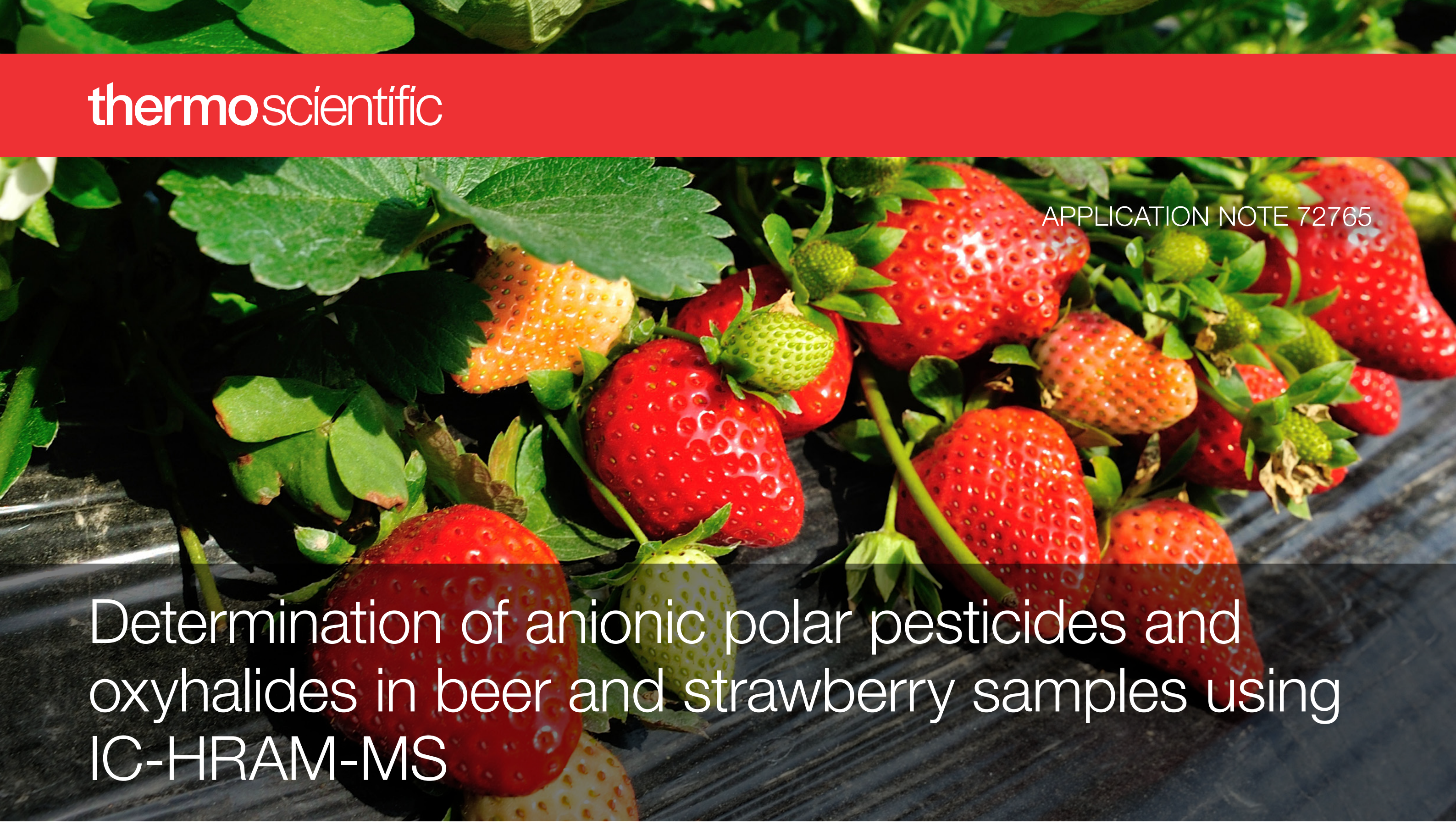

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APPLICATION NOTE 72765

Determination of anionic polar pesticides and oxyhalides in beer and strawberry samples using IC-HRAM-MS

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Keywords

IonPac AS19 column, RFIC, Reagent-Free IC, Integrion, accurate mass spectrometry, disinfection by-products

Goal

Provide a combined analytical method for polar pesticides and oxyhalide disinfection by-products determination using ion chromatography with the Thermo Scientific™ Q Exactive™ Focus mass spectrometer

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 are the polar pesticides, which include ionic post-emergent and desiccant herbicides, fungicides, growth-regulating chemicals, and the resultant metabolites of those compounds (Table 1).

Herbicides such as glyphosate and naturally occurring glufosinate and bialaphos are applied to prevent emerging weeds on fallow ground. If applied to growing crops, post-emergent herbicides are likely to kill the crops as well as weeds and therefore are unlikely to cause food contamination. However, these same herbicides are also applied before harvest to kill weeds and as a desiccant[1] to hasten drying of plants and grains. In both applications, the herbicide can contribute to food contamination. More recently, certain crops (soybean, maize, canola, and sugar beets) and seeds (alfalfa, canola, corn, cotton, sorghum, soybeans, sugar beets, and wheat) have been genetically modified to be more tolerant of glyphosate.[1-3] As a result, many farmers have replaced more toxic compounds with glyphosate. However, they have also applied more glyphosate on crops before harvest, and as a result, food safety concerns have increased.[4] A European analysis of popular breakfast cereals and beers confirmed that glyphosate is present as a food contaminant in most of the samples.[5]

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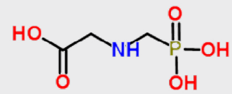
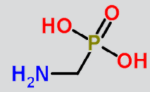
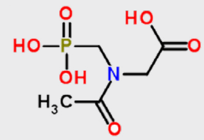
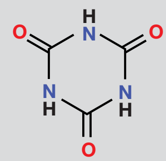
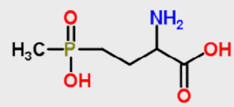
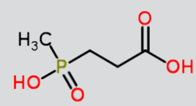
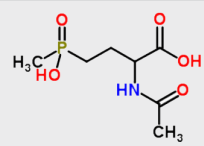
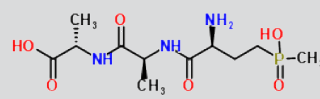
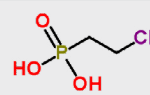
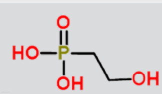
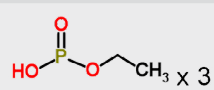
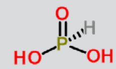
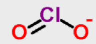
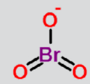
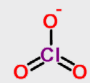
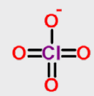
Table 1. Anionic polar pesticides, oxyhalides, and perchlorate

Compound	IUPAC Name*	Type	Structure**	Formula	Monoisotopic Mass**
Glyphosate	2-(phosphonomethylamino) acetic acid	Synthetic herbicide		$C_3H_8NO_5P$	169.0140
AMPA	aminomethylphosphonic acid	Glyphosate metabolite		$C_7H_{10}N_2O_4$	111.0085
N-Acetylglyphosate	2-[acetyl(phosphonomethyl) amino] acetic acid	Glyphosate metabolite		$C_5H_{10}NO_6P$	211.0246
Cyanuric acid	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione	Herbicide precursor		$C_3H_3N_3$	129.0174
Glufosinate	2-amino-4-[hydroxy(methyl) phosphoryl]butanoic acid	Natural herbicide (also bialaphos metabolite)		$C_5H_{12}NO_4P$	181.0504
3-MPPA	3-[hydroxy(methyl) phosphoryl]propanoic acid	Glufosinate metabolite		$C_4H_9O_4$	152.0238
N-Acetylglufosinate	2-acetamido-4-[hydroxy(methyl)phosphoryl] butanoic acid	Glufosinate metabolite		$C_7H_{14}NO_5P$	223.0610
Bialaphos	(2S)-2-[[(2S)-2-[[(2S)-2-azaniumyl-4-[methyl(oxido) phosphoryl]butanoyl] amino]propanoyl]amino] propanoate	Natural herbicide		$C_{11}H_{22}N_3O_6P$	323.1246
Ethephon	2-chloroethylphosphonic acid	Synthetic growth regulator		$C_2H_6ClO_3P$	143.9743
HEPA (and ethylene gas)	2-hydroxyethylphosphonic acid	Ethephon metabolite		$C_2H_7O_4P$	126.0082
Fosetyl-Al (without) aluminum	Aluminum tris(ethyl phosphonate)	Synthetic greenhouse fungicide		$C_6H_{15}AlO_9P_3^{+3}$	327.0167

