APPLICATION NOTE 73339

Determination of anionic polar pesticides by ion chromatography with serial detection by suppressed conductivity and mass spectrometry

Authors: Terri Christison and Jeff Rohrer
Thermo Fisher Scientific, Sunnyvale, CA

Keywords: AMPA, Dionex IonPac AS19-4µm column, glyphosate, IC-MS, Reagent-Free IC, RFIC, single quadrupole mass spectrometer

Goal

Demonstrate fast determinations of anionic polar pesticides using ion chromatography and single quadrupole mass spectrometry

Introduction

Food safety and perceived health risks from residual agricultural chemicals are ongoing public concerns, and these chemicals are under increasing regulatory scrutiny. One category is the polar pesticides, which include ionic post-emergent and desiccant herbicides, fungicides, growth-regulating chemicals, and the resultant metabolites of these compounds. Much of the attention is focused on glyphosate because of its extensive use and association with crops and seeds genetically modified to tolerate glyphosate.

Glyphosate, its metabolite aminomethylphosphonic acid (AMPA), naturally occurring glufosinate, its metabolite 3-[hydroxy(methyl)phosphoryl]propanoic acid (3-MPPA), and bialaphos, which has glufosinate as a metabolite, are broad-spectrum herbicides applied to prevent emerging weeds on fallow ground. These herbicides are also commonly used as a desiccant to hasten the drying of plants and grains prior to harvest. This application as a desiccant can result in residual contamination in food.[1,2] In addition, crops genetically modified for increased glyphosate tolerance (alfalfa, canola, corn, cotton, sorghum, soybeans, sugar beets, and wheat) are likely to have more concentrated and more frequent herbicide applications.[1–6] As a result, higher residual contamination would be expected in these GMO crops. Glyphosate has been detected in German wheat and pilsner beer samples,[7] U.S. corn and soybeans,[8,9] and U.K. oat cereals[10,11] though in µg/L or µg/kg (ppb) concentrations, well below the 30 mg/kg residue limits (U.S.).[12] The occurrences of these residual concentrations were independent of the crop's GMO status.

Most polar pesticides have relatively low toxicity as compared to the previous generation of herbicides and pesticides, such as DDT or 2,4,5-T. However, the toxicology of glyphosate is controversial and complicated by epidemiology studies including occupational exposures to multiple environmental chemicals. In 1991 and 1999, the Food and Agricultural Organization and World Health Organization (FAO/WHO), using animal studies, ruled that glyphosate was “unlikely to be a carcinogen”.[13,14] In 2015, the European Food Safety group and the U.S. Environmental Protection Agency (EPA) using a subset of epidemiology cases concluded that glyphosate was “unlikely to be a carcinogen”.[13] The WHO International Agency for Research on Cancer (IARC) using a different subset of epidemiology studies ruled that glyphosate was “likely a carcinogen”.[14] In 2017, along with the ruling by IARC, but in contrast to the U.S. EPA ruling, the U.S. State of California Office of Environmental Health Hazard Assessment (OEHHA) ruled that glyphosate should be added to the Proposition 65 list of compounds known to be carcinogens.[15] As of August 2019, glyphosate toxicity is currently in dispute between the two U.S. agencies.[16,17] The 2011 limit set by the U.S. EPA for residual contamination in food is 0.1 mg/kg.[18] In the EU, the Maximum Residue Limit (MRL) varies per food, but it is typically 0.1 mg/kg.[6] The long term (chronic) exposure assessment showed that the estimated exposure did not exceed the acceptable daily intake values (ADI) for any of the tested pesticides.

Also included in the polar pesticides’ category are the fungicides fosetyl aluminum and methyl bromide, and the growth regulator ethephon. Fosetyl aluminum is commonly applied to horticulture crops or as a dip treatment to prevent cross-contamination by transplanted plants. Methyl bromide is applied to strawberries to inhibit spoilage due to fungal contamination. Ethephon is applied pre-harvest to shorten ripening time in wheat, coffee, tobacco, cotton, and rice, and post-harvest to inhibit rooting in seed potatoes and greening in mature pineapples.[5,6] In 1996, the U.S. EPA set the recommended maximum ADI to 0.1–0.5 mg per kg body weight per day, which remains unchanged in 2019.[5]

Although determinations of ionic pesticides have been previously performed directly by LC-MS and indirectly (after derivatization) by GC-MS, ion chromatography (IC) with mass spectrometry is more suitable because these pesticides and their metabolites are ionic. Determinations of cationic and anionic pesticides have been previously demonstrated using IC with tandem MS (IC-MS/MS)[19–25] and high-resolution accurate MS (IC-HRAM),[26,27] however it has not been demonstrated using a single quadrupole MS. An IC system coupled to an economical and simple-to-use single quadrupole MS (IC-MS) can be used to screen for and confirm the presence of ionic pesticides. In addition to the polar pesticides, other ionic contaminant classes, such as municipal disinfection byproducts, can be determined at the same time.

