- Disinfection Byproducts
- Acrylamide

Food Safety Applications Notebook

Processing Contaminants

Thermo SCIENTIFIC

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Introduction to Food Safety

Food contamination stories in the news media have raised awareness of the fact that we live with a global food supply chain, and food safety is increasingly becoming an important concern. All types of fruits, vegetables, seafood, and meat can be purchased year round independent of the local growing season. For example, in many countries, well-stocked grocery stores carry cantaloupes from Guatemala, cucumbers from Mexico, shrimp from Vietnam, and fish from China. With fruit, vegetables, seafood, and meat traveling thousands of miles to reach far-flung destinations, and with poor or no knowledge of the agricultural practices, the need for food testing is increasingly important.

Thermo Fisher Scientific understands the demands of food safety related testing. Our separation and detection technologies, combined with experienced applications competence, and our best suited chemistries provide solutions for the analysis of inorganic ions, small drug molecules, pesticides to large components, such as polysaccharides. Your laboratory can now conduct reliable, accurate, and fast testing of food. This notebook contains a wide range of food safety related application notes that will help address your food safety issues.

Thermo Scientific and Dionex Integrated Systems

Dionex Products are now a part of the Thermo Scientific brand, creating exciting new possibilities for scientific analysis. Now, leading capabilities in liquid chromatography (LC), ion chromatography (IC), and sample preparation are together in one portfolio with those in mass spectrometry (MS). Combining Dionex's leadership in chromatography with Thermo Scientific's leadership position in mass spec, a new range of powerful and simplified workflow solutions now becomes possible.

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- Liquid Chromatography and Mass Spectrometry
- Sample Preparation and Mass Spectrometry

UltiMate 3000 UHPLC+ Systems

Best-in-class HPLC systems for all your chromatography needs

Thermo Scientific Dionex UltiMate 3000 UHPLC+ Systems provide excellent chromatographic performance while maintaining easy, reliable operation. The basic and standard analytical systems offer ultra HPLC (UHPLC) compatibility across all modules, ensuring maximum performance for all users and all laboratories. Covering flow rates from 20 nL/min to 10 mL/min with an industry-leading range of pumping, sampling, and detection modules, UltiMate™ 3000 UHPLC+ Systems provide solutions from nano to semipreparative, from conventional LC to UHPLC.

- Superior chromatographic performance
- UHPLC design philosophy throughout nano, standard analytical, and rapid separation liquid chromotography (RSLC)
- 620 bar (9,000 psi) and 100 Hz data rate set a new benchmark for basic and standard analytical systems
- RSLC systems go up to 1000 bar and data rates up to 200 Hz
- ×2 Dual System for increased productivity solutions in routine analysis
- Fully UHPLC compatible advanced chromatographic techniques
- Thermo Scientific Dionex Viper and nanoViper–the first truly universal, fingertight fitting system even at UHPLC pressures

Thermo Fisher Scientific is the only HPLC company uniquely focused on making UHPLC technology available to all users, all laboratories, and for all analytes.

Rapid Separation LC Systems: The extended flow-pressure footprint of the RSLC system provides the performance for ultrafast high-resolution and conventional LC applications.

RSLCnano Systems: The Rapid Separation nano LC System (RSLCnano) provides the power for high-resolution and fast chromatography in nano, capillary, and micro LC.

Standard LC Systems: Choose from a wide variety of standard LC systems for demanding LC applications at nano, capillary, micro, analytical, and semipreparative flow rates.

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A complete range of ion chromatography solutions for all customer performance and price requirements

For ion analysis, nothing compares to a Thermo Fisher Scientific ion chromatography system. Whether you have just a few samples or a heavy workload, whether your analytical task is simple or challenging, we have a solution to match your needs and budget. And with your IC purchase, you get more than just an instrument—you get a complete solution based on modern technology and world-class support.