* Pubmed[6]
** ChemSpider[7]

Table 1 (continued). Anionic polar pesticides, oxyhalides, and perchlorate

Compound	IUPAC Name*	Type	Structure**	Formula	Monoisotopic Mass**
Phosphonic acid	Phosphonic acid	Acid of Fosetyl-Al salt		PO_3H_3	81.9820
Chlorite		DBP		ClO_2^-	66.9522
Bromate		DBP		BrO_3^-	126.9030
Chlorate		DBP		ClO_3^-	82.9536
Methyl bromide	Analyzed as bromide	Fungicide		Br^-	78.9183
Perchlorate		Fireworks, ammunition byproduct		ClO_4^-	98.9490

* Pubmed[6]
** ChemSpider[7]

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.[8,9]

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. Naturally occurring compounds not exhibiting toxicity are exempt from toxicological evaluation. For example, limited toxicology studies have been conducted on naturally occurring glufosinate and bialaphos with EU maximum residue levels (MRL) of 0.03 to 0.3 mg/kg.[8-11] Glufosinate is suspected to be a reproductive toxin.[12,13]

In contrast, manufacturers of synthetic compounds such as glyphosate, AMPA, and *N*-acetyl AMPA, are typically required to provide toxicology results to the regulatory agencies. The results of glyphosate exposures in animal studies show low mutagenicity risk, resulting in rulings of "unlikely to be a carcinogen" in 1991 and 1999 by the Food and Agricultural Organization and World Health Organization (FAO/WHO), and a recommended maximum Adjusted Daily Intake (ADI) of 0.1–0.5 mg per kg body weight per day in 1996 by the U.S. Environmental Protection Agency (EPA).[14,15] The toxicity impact on humans is more controversial because occupational exposure and epidemiology cases are also included, which adds greater complexity. An evaluation of impact must decide on whether to include or discard cases with exposures to multiple compounds resulting in either unclear conclusions or reduced population studies. As a result, reviews in 2015 of one subset of epidemiology studies resulted in rulings of "unlikely to be a carcinogen" (the European Food Safety group and the EPA) and, using another subset, a ruling that it was "likely a carcinogen" (WHO International Agency for Research on Cancer (IARC)).[14,15] The U.S. state of California ruled along the lines of the IARC, adding glyphosate to the Proposition 65 list of compounds known to be carcinogens. This was then blocked by the U.S. judicial system.[16,17]

Determinations of ionic pesticides by LC-MS can be challenging because of their ionic nature, while GC-MS determinations require costly derivatization reagents. As previously demonstrated, these negatively charged ionic compounds are more suited for anion-exchange chromatography combined with the increased selectivity and sensitivity delivered by mass spectrometry.[18-22]

Additionally, determinations of perchlorate (from munitions production), and chlorate, chlorite, and bromate from disinfection byproducts are also needed in food and beverage analysis. Although routinely determined using IC, IC-MS, and IC-MS/MS, they have not been determined alongside polar pesticides.[23,24] In this application note we demonstrate fast determinations of multiple ionic pesticides and disinfection byproducts by anion-exchange chromatography and high-resolution, accurate-mass (HRAM-MS) mass spectrometry detection. All analytes of interest elute within 15 min and were detected by suppressed conductivity detection and HRAM-MS connected in series, with sensitivity to µg/L in full scan mode and sub µg/L in selected ion monitoring mode (SIM).

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, high-pressure capable and RFIC enabled, including:
  - Eluent generation capabilities
  - Column oven temperature control
  - Detector-Suppressor compartment temperature control
  - Tablet control
  - Consumable device monitoring
  - 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
  - Two Thermo Scientific™ Dionex™ AXP Auxiliary pumps (to pump solvent for desolvation and water for the suppressor regenerant), P/N 060684
  - 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 configured for eluent generation, suppressed conductivity detection, and mass spectrometry detection.

Software

- Thermo Scientific™ Foundation 3.0 software
- Thermo Scientific™ Xcalibur™ software or Thermo Scientific™ TraceFinder™ software with SII for Xcalibur software

Reagents and standards

- Deionized (DI) water, 18 MΩ·cm resistivity (ASTM™ Type I water)[23], 0.2 µm filtered
- Lab Instruments, QPP-Lab Standards (Polar Pesticides) Standard Mix 1.3, P/N CRM3G11L346 (http://www.labinstruments.org/en/catalogs/#qpp-lab), 5 mg/L
  - Ethephon
  - HEPA
  - Glyphosate
  - AMPA
  - Glufosinate
  - MPPA
  - *N*-Acetyl Glufosinate
  - Fosetyl-Aluminum
  - *N*-Acetyl AMPA
  - Phosphonic acid
  - Bialaphos
  - Perchlorate
  - Chlorate
  - *N*-Acetyl AMPA
  - Glyphosate
- Cambridge Isotope Laboratories, Inc.
  - Glyphosate (ISTD), 2-C13, 99%, 15N >98%, P/N CNLM-4666-10X-1.2
- Thermo Scientific Dionex
  - Perchlorate-ISTD, P/N 062923
- Fisher Scientific
  - Acetonitrile, Optima™ grade for desolvation, P/N A955-1
  - Sodium bromide, crystalline, ACS grade, P/N S255 (MW 102.89)
  - Sodium chlorite, J.T. Baker, P/N 02-004-050 (MW 90.44)

Table 2. Consumables list for the Dionex Integrion HPIC system

Product Name	Description	Part Number
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kits	Dionex IC PEEK Viper fitting assembly kit for the Integrion system configured for eluent generation and conductivity detection. Included with the Dionex Integrion HPIC System, RFIC model	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 KOH Eluent Generator cartridge	Eluent generator cartridge recommended for this application	075778
Thermo Scientific™ Dionex™ CR-ATC 600 Electrolytic trap column	Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge and required for the Dionex Integrion HPIC system	088662
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 Integrion RFIC model	075522
Thermo Scientific™ Dionex™ ASRS™ 300 suppressor	Recommended suppressor for 2 mm i.d. columns when determining AMPA	SP6948
Thermo Scientific™ Dionex™ IonPac™ AG19-4µm column	Anion guard column, 2 × 50 mm	083225
Dionex IonPac AS19-4µm column	Anion separator column, 2 × 250 mm	083223
IC-MS Installation Kit	IC-MS installation kit including tubing, mixing tee, and Dionex SRD 10 device	22153-62049
0.2 µm syringe filters	Syringe filters used when samples require removal of particulates	Fisher Scientific P/N 09-740-113
Centrifuge tubes	50 mL Centrifuge tubes	339652 or Fisher Scientific 12-565-270

Ion chromatography conditions

Columns:	Dionex IonPac AG19-4µm guard, 2 × 50 mm Dionex IonPac AS19-4µm separation, 2 × 250 mm
KOH Eluent:	15 mM KOH (0–0.2 min), 15–20 mM (0.2–4 min), 20–75 mM (4–10 min), 75 mM (10–11.5 min), 75–15 mM (11.5), 15 mM (11.5–15 min)
Eluent Source:	Dionex EGC 500 KOH eluent cartridge, Dionex CR-ATC 600 trap column and high pressure degas module
Flow Rate:	0.45 mL/min
Injection Volume:	100 µL
Column Temp.:	40 °C
Detection/Suppressor Compartment:	15 °C
Detection 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 2 mm, external water mode (driven by AXP-MS pump at 0.7 mL/min)
Conductance Background:	< 1 µS/cm
Conductance noise:	< 1 nS/cm
System backpressure:	~ 3300 psi

Mass spectrometry conditions

IC-MS Interface Makeup Solution:	Acetonitrile at 0.23 mL/min
Detection 2:	HRAM-MS, -ESI by HESI II probe
MS Scan Mode:	Full scan: 60–750 *m/z*, 30k resolution, AGC 1e6, max IT 100 s
Ion Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 Gas Flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 °C, Capillary 265 °C
MS-SIM Mode	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window
Run Time:	15 min

Figure 8 displays the SIM values.