In this application note, fast determination of multiple anionic pesticides and disinfection byproducts by IC-MS is demonstrated. All analytes of interest elute within 14 min and are detected serially by suppressed conductivity and mass spectrometry with sensitivity down to µg/L in full scan mode and selected ion monitoring mode (SIM).

Experimental

Equipment

Ion chromatography

- Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system*
  - Dual Pump DP module, isocratic configuration. The second pump is used to deliver suppressor regenerant.
  - Eluent Generator EG module
  - Detector Chromatography DC module with a second 6-port injection valve (P/N 075917) used as a diverter valve
  - CD Conductivity Detector (P/N 079829)

*or a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (RFIC model) with two injection valves, CD Detector, and a Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump, (P/N 060684).

- Thermo Scientific™ Dionex™ AS-AP autosampler with temperature control and 100 µL syringe (P/N 074305)

Table 1. Consumables list for the Dionex ICS-6000 HPIC System

Product name	Description	P/N
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kit	Dionex IC Viper fitting assembly kit for 2mm Dionex ICS-6000 HPIC system with CD	302965
Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge	Anion eluent generator cartridge for HPIC high pressure systems	075778
Thermo Scientific™ Dionex™ CR-ATC™ 600 Continuously Regenerated Anion Trap Column	Trap column used with Dionex EGC KOH 500 cartridge on Integrion and ICS-6000 HPIC systems	088662
Thermo Scientific™ Dionex™ HP EG Degasser module	Degasser installed after Dionex CR-TC trap column and before the injection valve, used with eluent generation	075522
Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor	Anion suppressor for polar pesticides application using 2 mm i.d. columns	SP6948
Thermo Scientific™ Dionex™ IonPac™ AG19-4µm Guard column	Anion guard column, 2 × 50 mm	083225
Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical column	Anion analytical column, 2 × 250 mm	083223
Thermo Scientific™ Dionex™ AS-AP Autosampler vial kit options (Glass vials should not be used)	0.3 mL polypropylene, package of 100 vials and caps (includes insert)	055428
	1.5 mL polypropylene, package of 100 vials and caps	079812
	10 mL polystyrene, package of 100 vials, caps, and septa	055058
ISQ EC Mass spectrometer calibration solution	Replacement Calibrant Solution, 250 mL	1R120590-6204
Thermo Scientific™ Dionex™ IC PEEK Viper™ sample loop	5 µL PEEK Viper sample loop	302897
ISQ EC HESI II needle	Replacement HESI II needle	80000-60317
IC-MS Installation kit	Contains fittings and tubing for IC-MS applications	22153-62049
Syringe filters	Syringe filters suitable for IC, 0.45 µm, PES	Fisher Scientific 725-2545

Mass spectrometry

- Thermo Scientific™ ISQ EC™ single quadrupole mass spectrometer
  - Thermo Scientific™ syringe pump (P/N 1245740) for method optimization (optional)
  - Thermo Scientific™ HESI-II probe (P/N 80000-60317, included with ISQ EC)
  - IC-MS Installation Kit, P/N 22153-62049
- Nitrogen generator with capacity for 3 L/min flow at 100 psi (110 V: P/N 1R77606-1120; 230V: P/N 1R77606-1230)

Table 1 lists the consumable products needed for the Dionex ICS-6000 system.

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software 7.2 with MS driver of ISQ EC

Reagents

- ASTM Type 1 deionized water (DI water) with 18 MΩ·cm resistivity[28]
- A fifteen-component standard mix (QPP-Lab STANDARD MIX 1.3, 5 mg/L, P/N CRM3G11L346) is available as a kit from Lab Instruments: http://www.labinstruments.org/en/qpp-lab/.

Chem Service, Inc.:

- Fosetyl-aluminum (fosetyl-Al), technical grade, 250 mg, (CAS# 39148-24-8, EC# 254-320-2), P/N N-12019-250mg

Fisher Scientific™:

- Cyanuric acid, ACS Certified, (CAS# 108-80-5, EC# 203-618-0), P/N AAA1544722
- Glufosinate, ammonium, 100 mg, (CAS# 77182-82-2; EC# 278-636-5), P/N NC1044687
- Sodium bialaphos, Alfa Aesar™, 85%, (CAS# 71048-99-2), P/N AAJ66802MC
- Sodium bromate, ACS Certified, P/N S253-500
- Sodium bromide, crystalline, ACS Certified, P/N S255-500
- Sodium chlorite, J.T. Baker, ACS Certified, P/N 02-004-050