- Thermo Scientific Dionex ICS-5000: The world's first capillary IC system
- Dionex ICS-2100: Award-winning integrated Reagent-Free™ IC system
- Dionex ICS-1600: Standard integrated IC system
- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

Ranging from the Dionex ICS-900 to the ICS-5000, these IC systems cover the entire range of IC needs and budgets and come with superior support and service worldwide.

Dionex ICS-5000: Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000 combines the highest sensitivity with convenience

Dionex ICS-2100: An integrated Reagent-Free IC (RFIC™) system for electrolytically generated isocratic and gradient separations with conductivity detection, now with electrolytic sample preparation.

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Single-point control and automation for improved ease-of-use in LC/MS and IC/MS

Thermo Fisher Scientific provides advanced integrated IC/MS and LC/MS solutions with superior ease-of-use and modest price and space requirements. UltiMate 3000 System Wellness technology and automatic MS calibration allow continuous operation with minimal maintenance. The Dionex ICS-5000 instrument and the family of RFIC systems automatically remove mobile phase ions for effort-free transition to MS detection.

- Thermo Scientific MSQ Plus mass spectrometer, the smallest and most sensitive single quadrupole on the market for LC and IC
- Self-cleaning ion source for low-maintenance operation
- Thermo Scientific Dionex Chromeleon Chromatography Data System software for single-point method setup, instrument control, and data management
- Compatible with existing IC and LC methods
- The complete system includes the MSQ Plus™ mass spectrometer, PC datasystem, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vaccum system

You no longer need two software packages to operate your LC/MS system. Chromeleon™ LC/MS software provides single-software method setup and instrument control; powerful UV, conductivity, and MS data analysis; and fully integrated reporting.

MS Systems and Modules: MSQ Plus Mass Spectrometer; MSQ18LA nitrogen gas generator; Thermo Scientific Dionex AXP-MS digital auxiliary pump

Chromeleon 7 Chromatography Data System Software

The fastest way to get from samples to results

Discover Chromeleon software version 7, the chromatography software that streamlines your path from samples to results. Get rich, intelligent functionality and outstanding usability at the same time with Chromeleon software version 7—the Simply Intelligent™ chromatography software.

- Enjoy a modern, intuitive user interface designed around the principle of operational simplicity
- Streamline laboratory processes and eliminate errors with eWorkflows, which enable anyone to perform a complete analysis perfectly with just a few clicks
- Access your instruments, data, and eWorkflows instantly in the Chromeleon Console
- Locate and collate results quickly and easily using powerful built-in database query features
- Interpret multiple chromatograms at a glance using MiniPlots
- Find everything you need to view, analyze, and report data in the Chromatography Studio
- Accelerate analyses and learn more from your data through dynamic, interactive displays
- Deliver customized reports using the built-in Excel-compatible speadsheet

Chromeleon software version 7 is a forward-looking solution to your long-term chromatography data needs. It is developed using the most modern software tools and technologies, and innovative features will continue to be added for many years to come.

The Cobra™ integration wizard uses an advanced mathematical algorithm to define peaks. This ensures that noise and shifting baselines are no longer a challenge in difficult chromatograms. When peaks are not fully resolved, the SmartPeaks™ integration assistant visually displays integration options. Once a treatment is selected, the appropriate parameters are automatically included in the processing method.

Chromeleon software version 7 ensures data integrity and reliability with a suite of compliance tools. Compliance tools provide sophisticated user management, protected database stuctures, and a detailed interactive audit trail and versioning system.

Process Analytical Systems and Software

Improve your process by improving your process monitoring with a Thermo Scientific Dionex on-line IC or HPLC system