Sample preparation

The beer samples were analyzed without dilution and diluted 10- to 100-fold with DI water. The strawberry samples were crushed and prepared according to the EURL Quick Polar Pesticides Extraction (QuPPe) method (Figure 1) and diluted 10-fold with DI water before analysis.

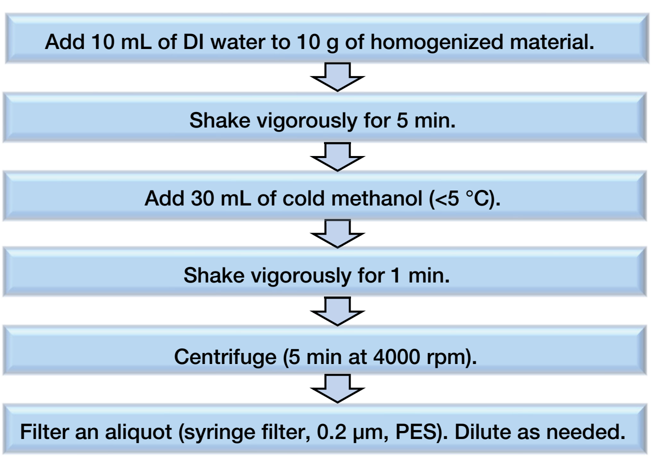


Figure 1. EURL Quick Polar Pesticides (QuPPe) extraction method

Standard preparation

Individual bromide (1000 mg/L, 12.8 mg of sodium bromide in 100 mL DI water) and chlorite (1000 mg/L, 13.24 mg of sodium chlorite in 100 mL DI water) stock standards were prepared. A combined intermediate standard was prepared by diluting the Lab Instrument standards and lab-prepared stock standards with DI water. The intermediate standard was diluted sequentially with DI water to create the working standards.

Instrument setup and installation

IC system

The Dionex Integrion HPIC system is a high-pressure integrated IC system. The Dionex Integrion HPIC system and the Dionex EGC 500 KOH cartridge and Dionex CR-ATC 600 consumable products are designed for high-pressure conditions up to 5000 psi. Configure and install the Dionex Integrion HPIC system according to Figure 2 and Thermo Scientific Technical Note TN175: Configuring the Dionex Integrion HPIC System for High-Pressure Reagent-Free Ion Chromatography.[24]

A second valve used as a diverter valve should be installed. Plumb the flow direction in valve Position A to the MS and valve Position B to waste.

In the SII for Xcalibur Instrument Configuration Manager, assign control of the injection valve to the autosampler, which will assign control of the second valve, named the HP_valve, to the Integrion system. The valve positions are programmed directly in the program script. Position the IC as close to the MS as possible and minimize the length of the tubing from the IC to the mass spectrometer to reduce backpressure and maintain analyte separation. The backpressure generated by the MS-interface after the suppressor should be below 100 psi, which translates into less than two feet of the red PEEK tubing length.

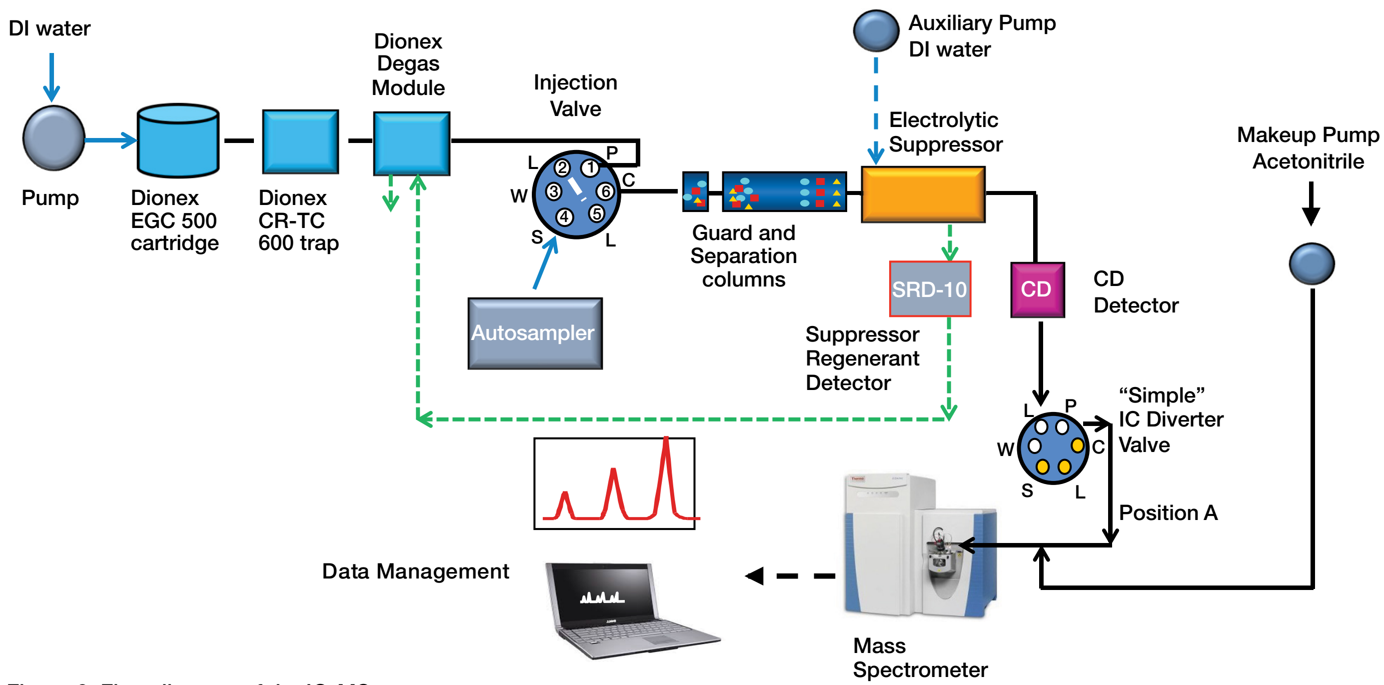


Figure 2. Flow diagram of the IC-MS system

Installing the Dionex SRD-10 Suppressor Regenerant Detector device

In addition to the second valve, the Thermo Scientific™ Dionex™ SRD-10 device is recommended when running IC-MS applications. The SRD-10 device is programmed to turn off the IC pump when the suppressor regenerant flow ceases for 5 min, and thus stop the eluent flow into the MS. This setup prevents KOH eluent from entering the MS. Install the SRD-10 device according to Figure 3 and the installation instructions manual.[28] Connect the USB cable to the back of the Integrion system. Install the TTL cable (twisted pair wire cable) into the 8-pin connector on the back of Integrion system with the red wire in the *TTL-1 IN* position and the black wire in the *D-GND* position. Install the SRD-10 fluid lines between the suppressor (Regen Out) and the Dionex CR-TC trap column (Regen In) ports. Align the liquid tubing in the door slots to prevent backpressure to the suppressor from inadvertently crimping the tubing.