IC-MS conditions			
Columns	Dionex IonPac AG19-4µm guard (2 × 50 mm) and IonPac AS19-4µm separation (2 × 250 mm)		
KOH gradient	15 mM KOH (-1 to 0.2 min), 15–20 mM (0.2–4 min), 20–55 mM (4–7.5 min), 55–57 mM (7.5–9 min), 57–75 mM (9–10 min), 75 mM (10–11.5 min), 15 mM (11.6–14 min)		
Eluent source	Dionex EGC 500 KOH eluent cartridge, Dionex CR-ATC 600 trap column and high pressure degas module		
Flow rate	0.375 mL/min		
Injection volume	2 µL, partial loop injection of 5 µL loop, 5 µL cut volume		
Temperatures	Column: 40 °C, Detection/Suppressor Compartment: 15 °C, Autosampler: 20 °C		
Detection 1	Suppressed conductivity, Dionex ASRS 300 suppressor, 2 mm, 65 mA, constant current and external water modes, 0.5 mL/min DI water for regenerant by the second pump		
IC background	<1 µS/cm		
IC noise	<1 nS/cm		
IC-MS system backpressure	~3100 psi (1000 psi = 6,895 MPa)		
IC-MS run time	15 min		
Inject valve 2 (as an IC diverter valve)	*Timing (min)*	*Valve position*	
	-1	DC.InjectValve_2.InjectPosition	
	0, start run	DC.InjectValve_2.LoadPosition	
	19.8	DC.InjectValve_2.InjectPosition	
	20.0, stop run	No additional commands	
Detection 2	ISQ EC mass spectrometer, -ESI, -2500 V, full scan 60–300 *m/z*, CID: 10 V		
Flow (N_2)	Sheath: 40 psi, Aux: 2 psi, Sweep: 1.0 psi		
MS temperatures	Vaporizer: 390 °C, Ion transfer tube: 350 °C		
Chromatography width, SIM filter	10 points at 15 s, 0.3 amu		
Desolvation solution	None		
SIMs	*Anion*	*Mass m/z*	*SIM window (min)*
	AMPA	110.0	11–14
	Bialaphos	322.1	7–9
	Bromate	126.9	3–6.5
	Bromide	78.9	7–9
	Chlorite	66.96	3–6.5
	Cyanuric acid	128.0	11–14
	Fosetyl	63.0	3–6.5
	Glyphosate	169.1	7–14
	Glufosinate	180.1	7–9
	3-MPPA	151.0	11–14
CID (V)	10 V for most anions		

Millipore Sigma:

- Ethephon, PESTANAL®, analytical grade (CAS # 16672-87-0, EC# 240-718-3), P/N 50-185-7392

- 3-[hydroxy(methyl)phosphoryl]propanoic acid (3-MPPA), PESTANAL, analytical grade (CAS# 15090-23-0, EC# 239-144-6), P/N 31264

Cambridge Isotopes Laboratories, Inc.:

- Glyphosate unlabeled, 100 µg/mL in water (CAS# 1071-83-6, EC# 213-997-4), P/N ULM-6876-1.2

- Glyphosate, 95%, labeled, (13C3, 99%; 15N, 98%) 100 µg/mL in water, P/N CNLM-6792-1.2

- Aminomethylphosphonic acid (AMPA) unlabeled, 100 µg/mL in water (CAS# 1066-51-9), MW=173.04, P/N ULM-10880-1.2

- AMPA, labeled (13C, 99%;15N, 98%;methylene-D2,98%), 100 µg/mL in water, MW=115.04, P/N CDNLM-6786-1.2

Standard and sample preparation

Standards

Mixed working standards, 10, 50, 100, 500, 1000, and 5000 µg/L of 10 pesticides and disinfection byproducts were prepared from individual stock standards or the pesticide kits. Glyphosate and glufosinate standards were prepared daily.

Samples

The samples were prepared using a simplified Quick Polar Pesticide Extraction method (Figure 1). The supernatant portions of the extracted samples were diluted 2-fold and 5-fold with DI water. The supernatant of the pea sample was filtered before dilution. A portion of each diluted extracted sample was spiked with 100 µg/L standard with 50 µg/L glyphosate ISTD for recovery experiments.