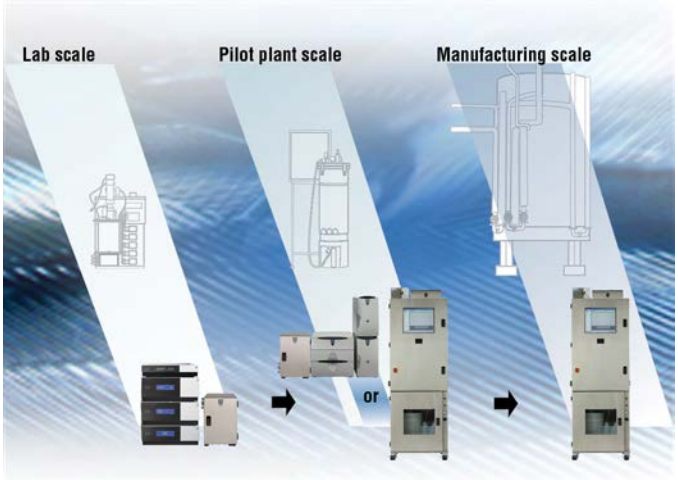
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, precise, accurate information faster than is possible with laboratory-based results. From the lab to the factory floor, your plant's performance will benefit from the information provided by on-line LC.

- Characterize your samples completely with multicomponent analysis
- Reduce sample collection time and resources with automated multipoint sampling
- Improve your process control with more timely results
- See more analytes with unique detection capabilities
- 25 years of experience providing on-line IC and HPLC capabilities to a wide range of industries
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best meets your needs

The Integral Migration Path™ approach enables on-line IC/HPLC to generate timely, high-resolution information when monitoring a small-scale reactor in a process R&D lab, in a pilot plant, or improving current manufacturing plant processes. No matter what the application, the Integral™ process analyzer has the versatility to place a solution using on-line IC/HPLC, whenever and wherever it is needed.

Integral: The Integral Migration Path approach: System solutions wherever you need them: lab, pilot plant, or manufacturing

Chromeleon Process Analytical (PA) Software: Chromeleon PA software provides unique capabilities to support on-line IC or HPLC analysis



Automated Sample Preparation

ACCELERATED SOLVENT EXTRACTORS

Two new solvent extraction systems with pH-hardened Dionium components

We offer two solvent extraction systems. The Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor is an entry-level system with a single extraction cell, for laboratories with modest throughput. The Dionex ASE™ 350 system is a sequential extraction system capable of automated extraction of up to 24 samples. Both systems feature chemically inert Dionium components that allow the extraction of acid- or base-pretreated samples.

SOLID-PHASE EXTRACTION SYSTEMS

Faster, more reliable solid-phase extraction while using less solvent

The Thermo Scientific Dionex AutoTrace 280 Solid-Phase Extraction (SPE) instrument unit can process six samples simultaneously with minimal intervention. The instrument uses powerful pumps and positive pressure with constant flow-rate technology. Current analytical methods that require SPE sample preparation include gas chromatography (GC), GC-MS, LC, and LC-MS, IC and IC-MS. The Dionex AutoTrace™ 280 instrument is approved or adapted for U.S. EPA clean water methods and safe drinking water methods (600 and 500 series) and can extract the following analytes:

- PCBs (polychlorinated biphenyls)
- OPPs (organophosphorus pesticides), OCPs (organochlorine pesticides), and chlorinated herbicides
- BNAs (base, neutral, acid semivolatiles)
- Dioxins and furans
- PAHs (polyaromatic hydrocarbons)
- Oil and grease or hexane extractable material

With SPE, large volumes of liquid sample are passed through the system and the compounds of interest are trapped on SPE adsorbents (cartridge or disk format), then eluted with strong solvents to generate an extract ready for analysis. Automated SPE saves time, solvent, and labor for analytical laboratories.

Dionex AutoTrace Systems: The new Dionex AutoTrace 280 system provides fast and reliable automated solid phase extraction for organic pollutants from liquid samples

Dionex AutoTrace Accessories: High-quality parts and accessories are available for Dionex AutoTrace 280 instruments

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Analysis of Processing Contaminants

Fast Determination of Acrylamide in Food Samples Using Accelerated Solvent Extraction (ASE®) Followed by Ion Chromatography with UV or MS Detection