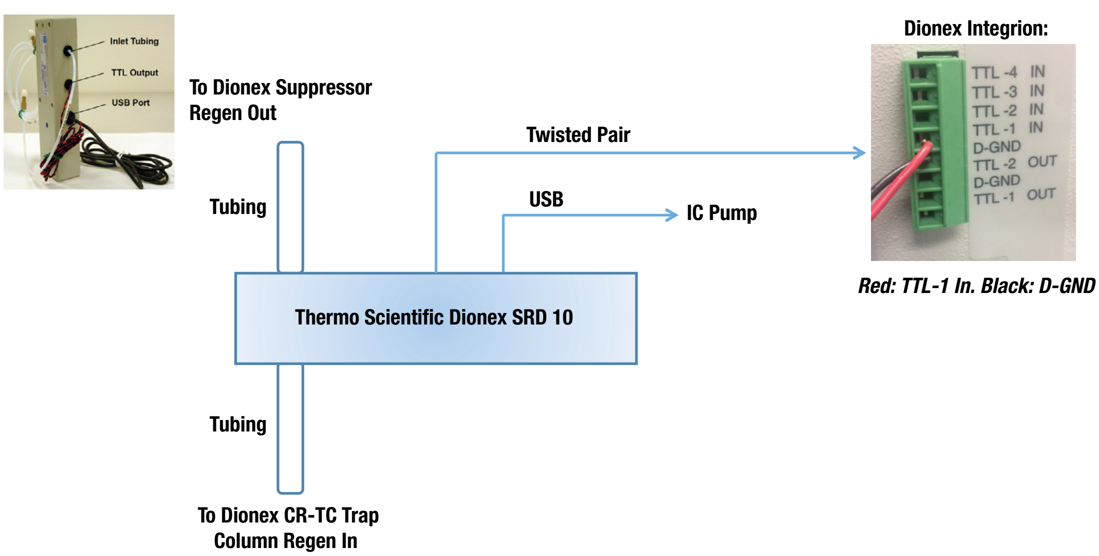


Figure 3. Installing the SRD-10 device

To verify that the SRD-10 device is working, first complete the configuration as described in the *Configuring the modules section.* Then, turn off the Regenerant pump, de-install the SRD-10 device, and blow out all liquid from the SRD-10 device using a compressed gas line. Reinstall the SRD-10 device. After 5 min, the SRD-10 device will trigger the IC pump to turn off. Restart the IC, but do not turn on the suppressor until the SRD-10 tubing is refilled with DI water. As a safety precaution in addition to the SRD-10 device, it is recommended to add a high conductivity trigger in the Instrument Method. Figure 5 shows a trigger to stop the IC system pump flow and rotate the diverter valve (HP_Valve) to waste when the total conductivity is at 50 µS or higher (CDet.CD_Total $\geq$ 50) for longer than 180 s.

IC-MS interface

The IC-MS interface includes a pump (AXP pump) delivering DI water to the suppressor regenerant channel, a second pump (AXP pump) providing the desolvation solvent, a mixing tee (blends the eluent containing the separated ions with the desolvation solvent), and tubing from the diverter valve to the mixing tee and to the MS. The mixing tee and tubing are included in the IC-MS installation kit. To reduce contamination to the MS from solvent extractable compounds, the following modifications are recommended to the pump delivering the desolvation solvent:

- If using the AXP or AXP-MS Auxiliary Pump, disassemble the pump head and remove the diaphragm located between the Self-Flush Housing and the Piston Retainer. Re-assemble the pump head assembly without the diaphragm. Follow the instructions in the AXP/AXP-MS Metering Pump Operator's Manual.[27]
- Use only glass bottles to hold the organic solvent to prevent possible leaching of plasticizers. Plastic containers, bottles, and vials should be used elsewhere.
- Replace as much as possible of all ¼" o.d. white tubing that comes in contact with the solvent with green (0.03" i.d.) PEEK tubing, particularly the tubing in the solvent bottle.

Configuring the modules in Xcalibur software with SII for Xcalibur software

To configure the IC system, first close all Xcalibur programs. Open the Configuration program (gear symbol), select the SII module, select configure, and then add the IC modules. Five modules are added to this instrument configuration: Dionex Integrion HPIC system (Integrated IC systems), Integrion HPIC Pump Wellness, Dionex AS-AP Autosampler, and two AXP auxiliary pumps. Figure 4 illustrates the configuration needed for the SRD-10 device. The configuration parameters are described in Table 3 and TN175.

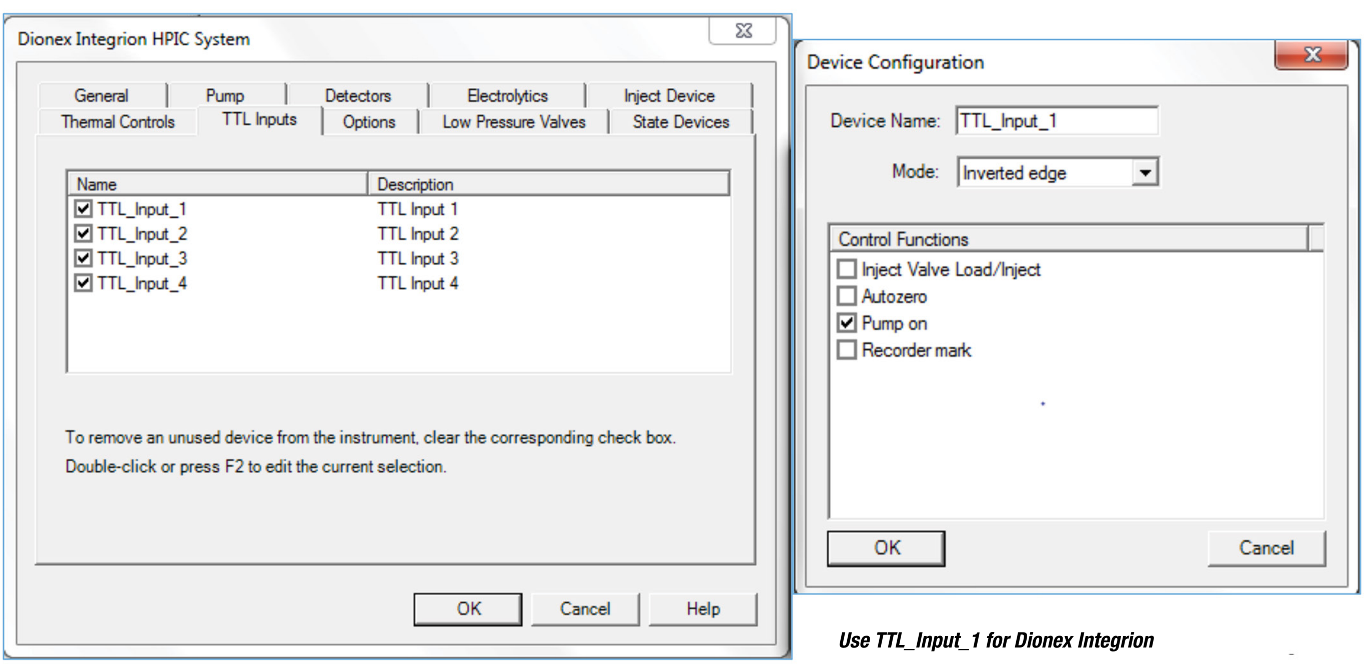
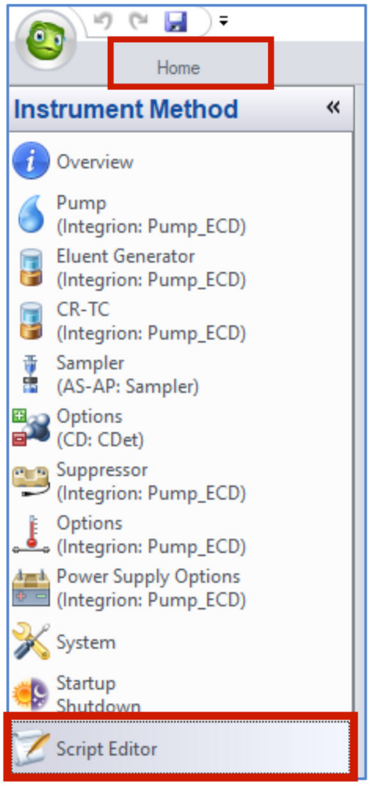
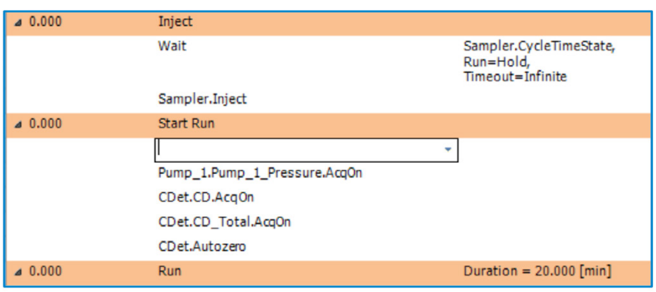


Figure 4. Configuring the SRD-10 device

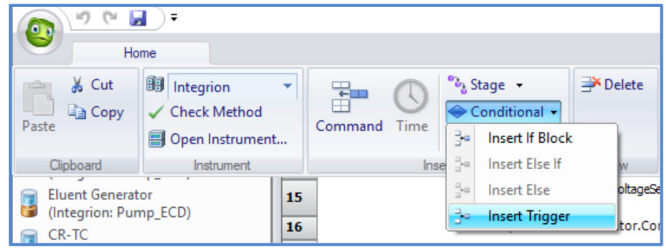


2. Select Home to open command ribbon.

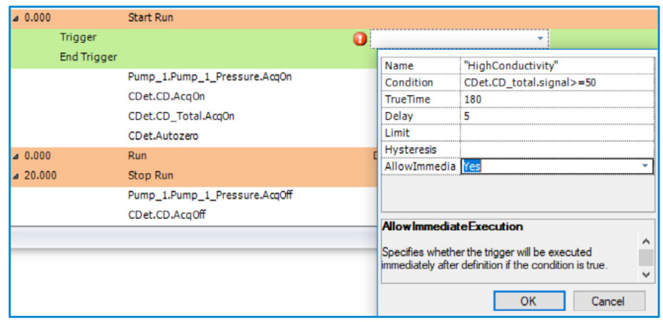
1. Open Script Editor of IC Instrument Method.