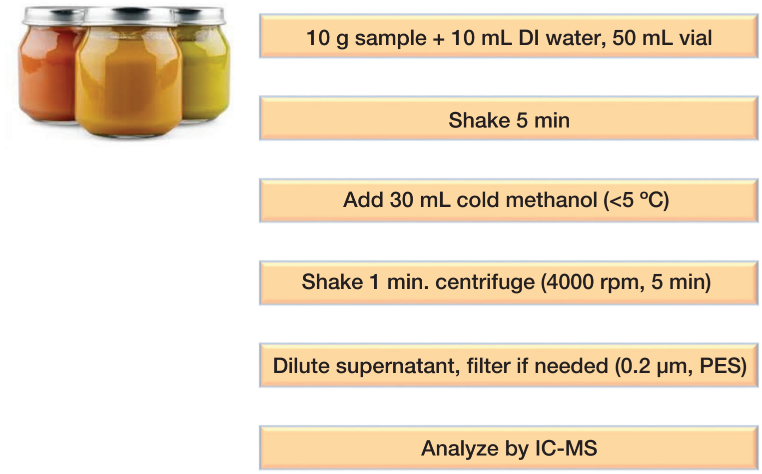


Figure 1. Simplified EURL-FV Quick Polar Pesticide (QuPPe) Extraction Method

Instrument setup and installation

Physical and electronic configuration

The application runs on System 1 with the second pump (Pump 2) and second injection valve (Valve 2) facilitating the flow to the suppressor regenerant (Load mode) and the MS (Inject mode) (Figure 2). Valve 2 in the Inject position directs the effluent exiting the CD detector to the ISQ EC mass spectrometer and directs the Pump 2 flow of ASTM Type I deionized (DI) water to the suppressor Regen In, suppressor Regen Out to the CR-ATC Regen In, and Regen Out to waste. Valve 2 in the Load position, directs the DI water to the MS and the CD effluent to the suppressor Regen In.

The following instructions assume a field service engineer has already installed the Dionex ICS-6000 system. To set up the IC-MS system, position the Dionex ICS-6000 system near the MS source. Install the power and USB cables, and turn on the IC, autosampler, and computer. Add ASTM Type I DI water to the eluent bottles and prime the pumps.

Installing the ISQ EC mass spectrometer

First, remove the shipping material located under the power supply inside the MS and then follow the installation instructions in the ISQ EC instrument manual.[29]

1. Install the nitrogen gas generator (must be capable of 100 psi) by removing the shipping brackets inside the generator. Complete the installation and conditioning process according to the installation instructions.
2. Install the foreline pump including the vacuum hose, mist filter, vacuum oil, and oil return assembly. Connect the vacuum hose and power cord to the MS.
3. Verify that the voltage of the foreline pump is the same as the mass spectrometer and that the foreline pump is set to high vacuum.
4. Install the solvent drain container and hoses.
5. Install the sweep cone and MS probe in the MS source.
6. Install the MS calibrant. Store the container(s) of calibrant at 4 °C when not in use.
7. Install the power cables for the MS and the foreline pump. Install the LAN cable from the MS to the PC.
8. Power-up the nitrogen generator, the MS (which turns-on the foreline pump), and the PC.

To electronically configure the IC system, start the Thermo Scientific™ Chromeleon™ Instrument Services program, then start the Instrument Controller program by selecting the *Configure instruments* link. Add the ICS-6000 system DP module, EG module, DC module, and the AS-AP Autosampler module, as described in Table 2. Add the ISQ EC module by selecting mass spectrometry, ISQ ICMS Family. Check and correct the configuration for any errors. Save and close the configuration program.

Table 2. Electronic configuration parameters

Module	Tab	Action
DP	General	Select Browse, and choose the serial number to link module to Instrument
	Device	Link pump to instrument
EG	General	Select the 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
	Thermal controls	Check Compartment_TC and Column_TC
	Suppressors	Double click Suppressor1, Link to Pump 1
	High pressure valves	Double click InjectValve1, Link to Pump 1, select control by autosampler Double click InjectValve2, Link to Pump1, select control by DC
	Low pressure valves	Not used in this application
AS-AP autosampler	General	Select serial number to link module to instrument
	Sharing	This option is present, select instrument
	Segments/pump link	Select 10 mL PolyVials or 1.5 mL vials for “Red”, “Blue”, and "Green". Leave the pump and TTL link boxes empty.
	Options	Select 1200 buffer loop, 100 µL syringe, enter “5” µL in sample loop, temperature control, push mode.
	Relays	Not used in this application
	Inputs	Not used in this application
ISQ EC mass spectrometer	Add module	Mass spectrometry, ISQ ICMS family
	General	Select ISQ EC module
		Deselect the hardware inject synchronization. This is not needed for Chromeleon CDS.
		Select *ActiveLow* for Remote Start. (Setting for Dionex AS-AP autosampler)
		Deselect *split flow, fraction collection, Warn on source change* and *Simulation mode* boxes.
	Maintenance	Enable all boxes. Select OK
	Associate pump info	Optional: Select Associate a pump box will automatically enter flow rate when setting source conditions.
		If using this option, select pump used for eluent flow
		Select OK

