

Insert the high conductivity trigger into all IC-MS Instrument Methods. These conditions and commands are specifically for the Dionex Integrion HPIC system. Other systems have slightly different commands. The trigger is active when the instrument program is active during a running sequence.

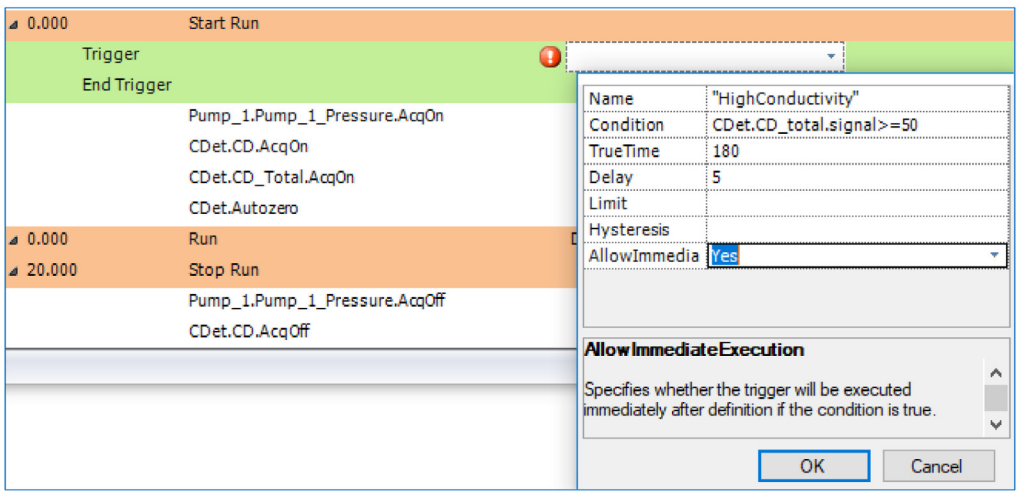


Figure 4. Creating the HighConductivity trigger in Script Editor

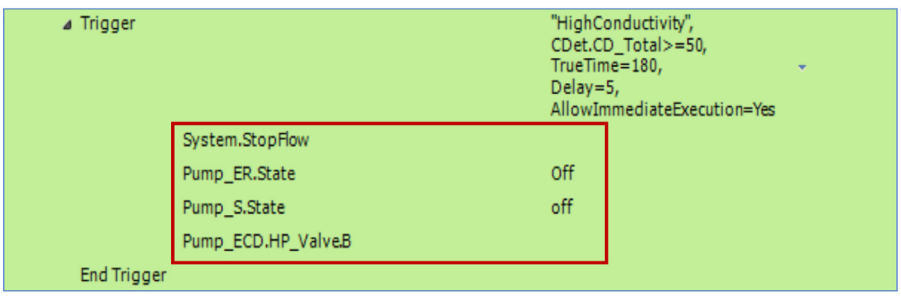


Figure 5. Completing the HighConductivity trigger to initiate commands

Virtual Channel

A Virtual Channel can be created to monitor the Dionex CERS 500e suppressor voltage and to provide guidelines when the suppressor should be cleaned. (Typically, when the voltage is 1 V greater than the initial voltage of the new suppressor.) To create a Virtual Channel of the suppressor voltage, follow the instructions in the IC-MS Installation Guide, and open the IC instrument method and the Script Editor. Insert a command (inserts a line) after the Start Run command and type in VirtualChannel. Tab to the right and click on the arrow to bring up the VirtualChannel window. Name the channel "SuppressorVoltage", enter in the formula (electrolytics.suppressor.voltage), and select digital, enter "V", and select No for the type, unit, and evaluate parameters (Figure 6).

Contamination

To minimize contamination for IC-MS applications one must consider both typical IC and MS contamination concerns. For example, use a glass bottle for the acetonitrile, use plastic vials for samples, use only Optima grade solvents, and rinse water eluent containers with methanol and then several times with DI water. For more detailed information, refer to TN72611 and the IC-MS Installation Guide.[28,29]

Consumables Device Tracking

The Consumables Device Tracking feature monitors and logs information of the installed consumable products (that have device tracking tags or wires) and warns if incompatible consumables are installed, such as anion suppressor with a cation-exchange column.

Consumables Device tracking requires a review and approval of the consumable devices prior to starting the sequence. Typically, a response is needed if new consumables have been installed, the IC instrument doors have been opened and closed, or there is a warning message. To approve the consumables and release the hold by the consumables monitoring, follow the instructions in TN72611 by opening direct control of the IC system, accessing the Consumables Tracking, approving the inventory, and releasing control. After approving the consumables, the sequence can be started or restarted.