3. Place cursor on row after 0.000 Start Run and select Insert Command.



4. Select Conditional and Insert Trigger.

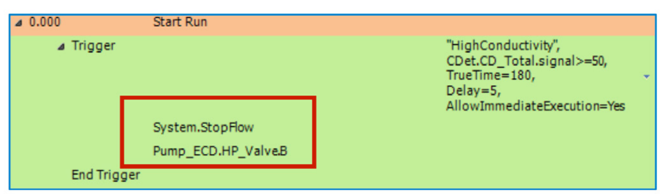


5. Enter the name and conditions for the trigger.*

- Name: “HighConductivity”
- Condition: CDet.CD_total.signal>=50
- TrueTime: 180
- Delay: 5
- AllowImmediate: yes

Press OK.

*The Trigger commands will be slightly different for other IC systems.



6. Place cursor on the End Trigger row and Command Column, and select Insert Command. Enter commands to stop the IC flow and to divert flow to waste (B position).

Save Instrument Method.

Figure 5. Creating the HighConductivity Trigger (> 50 µS for 180 s) in Script Editor

Table 3. Summary of system configuration for high-pressure Dionex Integrion HPIC system, RFIC model

Tab	Action	Result
Dionex Integrion HPIC Module		
General		Described in TN175
Pump	Verify that the pressure maximum limit is set to 5000 psi	Flow rate and pressure limitations are displayed
Detectors		Automatically detects CD
Electrolytics	Verify that the devices are detected	Automatically detects Dionex eluent generator cartridge, Dionex CR-TC 600 trap column, and suppressor
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 Dionex Integrion IC system
TTL Inputs	Click on the box next to TTL_Input 1	*Activates TTL_Input I. The other TTL channels are not needed for this application.* (Figure 4)
	Double left-click on TTL_Input 1	Opens Device Configuration box. (Figure 4)
	Select Pump On Select Inverted edge mode	Directs (IC) Pump to turn off when this channel is activated (no suppressor regen flow > 5 min) (Figure 4)
Pump Wellness module: Described in TN175		
Add Dionex AS-AP Autosampler: Described in TN175		
AXP auxiliary pump to pump DI water through the suppressor's Regenerant-In channel		
Add Module		Select AXP Pump under IC: Dionex modules
General	Rename Device Name to Pump_Regen	Name pump with a unique name
	Select COM Port from drop down menu	Typically COM 5, COM 6, or COM 7
Pump Type	Select pump type	AXP or AXP-MS, Press OK
AXP auxiliary pump to pump desolvation solvent to mixing tee		
Add Module		Repeat for another AXP pump
General	Rename Device Name to Pump_Solv	Name pump with a unique name
	Select COM Port	Use the remaining available COM channel
Pump Type	Select pump type Press OK	AXP or AXP-MS

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-ATC 600 Continuously Regenerated Trap column according to TN175, product manuals, or the instructions in the drop-down menu (Consumables, Conditioning on the Chromeleon Console panel after accessing direct control). Condition the columns 30 min according to the instructions found in the same resources.

Hydrate the Dionex ASRS 300 suppressor for 20 min as previously described in TN175 for the Dionex AERS 500 suppressor. As a precaution, do not turn on the suppressor until liquid is observed flowing out of each stage of the flow path: the AXP Auxiliary pump, the suppressor Regen Out port, and the SRD-10 device.

Consumables Device Tracking

The Consumables Device Tracking feature monitors and logs information of the installed consumable products (that have device tracking tags or wires), including: serial and lot numbers, use-by dates, number of injections, and operating history. Additionally, if incompatible consumables (e.g., cation-exchange column and an anion suppressor) are installed, this feature will issue a warning message in the Consumables Tracking panel.

The Consumables Device Tracking will also issue a warning message and prevent the sequence from starting when the instrument door has been opened recently, the sequence is new, or a new device or column has been installed. To correct any error and clear the messages:

1. Open Xcalibur software, select the Sequence Setup button, select the Status, Ready to Download under the Thermo Scientific SII for Xcalibur listing, and select Direct Control, which will open the Chromeleon Instrument Panel (Figure 6).

2. On the Chromeleon Instrument Panel, select Take Control, Consumables, and then Tracking, and Inventory. Correct any incompatibility warnings, rescan, approve, and close.

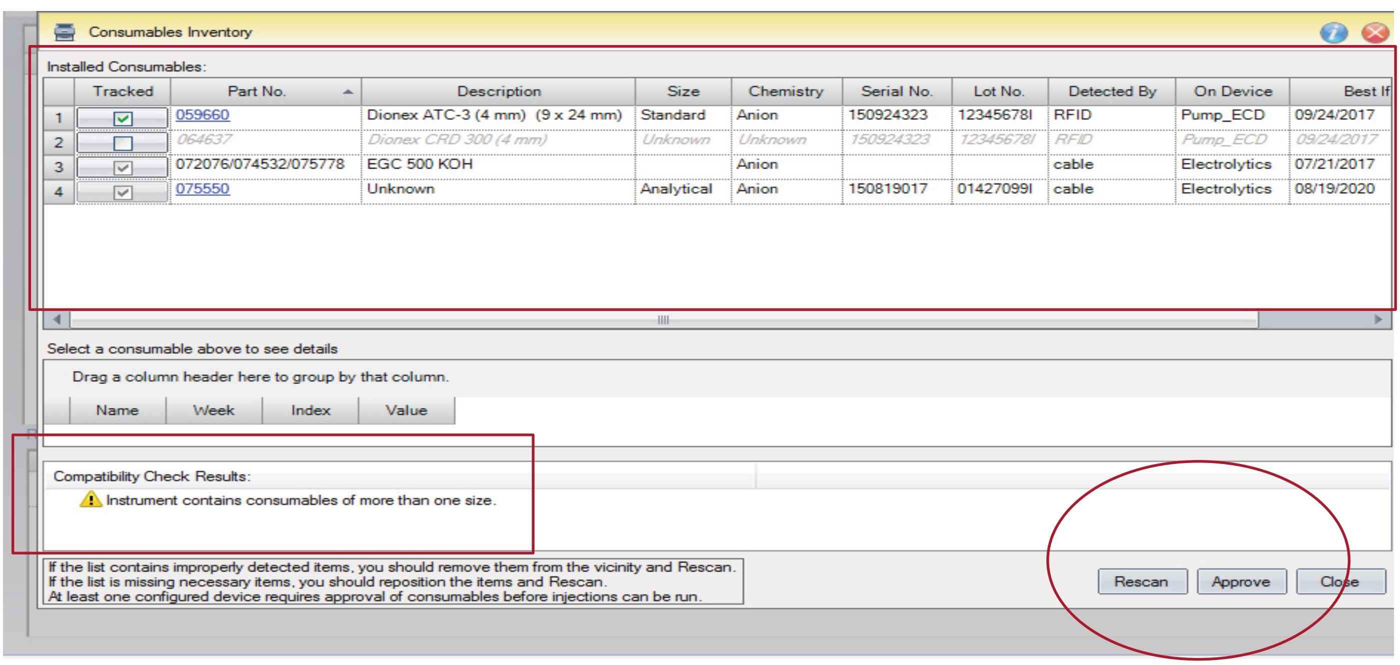


Figure 6. Approving consumables inventory and clearing errors in consumables tracking inventory

Results and discussion

Previously published methods for ionic pesticides determinations by IC-MS used the Dionex IonPac AS24 anion-exchange column, a column optimized for haloacetic acid separations at low temperatures.[20-24] We investigated other columns for this application and selected the Dionex IonPac AS19-4µm column, which is compatible with higher flow rates and temperatures above ambient. This column is optimized for the determinations of oxyhalide disinfection byproducts. To obtain a fast 15-min method for anionic pesticides, perchlorate, and oxyhalides, the conditions were modified from those used for oxyhalides by increasing the flow rate, column temperature, and the slope of the gradient. To determine the optimum flow rate of the makeup solvent, the MS response was evaluated with 1 mg/L glyphosate and AMPA (Figure 7).