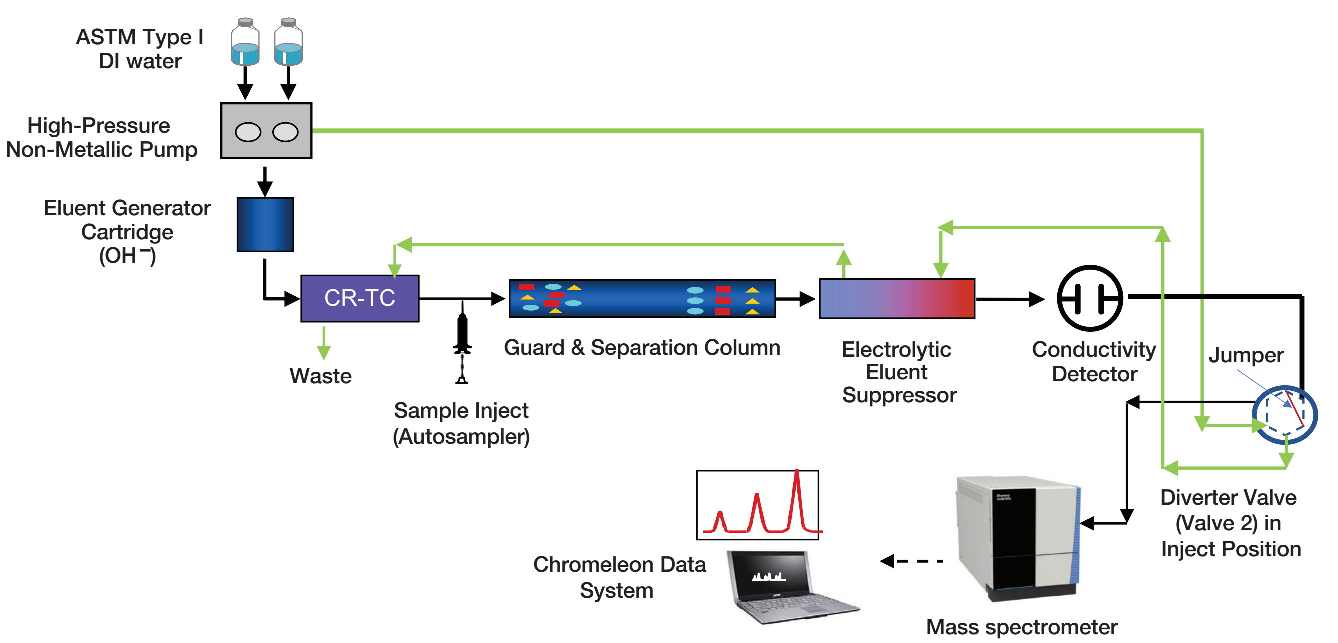


Figure 2. IC-MS flow diagram

Plumbing the Dionex ICS-6000 HPIC

Plumb the Dionex ICS-6000 IC as a standard Reagent-Free™ IC (RFIC™) system using the IC PEEK Viper fittings as indicated on the fitting labels, and as shown in Figure 2. IC PEEK Viper fittings should also be used between the second valve acting as the diverter valve and the MS to minimize the backpressure to the suppressor. Temporarily direct the liquid flow away from the ISQ EC mass spectrometer until the IC and IC consumables are fully conditioned. The schematics are also illustrated on the inside doors of the Dionex ICS-6000 IC system. Further information can be found in the product manual and the AXP Metering Pump manual.[30,31] Direct the waste lines to the waste containers.

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 KOH eluent generator cartridge and Dionex CR-ATC 600 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] Condition the columns as described in the Dionex IonPac AS19-4µm product manual[34] or Consumables Conditioning instructions (Chromeleon Console, under Consumables drop-down menu), 20 mM KOH, 30 °C at 0.25 mL/min for 30 min while directing the effluent to waste. Install the conditioned columns according to Figure 2.

To hydrate the Dionex ASRS 300 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 suppressor is within backpressure specifications. The backpressure by the suppressor (1) should be <50 psi, whereas the backpressure applied after the suppressor (2) should be <100 psi. The operations of the ASRS suppressor are thoroughly discussed in the suppressor manual.[36]

System startup, conditioning, and consumables device tracking

To condition the IC consumables and IC system, temporarily direct the IC effluent to waste (instead of to the MS) and set the ISQ EC mass spectrometer to standby mode (vaporizer temperature =“0”, ion transfer temperature = 150 °C, and sheath = 20 psi). Initially, equilibrate the IC using the Quality Assurance Report (QAR) conditions for the Dionex IonPac AS19-4µm column. To create an IC-only instrument program, use the Chromeleon Instrument Method Wizard program, enter the QAR conditions and click on the “to remove from method” box on the ISQ EC page. To start the sequence, select the IC-only instrument method and approve the consumables in the Consumables Tracking panel (Chromeleon console) as described in Thermo Fisher Scientific TN175.[37] Compare the results against the QAR report. Continue to equilibrate the IC until the total conductivity is <2 µS/cm. Connect the flow 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

To create an IC-MS method, use the Chromeleon Instrument Method Wizard program and enter the parameters listed in the Conditions section, including the InjectValve_2 timing. Uncheck the “to remove from method” box on the ISQ EC page. Select partial loop mode to minimize sample and standard consumption. Save the instrument method.