Results and discussion

Cationic pesticide determinations were previously demonstrated by IC-MS/MS using an electrolytically generated shallow MSA gradient from 1 to 40 mM at 0.40 mL/min and 40 °C on the Dionex IonPac CS17 cation-exchange column with a total run time of 20 min.[11] The authors selected the IonPac CS17 column for the pesticide application because of the selectivity for hydrophobic and multivalent amines. The method had good chromatographic resolution of all cationic pesticides of interest except for diquat and paraquat and because they had similar characteristics also could not be resolved by MS/MS.

In this application, the previous method was modified by increasing the gradient slope (2 to 60 mM MSA over 8 min), resulting in a 10-min run time for the six cationic pesticides. The analytes were detected by high-resolution, accurate-mass spectrometry detection, which was able to resolve diquat and paraquat.

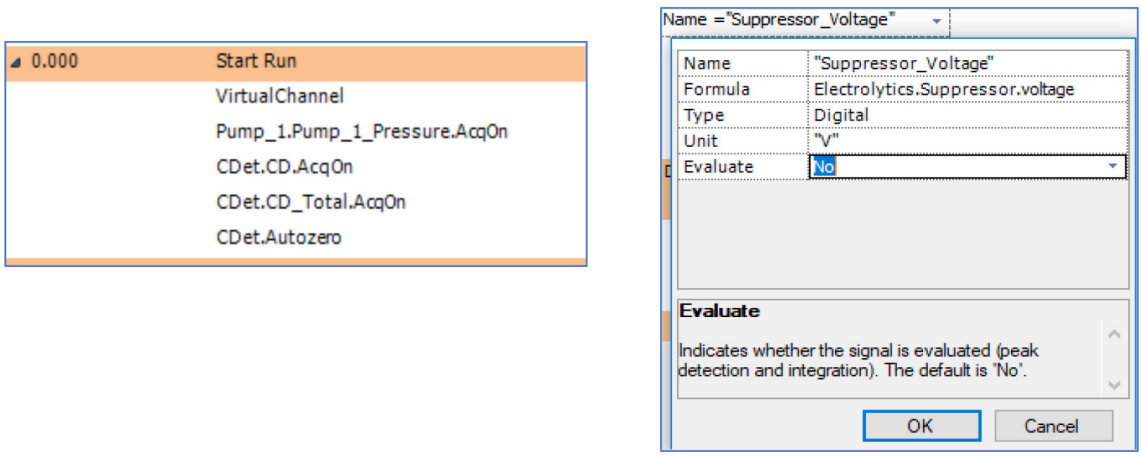


Figure 6. Creating a Virtual Channel to monitor voltage in the ERS type suppressor

To optimize the MS conditions, the ion source conditions were optimized by infusing a 10 ppm standard of morpholine at 30 µL/min with the suppressed eluent flowing from the IC at 0.4 mL/min combined with acetonitrile desolvation solvent at 0.023 mL/min. Figure 7 shows the cationic pesticides (1 mg/L) in full scan mode using the optimized IC-MS conditions.

To optimize the normalized collision energy (NCE) and determine the confirming ions in ddMS, PRM mode conditions, a 10 ppm standard of each analyte was again infused with the suppressed eluent and acetonitrile. The optimized PRM mode conditions are shown in Table 4.

Figure 8 shows the PRM chromatograms of the 10 µg/L of six cationic pesticides of interest.