Figures 8A and 8B show the SIMs of 18 analytes of interest. Figure 8A shows the SIMs of chlorite, chlorate, and bromate (all disinfection byproducts), bromide, and the quantitative and confirming ions of perchlorate. Bromide is the quantitative ion of the fungicide methyl bromide, but also the precursor of bromate in disinfection processes. Figure 8B shows 12 polar pesticides and metabolites.

Columns:	Dionex IonPac AG19-4µm, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
Eluent:	KOH gradient (not final conditions)
Detector 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 15 °C, external water mode, 0.7 mL/min by Dionex AXP-MS pump
IC-MS Interface:	Acetonitrile make-up solution
MS Detector:	HRAM MS, -ESI by HESI II probe
MS Scan Mode:	Full scan: 60–750 *m/z*, 30k resolution, AGC 1e6, max IT 100 s
MS Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 Gas flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 °C, Capillary 265 °C
MS SIM mode:	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window

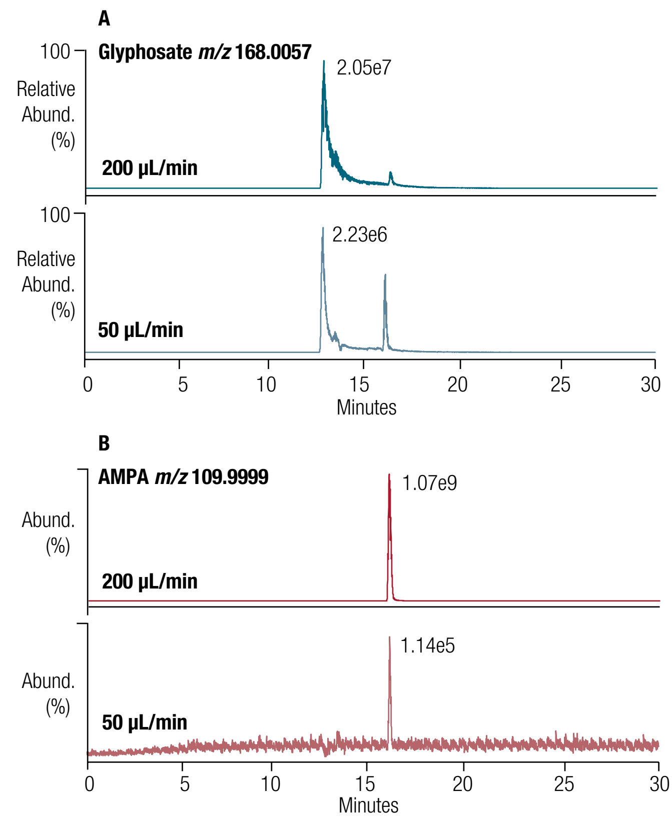
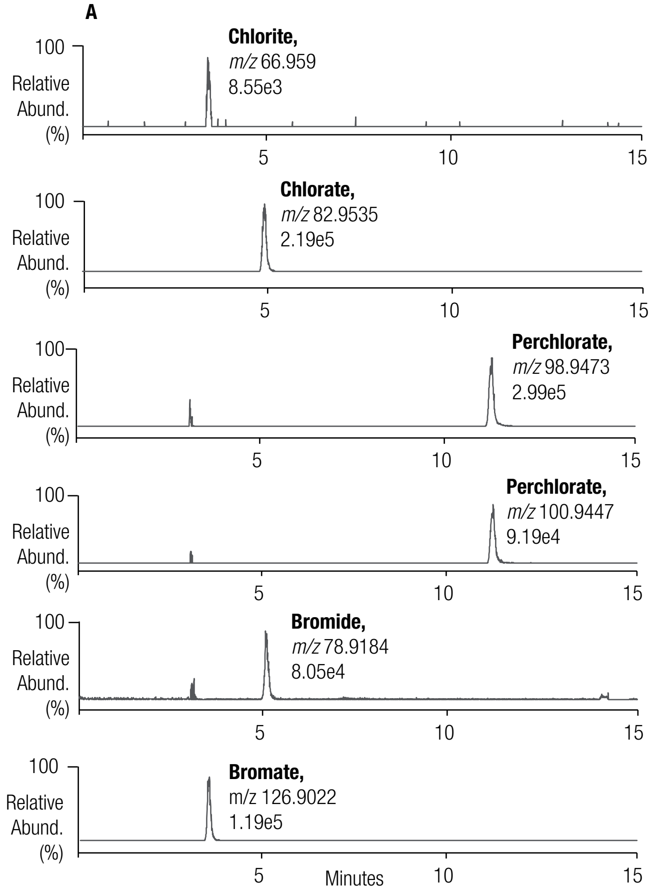


Figure 7. (A) Optimizing the acetonitrile desolvation solvent flow rate for 1 mg/L glyphosate and (B) for 1 mg/L AMPA



Columns:	Dionex IonPac AG19-4µm, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
KOH Gradient:	15 mM KOH (0–0.2 min), 15–20 mM (0.2–4 min), 20–75 mM (4–10 min), 75–75 mM (10–11.5 min), 75–15 mM (11.5), 15 mM (11.5–15 min),
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate:	0.45 mL/min
Injection Vol.:	100 µL
Column Temp.:	40 °C
Detector 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 75 mA, 15 °C, external water mode, 0.5 mL/min by Dionex AXP-MS pump
IC-MS Interface:	Acetonitrile make-up solution at 0.23 mL/min
MS Detector:	HRAM MS, -ESI by HESI II probe
MS Scan mode:	Full scan: 60–750 *m/z*, 30k resolution, AGC 1e6, max IT 100 s
MS Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 gas flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 ºC, Capillary 265 ºC
MS SIM mode:	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window
Run time:	15 min
Standard:	1 µg/L of oxyhalide mixed standard added