Unexpected failures in the suppressor or in the regenerant pump directing water to the suppressor can damage the mass spectrometer. For IC-MS applications, at the end of each run the diverter valve should be rotated to direct the CD effluent away from the MS. In this configuration, the Inject Valve 2 is in the Inject position directing DI water to the MS and the suppressor is in the recycle mode. This has the additional benefit of rinsing the diverter valve.

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 Valve_2 to direct water to the MS and the CD flow to recycle mode. This could be due to an unexpected suppressor failure. (Note: when the suppressor fails, 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 sample.

To create an emergency trigger:

1. Open the IC-MS instrument method.

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.

	DC.InjectValve_2.LoadPosition

6. Save the trigger.

8. Save the instrument method.

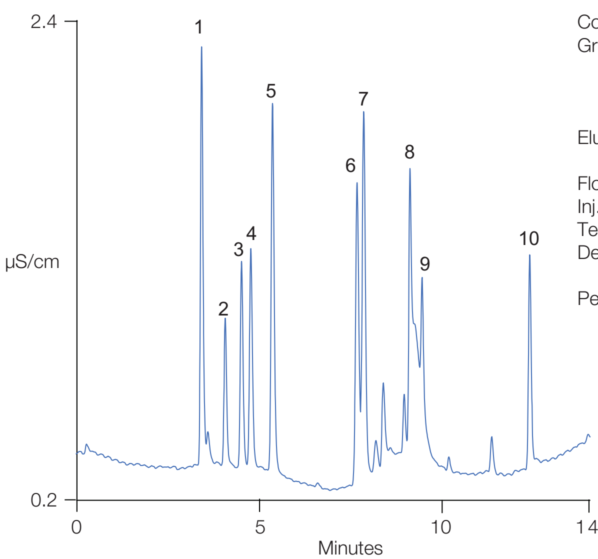
Second high conductivity emergency trigger

The suppressor can fail gradually over time, resulting in increased high conductivity as it fails. Therefore, it is prudent to use a second trigger for a slowly failing suppressor. Insert a second trigger after the high conductivity trigger following the same steps using the following conditions.

Name	"HighConductivity2"
Condition	CDet.CD_1_total.signal>=5
TrueTime	120
Delay	5
AllowImmediate	Yes

Results and discussion

In this application, a 2 µL injection of µg/L to mg/L concentrations of seven polar pesticides and three anions related to disinfection processes (chlorite, bromide, and bromate) were determined in 2-fold and 5-fold diluted extractions of homogenized pears and peas using the previously demonstrated separation method.[26] Partial loop injection mode (2 µL injection of a 5 µL loop) was selected to minimize standard consumption. The ten analytes of interest were separated by anion-exchange chromatography using an electrolytically generated KOH gradient from 15 to 75 mM KOH, at 40 °C and 0.375 mL/min.[26] The ions eluted within 14 min and were detected serially using suppressed conductivity and ESI mass spectrometry detection, and quantified using mass spectrometry. Figures 3A and 3B show the conductivity chromatogram and SIM traces, respectively, of 5 mg/L standards. Glufosinate-AMPA-Bialaphos-3-MPPA and glyphosate-cyanuric acid were not chromatographically separated but were easily resolved by MS, demonstrating the advantages of using MS detection with IC. All ions, except glufosinate, AMPA, and bialaphos, show good peak shape with no or slight tailing. The Dionex SRS type suppressor was selected for this application because it provides better peak shapes for glufosinate, AMPA, and bialaphos compared to other suppressor types. Despite the use of this suppressor, glufosinate, AMPA, and bialaphos had less than ideal shape with larger peak widths.



Columns:	Dionex IonPac AG19-4µm, Dionex IonPac AS19-4µm, 2 mm i.d.
Gradient:	15 mM KOH (-1 to 0.2 min), 15–20 mM (0.2–4 min), 20–55 mM (4–7.5 min), 55–57 mM (7.5–9 min), 57–75 mM (9–10 min), 75 mM (10–11.5 min), 15 mM (11.6–14 min)
Eluent source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser device
Flow rate:	0.375 mL/min
Inj. volume:	2 µL partial injection mode of 5 µL loop, 5 µL cut volume
Temperatures:	Column: 40 °C, Detector: 20 °C
Detection:	Suppressed conductivity, Dionex ASRS 300, 2 mm constant current and recycle modes, 65 mA
Peaks:	1. Fosetyl 2. Acetate 3. Chlorite 4. Bromate 5. Chloride 6. Bromide 7. Chlorate 8. Bialaphos, AMPA, glufosinate 9. 3-MPPA 10. Cyanuric acid, Glyphosate