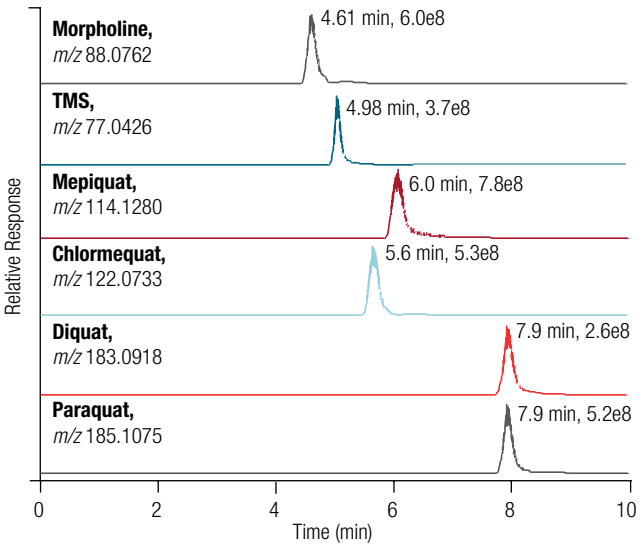


Figure 7. Full Scan MS chromatograms

Method evaluation

To evaluate this method, the mass accuracy, limits of detection (SANTE 20% RSD), and response to concentration via calibration curves were determined in PRM mode. Mass accuracy is defined as the difference between theoretical and measured *m/z*. Table 5 shows that the HRAM results (-0.4 to 1.1 ppm) are well within the SANTE limits of <5 ppm. Paraquat and d8-paraquat had the least mass accuracy, 1.1 and 3.0 ppm, respectively. These results show that the economical Q Exactive Focus mass spectrometer can meet the SANTE mass accuracy requirements. The Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap mass spectrometer is recommended if greater mass accuracy is needed. The peak area response to concentration was evaluated using seven calibration standards (0.2, 0.5, 1.0, 2.0, 5.0, 10, 20 µg/L) spiked with 1.0 µg/L of ISTD with triplicate injections. The results showed coefficients of detemination (r^2) >0.98 for the six cationic pesticides. Limit of detections, defined as 20% RSDs (SANTE23) ranged from 0.1 to 1.1 µg/L.

Table 4. PRM mode conditions

	Formula	Ret. Time (min)	Accurate Mass (*m/z*)[24]	PRM Confirming Ions (*m/z*)	NCE (V)	PRM Window
Morpholine	$O(CH_2CH_2)2N^+$	4.84	88.0764	70.0658 68.050	140	3-6
Trimethylsulfonium (TMS)	$C_3H_9OS^+$	5.18	77.0426	62.019 61.011	50	3-6
Mepiquat	$C_7H_{16}N^+$	6.31	114.1283	98.0985 58.0659	70	3-6
Chlormequat	$C_5H_{13}N^+$	5.91	122.0736	58.0659 59.0737	120	3-6
Diquat	$C_{12}H_{12}N_2^{+2}$	8.04	183.0920	157.076 143.081	50	6-9
Paraquat	$C_{12}H_{14}N_2^{+2}$	8.05	186.1146	171.0915 144.0807	30	6-9
ISTD Paraquat-d8	$C_{12}H_6D_8N_2^{+2}$	8.05	194.1663 97.0832	77.0091 79.0065	50	6-9

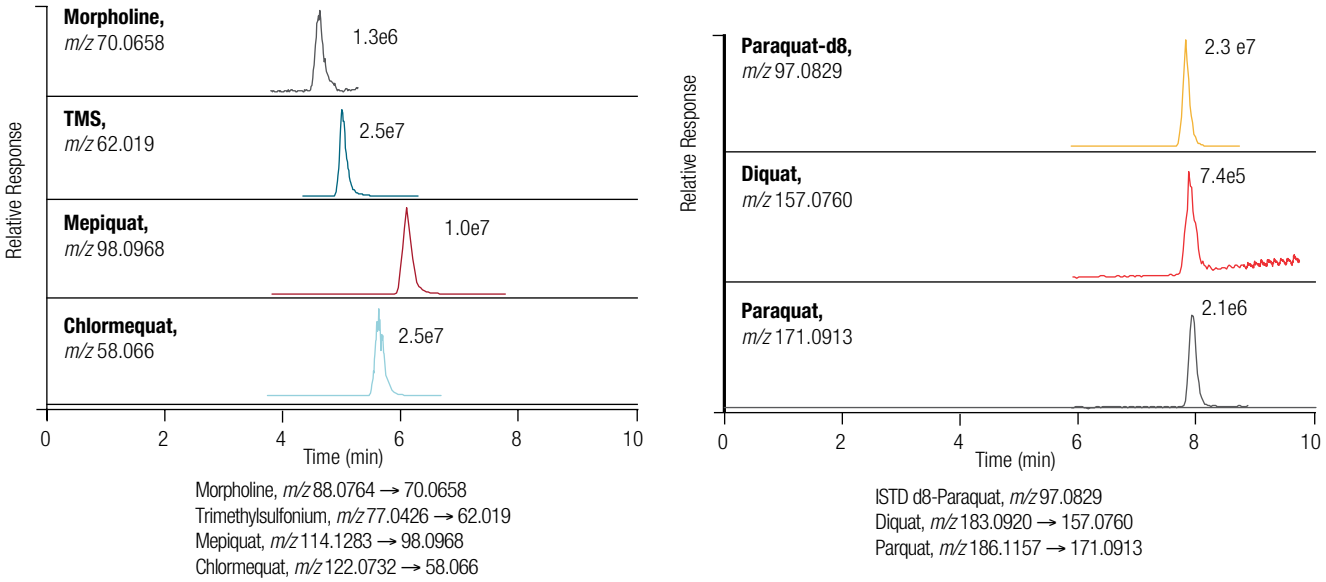


Figure 8. PRM mode chromatograms

Table 5. Summary of accurate mass determinations, coefficient of determination, and LOD results