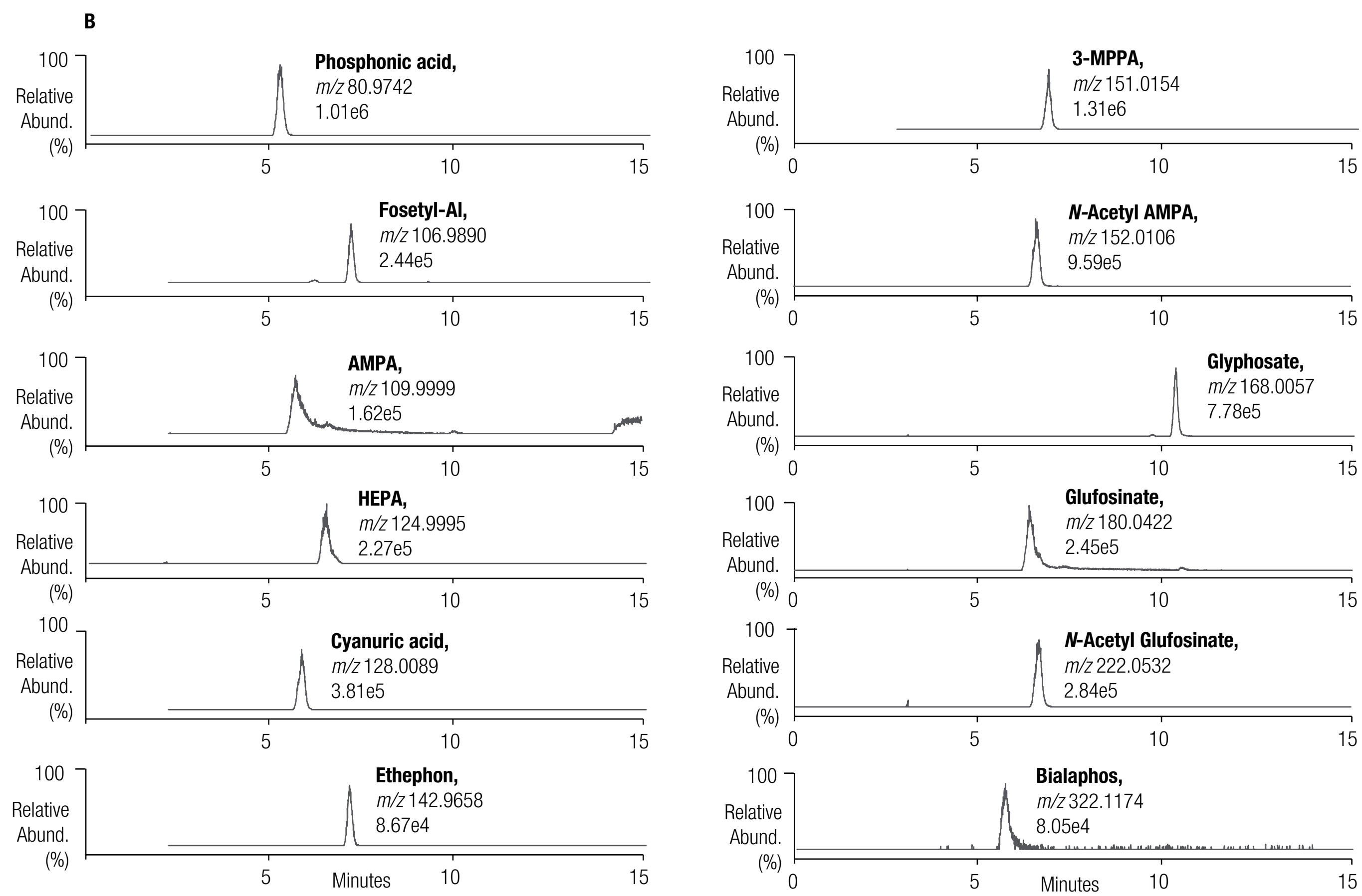


Figure 8. (A) 1 µg/L oxyhalides, perchlorate, and bromide and (B) 10 µg/L polar pesticides

Method evaluation

To evaluate this technique for sample analysis, calibration curves were generated from 50 to 2000 ng/L using triplicate injections. Limit of detections were defined using the SANTE definition of 20% RSDs.[11] The LODs were 0.1 to 0.5 µg/L for the oxyhalides, bromide, and perchlorate; ~1 µg/L for AMPA and bialaphos; and 0.05 to 0.1 µg/L for the other polar pesticides.

Sample analysis

The strawberry extracts were scanned for the 18 analytes. Only bromide (Figure 9) and chlorate (not shown) were detected. While bromide was detected at ng/L concentrations in the strawberry samples, no bromate was detected (Figure 9). Methylbromide is commonly applied to strawberries to prevent fungal growth, and the lack of bromate suggests that the bromide is possibly a pesticide residue.

Accuracies were determined by recovery determinations of 1 µg/L combined standard (oxyhalides, polar pesticides, perchlorate) added to the strawberry sample extracts after sample preparation and the diluted beer samples (Figures 9–12). Recoveries were 90–95% for all three samples, showing good method accuracy.

Perchlorate contamination is commonly due to irrigation with perchlorate-contaminated water. Figure 10 shows perchlorate determinations in the strawberry sample before (Figure 10A) and after adding the mixed standard (Figure 10B). Perchlorate ion *m/z* 98.947 and the confirming ion *m/z* 100.9447 must both be present for the confirmation of the presence of perchlorate. Figure 10A shows that perchlorate is not present in the strawberry sample. Figure 10B shows the presence of both isotopes when perchlorate is present.

Columns:	Dionex IonPac AG19-4µm, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
KOH Gradient:	15 mM KOH (0–0.2 min), 15–20 mM (0.2–4 min), 20–75 mM (4–10 min), 75–75 mM (10–11.5 min), 75–15 mM (11.5), 15 mM (11.5–15 min),
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate:	0.45 mL/min
Injection Vol.:	100 µL
Column Temp.:	40 °C
Detector 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 75 mA, 15 °C, external water mode, 0.5 mL/min by Dionex AXP-MS pump
IC-MS Interface:	Acetonitrile make-up solution at 0.23 mL/min
MS Detector:	HRAM MS, -ESI by HESI II probe
MS Scan mode:	Full scan: 60–750 *m/z*, 30k resolution, AGC 1e6, max IT 100 s
MS Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 gas flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 °C, Capillary 265 °C;
MS SIM mode:	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window
Run time:	15 min
Sample Prep.:	QuPPe, 5-fold diluted
Sample:	A) Strawberry extract B) 1 µg/L of oxyhalide mixed standard added
SIMS:	Bromide: *m/z* 78.9814; Bromate: 126.9023

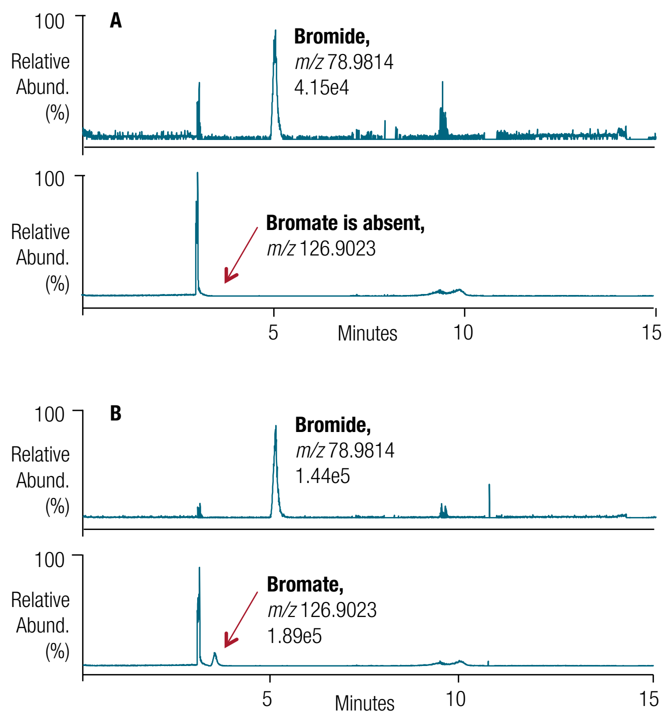


Figure 9. Bromide detected in a strawberry sample: A) present at ~100 ng/L, (B) 1 µg/L bromate added. Bromate is absent in sample.

Columns:	Dionex IonPac AG19-4µm, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
KOH Gradient:	15 mM KOH (0–0.2 min), 15–20 mM (0.2–4 min), 20–75 mM (4–10 min), 75–75 mM (10–11.5 min), 75–15 mM (11.5), 15 mM (11.5–15 min),
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate:	0.45 mL/min
Injection Vol.:	100 µL
Column Temp.:	40 °C
Detector 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 75 mA, 15 °C, external water mode, 0.5 mL/min by Dionex AXP-MS pump
IC-MS Interface:	Acetonitrile make-up solution at 0.23 mL/min
MS Detector:	HRAM MS, -ESI by HESI II probe
MS Scan mode:	Full scan: 60–750 *m/z*, 30k resolution, AGC 1e6, max IT 100s
MS Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 gas flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 °C, Capillary 265 °C;
MS SIM mode:	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window
Sample Prep.:	QuPPe, 5-fold diluted
Sample:	A) Strawberry extract; B) 1 µg/L of perchlorate standard added
SIMS:	Perchlorate: *m/z* 98.9478, 100.9447

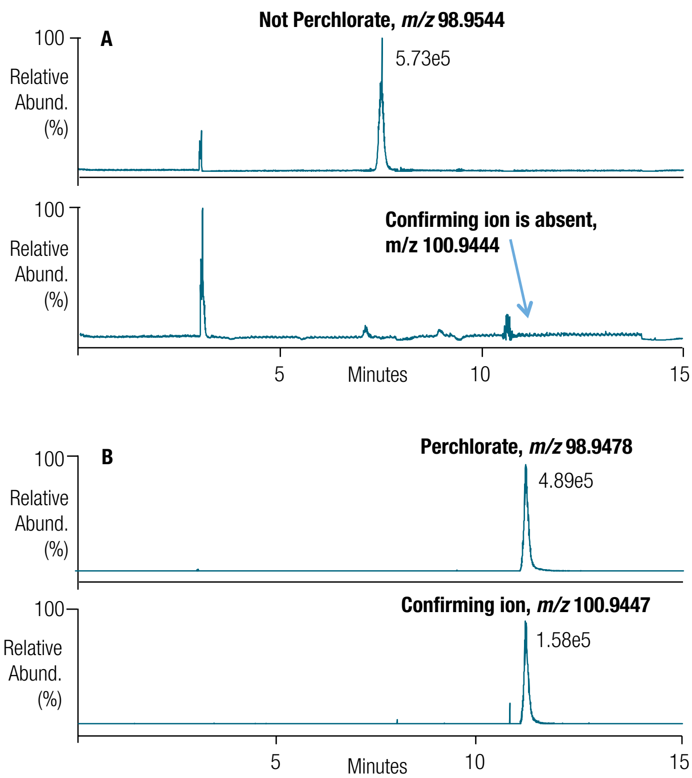


Figure 10. Perchlorate determinations in a strawberry sample: (A) not present; (B) 1 µg/L added

Seven beer samples (a barley-rice beer, gluten-free beer using sorghum, a non-alcoholic barley beer, two other barley beers, a wheat beer, and a maize-barley beer) were analyzed for polar pesticides. Glyphosate was found in the barley-rice beer, gluten-free sorghum beer, and the non-alcoholic barley beer (Figure 11). Figure 12 shows SIMs of eight polar pesticides added to a 10-fold diluted non-alcoholic barley beer.