Figure 3A. Suppressed conductivity chromatogram of a 5 mg/L standard

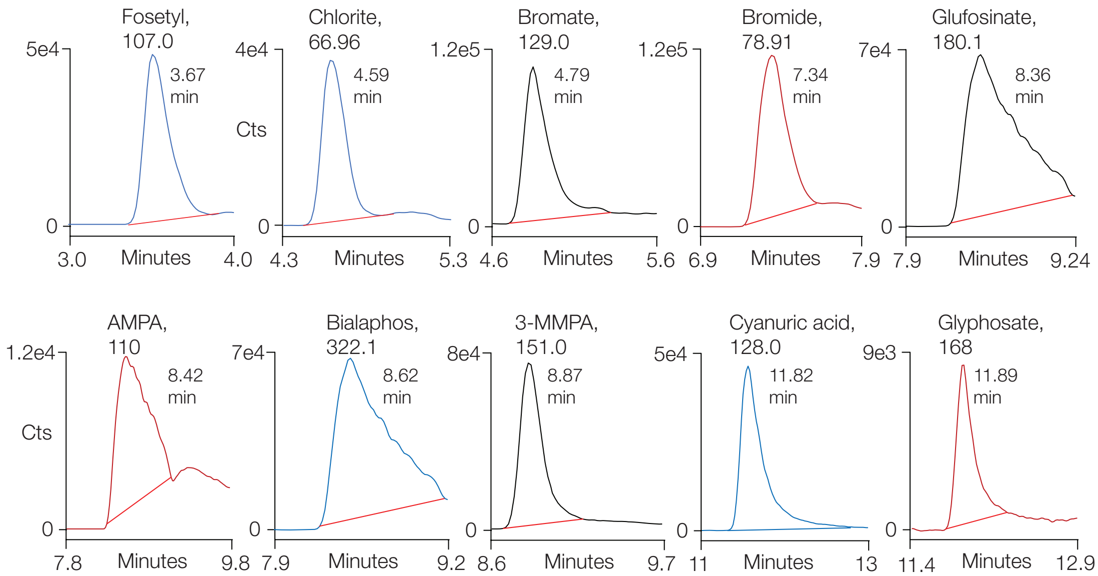


Figure 3B. SIMs of 5 mg/L standards

Method qualification

To evaluate the method, the calibration responses were evaluated over 10 to 10,000 µg/L, the method detection limits were determined, and the method stability was determined. The calibration ranges of each anion were determined by comparing the MS responses from triplicate injections of seven standards with the best mathematic fit to a linear or secondary equation. The results, summarized in Table 3, show that glyphosate, AMPA, glufosinate, 3-MPPA, and bialaphos had linear responses as indicated by the coefficient of determinations ($r^2 > 0.99$). Fosetyl, chlorite, bromate, bromide, and cyanuric acid had responses that are best fit with a quadratic equation. The method detection limits (MDL) were determined using seven replicate injections with 3 × S/N. Most of the analytes had estimated MDLs ranging from 2 to 30 µg/L with glyphosate at 136 µg/L.

The retention time and MS peak area reproducibilities were evaluated by determining the RSDs of triplicate injections of 50 µg/L standard. The reproducibility results were good as indicated by the retention time RSDs <0.5% and peak area RSDs <3%.

Table 3. Summary of calibration range and MDL results

	Calibration range (mg/L)	Type	Coefficient of determination (r^2)	MDL* (µg/L)
AMPA	0.10 to 10	Linear	0.9987	27.3
Bialaphos	0.06 to 10	Linear	0.9953	18.6
Bromate	0.03 to 10	Quadratic	0.9994	9.7
Bromide	0.01 to 10	Quadratic	0.9995	1.5
Chlorite	0.01 to 10	Quadratic	0.9999	2.0
Cyanuric acid	0.01 to 10	Quadratic	0.9997	5.4
Fosetyl	0.08 to 10	Quadratic	0.9990	24
Glufosinate	0.06 to 10	Linear	0.9997	19.7
Glyphosate	0.3 to 10	Linear	0.9998	136
3-MPPA	0.05 to 10	Linear	0.9996	16

*n = 3, MDL = 3 × S/N

Table 4. Summary of reproducibility results of 50 µg/L standard

	Retention time (min)	RSD	Average peak area × (µS-min)*	RSD
AMPA	8.80	0.24	16,450	0.80
Bialaphos	7.90	0.31	69,340	1.82
Bromate	5.05	0.14	89,850	2.35
Bromide	8.04	0.10	20,460	3.06
Chlorite	4.81	0.23	45,230	1.99
Cyanuric acid	12.54	0.12	21,350	3.20
Fosetyl	3.76	0.47	6,890	0.69
Glufosinate	8.63	0.18	87,580	0.37
Glyphosate	12.33	0.36	201,30	0.32
3-MPPA	9.15	0.08	288,900	1.99