INTRODUCTION

Acrylamide, a known genotoxic compound, was recently detected in carbohydrate-rich fried or baked food samples by a Swedish research group, Tareke, et al.[1] The content of acrylamide was as high as several mg/kg for typical samples such as hash browns and french fries. Published methods for acrylamide include U.S. EPA Method 8032A that uses liquid extraction and GC-ECD for determinations in water[2] and a method by the German Health Agency (BGVV) that uses HPLC with UV detection for migration analysis of acrylamide from food packing materials.[3]

The method presented here consists of a fast, automated extraction method using accelerated solvent extraction (ASE).[4] Samples were extracted in 20 min using pure water, water with 10 mM formic acid, or acetonitrile. The extracts were directly analyzed by ion chromatography (IC) using a 4-mm ion-exclusion column and both UV and MS detection. With this column, acrylamide is retained longer than on conventional reversed-phase columns, allowing separation from the many coextractable compounds present in food samples. Results were obtained for acrylamide in french fries, potato chips, and crisp bread. The benefits of this method are simplicity, speed of analysis, and a degree of automation that allows the analysis of large numbers of samples with minimal labor.

CONDITIONS

Extraction Conditions

Solvent:	Water, 10 mM formic acid, or acetonitrile
Temperature:	80 °C
Pressure:	10 MPa
Heatup Time:	5 min
Static Time:	4 min
Number of Static Cycles:	3
Flush Volume:	60%
Purge Time (N_2):	120 s

Chromatographic Conditions

Column:	IonPac® ICE-AS1, 4 × 250 mm, 7.5 µm, SP6003
Eluent:	3.0 mM formic acid in acetonitrile/water 30/70 (v/v)
Flow Rate:	0.15 mL/min
Inj. Volume:	25 µL
UV Detection:	202 nm
MS Detection:	ESI+: 3.0 kV, cone 50 V, probe temp. 300 °C, scan 50–250 *m/z,* SIM 72 *m/z*

EXPERIMENTAL

Extraction

Samples of 5 g were extracted using an accelerated solvent extraction system, (ASE 100 or ASE 200, Dionex, Sunnyvale, CA, USA) with 34-mL cells for the ASE 100, and 33-mL cells for the ASE 200.

Chromatography

Chromatographic analyses were performed on an DX-600 ion chromatograph, (Dionex, Sunnyvale, CA, USA) that included a GS50 gradient pump, PDA-100 photodiode array detector set at 202 nm, an MSQ™ single quadrupole mass spectrometer, and an AS50 Autosampler. A 250 × 4 mm i.d. IonPac ICE-AS1 (Dionex, Sunnyvale, CA, USA) analytical column (7.5-µm cross-linked polystyrene divinylbenzene functionalized with sulfonate functional groups) was used to separate acrylamide from the matrix compounds. All measurements were made at 30 °C and all samples were filtered through 0.45-µm filters. A 25-µL sample loop was used for all the determinations. Data collection and the operation of all components in the system was controlled by Dionex Chromeleon® 6.40 chromatography software.

Reagents and Standards

All reagents were analytical-grade. Formic acid was Suprapur (Merck, Darmstadt, Germany), and acetonitrile was HPLC reagent-grade (Novachimica, Milano, Italy). Ultrapure water with conductivity <0.1 ΩS (DI water) was obtained from a Milli-Q® system (Millipore, Bedford, MA, USA). Working standard solutions of acrylamide were prepared by serial dilution of a 1000-mg/L stock standard solution.

Samples

French fries, potato chips, tortilla chips, wheat snacks with bacon flavor, and crisp bread were obtained from a local food store. Representative samples (5 g) were loaded into 34-mL extraction cells onto a glass fiber filter. Samples like wheat snacks with bacon flavor or bread samples, which have a tendency to dissolve or swell, were loaded into Soxhlet thimbles that were then placed in the extraction cells. Any void volumes were filled with glass beads (1-mm i.d.) to reduce the volume of the extraction solvent.