Columns:	Dionex IonPac AG19-4µm, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
KOH Gradient:	15 mM KOH (0–0.2 min), 15–20 mM (0.2–4 min), 20–75 mM (4–10 min), 75–75 mM (10–11.5 min), 75–15 mM (11.5), 5 mM (11.5–15 min),
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate:	0.45 mL/min
Injection Vol.:	100 µL
Column Temp.:	40 °C
Detector 1:	Suppressed conductivity, Dionex ASRS 300 suppressor, 75 mA, 15 °C, external water mode, 0.5 mL/min by Dionex AXP-MS pump
IC-MS Interface:	Acetonitrile make-up solution at 0.23 mL/min
MS Detector:	HRAM MS, -ESI by HESI II probe
MS Scan mode:	Full scan: 60–750 m/z, 30k resolution, AGC 1e6, max IT 100s
MS Source:	Spray: 2.5 kV; S-Lens level, 50
MS N_2 gas flow:	Sheath: 50, Aux: 13; Sweep: 3 arbitrary units
MS Temperatures:	Aux: 425 °C, Capillary 265 °C;
MS SIM mode:	t-SIM mode, 15k resolution, AGC 3e5, max IT 100 ms, 2 amu window
Sample Prep.:	A) 10-fold diluted, B-C) Undiluted
Sample:	A) Rice-barley beer B) Gluten-free sorghum beer, C) Non-alcoholic barley beer D) 10 µg/L of glyphosate
SIMS:	Glyphosate: *m/z* 168.0057

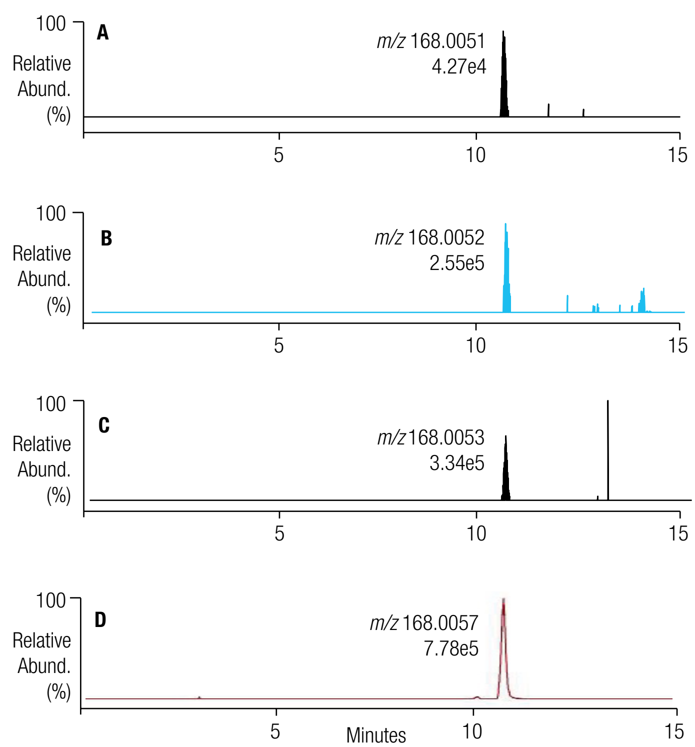


Figure 11. Native glyphosate determinations in beer samples (A–C), and (D) 10 µg/L standard

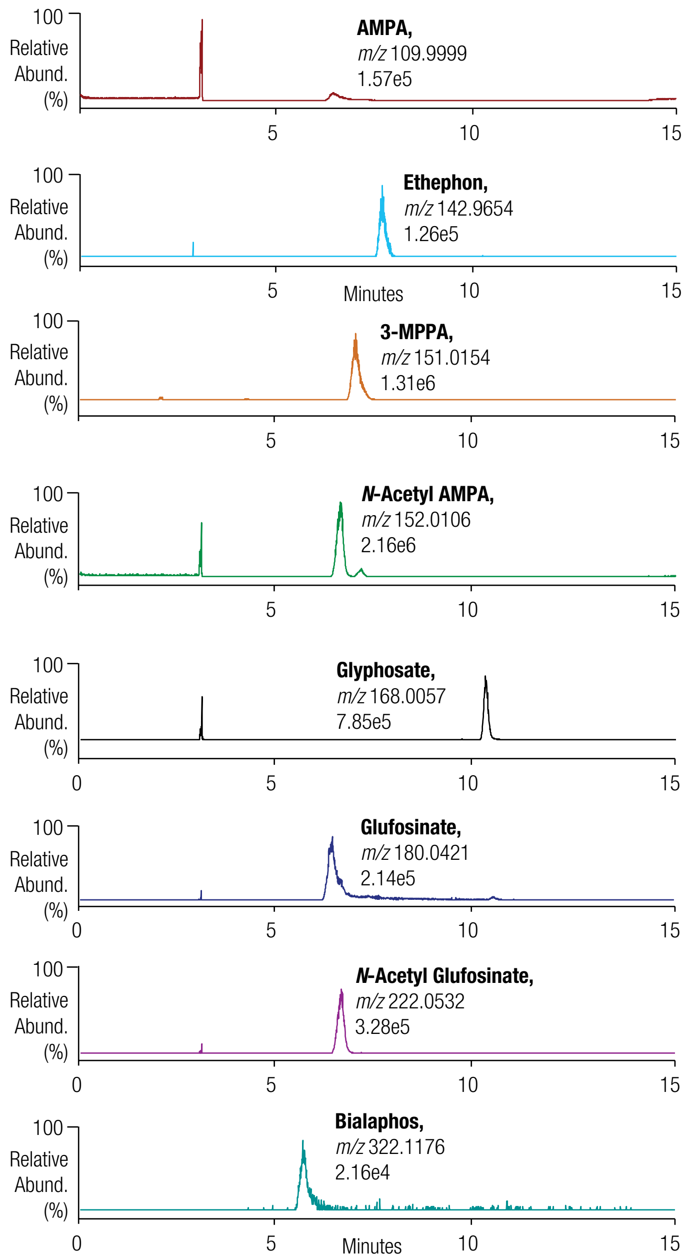


Figure 12. Eight (10 µg/L) polar pesticides added to 10-fold diluted non-alcoholic barley beer

Accuracies were determined by recovery determinations of 1 µg/L of glyphosate and AMPA added to one beer sample (non-alcoholic barley beer). Recoveries were 78–83% for AMPA and 88–92% for glyphosate, showing acceptable method accuracy.

Conclusion

This application note demonstrates fast direct determinations of 18 analytes that include 14 residual polar pesticides, three DBPs, and perchlorate using IC-HRAM-MS. The method uses a Dionex Integrion HPIC system and Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer. These determinations are essential to the food industry and food regulatory industry to ensure food safety or investigate possible food safety issues.

The method was applied to homogenized strawberry samples and seven diluted beer samples. Bromide at sub-ppb concentrations was found in the strawberries, which could be due to methylbromide, a common strawberry fungicide. Glyphosate at sub-ppb concentrations was determined in three of the beer samples: a rice-barley beer, a non-alcoholic barley beer, and a gluten-free sorghum beer. It is interesting to note that glyphosate was not found in any of the wheat beers despite the common application of glyphosate as a desiccant to wheat crops.

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