*Rounded to 4 significant digits
n = 3

The method was applied to two samples, homogenized pears and peas. The samples were extracted with methanol and agitation, and diluted before analysis. The pea sample required filtration as particles were observed. To determine the method accuracy, the recoveries of an added mixed standard were determined. Figure 4 shows the SIM traces of the 10 compounds in the 5-fold diluted extract from peaks with and without the addition of standards. Table 5 summarizes the results from the recovery experiments. No native glufosinate and glyphosate were found in the homogenized food samples; however, compounds having a common mass and retention time as fosetyl, chlorite, cyanuric acid, and bialaphos were detected at below the MDL concentrations. The recoveries of the 100 µg/L spiking standard were good, within 80–120%, except for AMPA in pears, 69–76% recovery. In general, the 5-fold diluted samples had better recoveries than the 2-fold diluted samples.

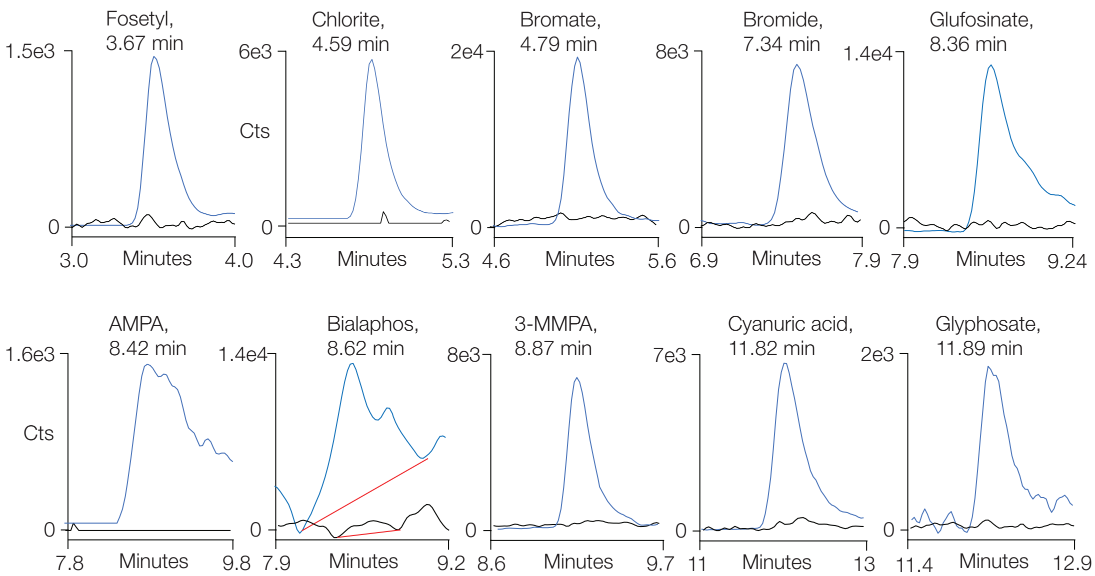


Figure 4. SIM traces of 5-fold diluted pear extract with and without 100 µg/L spiked standard

Table 5. Summary of recovery experiments of 100 µg/L added standard

Sample extract	Fosetyl (%)	Chlorite (%)	Bromate (%)	Bromide (%)	Chlorate (%)	Glufosinate (%)	AMPA (%)	3-MPPA (%)	Cyanuric acid (%)	Glyphosate (%)
Pears, 2-fold dilution	80.3	76.7	80.6	111	111	95.8	69.3	117	85.1	86.7
Pears, 5-fold dilution	87.9	92.1	106	111	111	104	76.6	114	91.7	94.8
Peas, 2-fold dilution	110	99.6	104	106	110	92.0	80.3	102	99.5	106
Peas, 5-fold dilution	98.4	98.1	95.9	101	102	89.7	88.1	99.4	98.8	111

Conclusion

Determination of anionic polar pesticides, such as glyphosate and glufosinate, with disinfection byproducts was demonstrated in extracts of homogenized pears and peas using anion-exchange chromatography with ESI-MS using the Dionex ICS-6000 Dual HPIC system coupled with the ISQ EC single quadrupole mass spectrometer. The method using a small sample loop (2.5 µL) had MDLs of single to triple-digit µg/L in solution with good recoveries, 80–120%. However, AMPA recovery in the pear extract was lower than desired, 69–76%. The single quadrupole MS provides sufficient selectivity and limits of detection for screening foods for polar pesticides. For more selective detection of polar pesticides and their quantitation at lower limits of detection required by most regulatory entities, an ion chromatograph coupled to either a triple quadrupole (IC-MS/MS) or high-resolution accurate mass (IC-HRAM-MS) mass spectrometer is recommended.

More information on other IC and IC-MS methods is available on Thermo Fisher Scientific AppsLab digital library.[38]

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