	Confirming	Accurate Mass (*m/z*)			Coef. of Determination (r^2)	LOD (µg/L)
		Reference	Measured	Accuracy (<5 ppm)**		
TMS*	62.019 61.011	77.0043	77.0043	–	0.989	0.14
Morpholine	70.066 68.050	88.0764	88.0763	-1.0 ppm	0.989	0.46
Mepiquat	98.097 58.066	114.1283	114.1280	-0.3 ppm	0.986	0.097
Chlormequat	124.070 58.066	122.0736	122.0736	–	0.987	0.089
Diquat	157.076 143.081	183.0920	183.0917	-0.3 ppm	0.980	1.1
Paraquat	185.107 171.092	186.1146	186.1157	+1.1 ppm	0.980	0.47
ISTD d8-Paraquat	97.5844 96.5797	97.0832	97.0829	-3.0 ppm	–	–

*Trimethylsulfonium (TMS)
**SANTE accuracy limit of <5 ppm[23]

Sample analysis

The cationic pesticides were determined in the 10-fold diluted aqueous phase (simplified QuPPe extraction) of the homogenized fruit and vegetable samples. Accuracies were determined by determining the recovery of 10 µg/L mixed standards spiked into the diluted and extracted homogenized food samples. Trace levels of TMS, morpholine, and mepiquat were found in the green bean sample, 0.22, 0.15, and 0.23 µg/L, respectively. Similar results for morpholine and TMS were found in the apple sample, 0.19, and 0.17 µg/L, respectively. Paraquat and diquat were not detected in any of the neat samples. Recoveries were acceptable for the green bean, pear, and apple samples, 80–120%, however low recoveries were obtained from the squash sample (Table 6). Figure 9 shows the PRM chromatograms of green bean sample with and without 10 µg/L added standards.

Diquat and paraquat were resolved using the high-resolution, accurate-mass capabilities of the Q Exactive mass spectormeter. To further test interfering ion suppression of the ions on each other, 20 µg/L of paraquat and diquat was added to the diluted green bean and pear extracts. Figure 10 shows that HRAM can fully resolve diquat and paraquat in the diluted, extracted pear sample using the confirmatory ions. The responses were comparable and therefore not suppressed.

Table 6. Summary of recovery results of 10 µg/L mixed standards

	Green Bean (%)	Pear (%)	Squash (%)	Apple (%)
Trimethylsulfonium (TMS)	102	97.4	78.5	101
Morpholine	105	99.0	79.1	107
Mepiquat	102	87.8	77.2	98.4
Chlormequat	97.1	92.2	70.6	99.3
Diquat	93.1	99.9	73.2	99.8
Paraquat	98.6	97.8	72.7	101

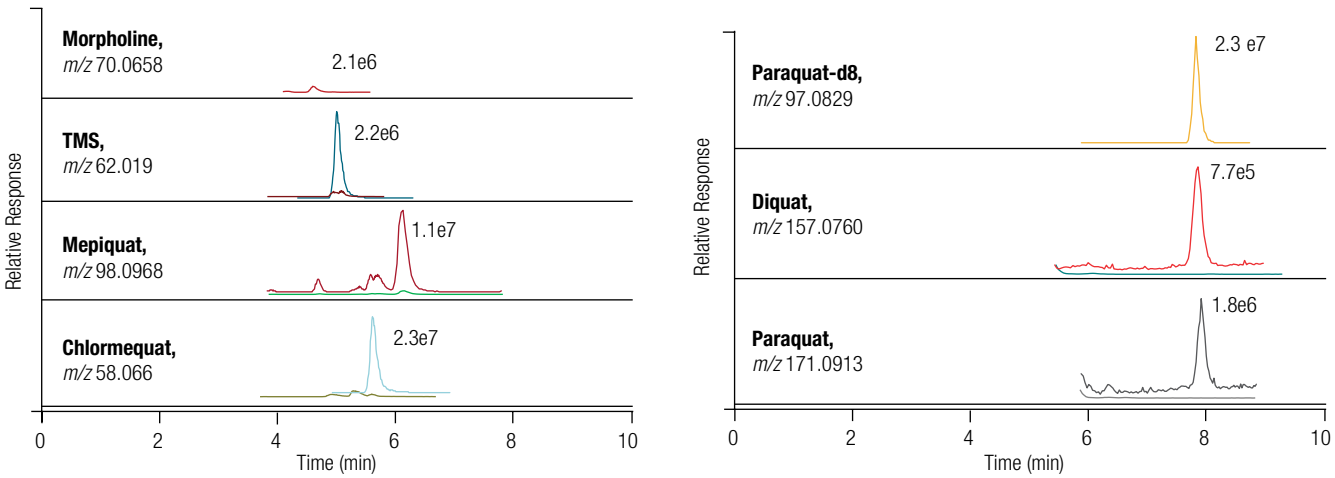


Figure 9. PRM mode chromatograms of 10 µg/L pesticides in 10-fold diluted, QuPPe extracted green bean sample

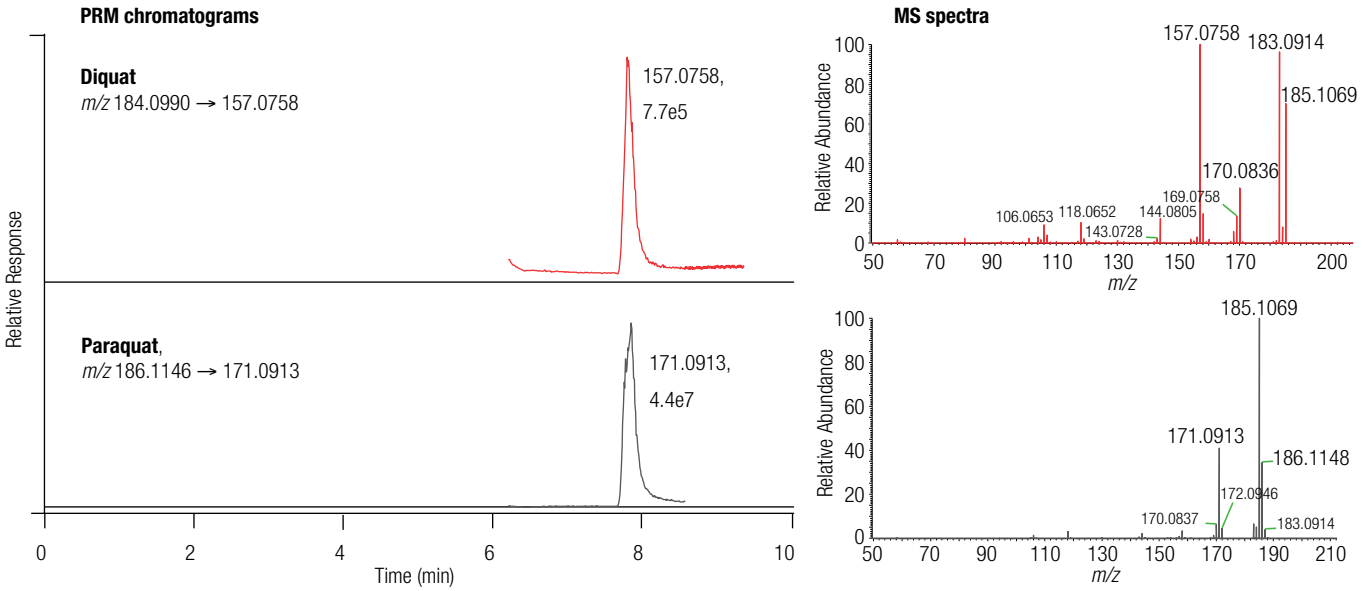


Figure 10. HRAM resolution of 20 µg/L diquat from paraquat in 10-fold diluted QuPPe extracted pear sample

Conclusion

The determinations of six cationic pesticides using IC-HRAM spectrometry were demonstrated in diluted, extracted homogenized food samples.

- In these experiments, the ddMS Parallel Reaction Monitoring mode used to extract ions of interest from the matrix was effective for qualitative and quantitative determinations in homogenized food samples.
- The IC method demonstrated high accuracy (80% recoveries for most of the samples, with the exception of squash) and sensitivity (LOD <1.1 µg/L). The squash sample exhibited recoveries <80%.
- The HRAM capability easily resolved co-eluting diquat and paraquat using the confirmatory ions, without any indication of suppression.
- Additionally, a simplified Quick Polar Pesticide (QuPPe) extraction method was demonstrated for multiresidue determinations of cationic pesticides.
- This application note and other applications can be found in the Thermo Fisher Scientific Appslab Digital Library.[33]

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