RESULTS AND DISCUSSION

ASE Extraction

Pure water, water with 10 mM formic acid, and acetonitrile were tested as the extraction solvent. Pure water extracts showed lower recoveries than the formic acid, but the formic acid extracts had lower stability. Extracts produced using acetonitrile were cleaner, as less material was coextracted from the sample matrix. The extraction temperature of 80 °C was chosen, because acrylamide starts to decompose at temperatures above 83 °C. With three extraction cycles of 4-min durations, a spiked french fries sample had a yield of 95% in the first extract and an additional 8% in the second extraction of the same sample using 10 mM formic acid.

Cleanup

The extract volume was determined using a volumetric flask. Afterward, the extracts were filtered using a 0.22-µm nylon filter. Further cleanup using solid phase extraction or liquid extraction did not exhibit any significant improvements for the subsequent chromatographic analyses.

Analysis of Acrylamide Using IC/UV

The separation of acrylamide was performed using an IC system with a UV detector. Formic acid was chosen as the eluent instead of sulphuric acid because it is more compatible with MS detection. The amount of acetonitrile was optimized to 30% v/v to reduce the total run time and avoid interferences with matrix components.

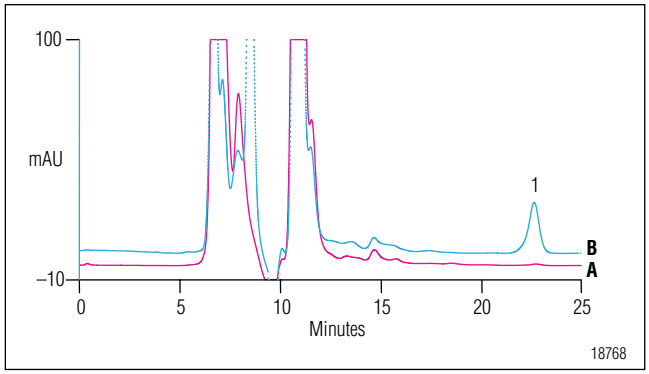

Figure 1. Comparison of french fry samples. Chromatographic conditions are listed under "Conditions". UV detection. (a) neat; (b) spiked with 40 µg of acrylamide. Peak: 1. Acrylamide.

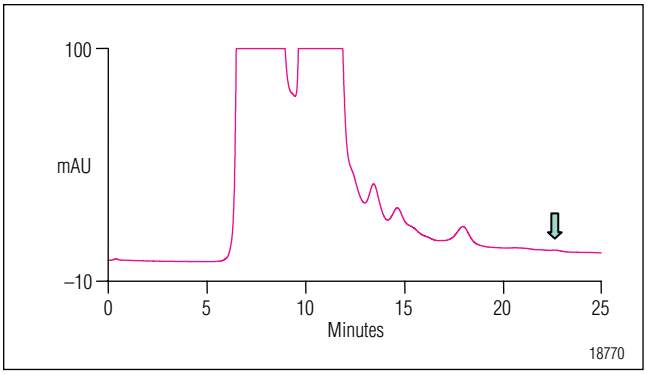

Figure 3. Chromatogram of a crisp bread sample with low acrylamide content. Chromatographic conditions are listed under "Conditions". UV detection. Peaks: arrow indicates acrylamide retention time.

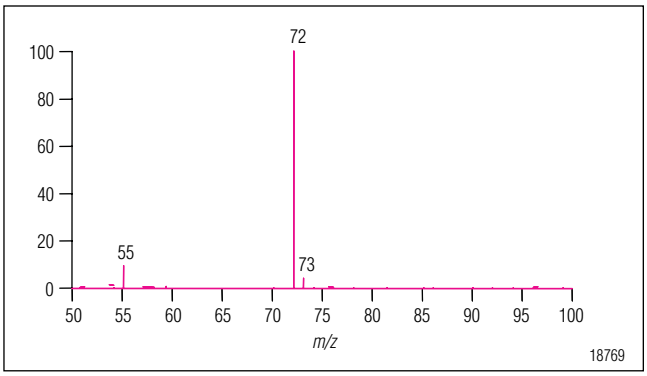

Figure 2. Mass spectrum of acrylamide in the range 50–100 m/z. Mass spectrometric conditions are listed under "Conditions".

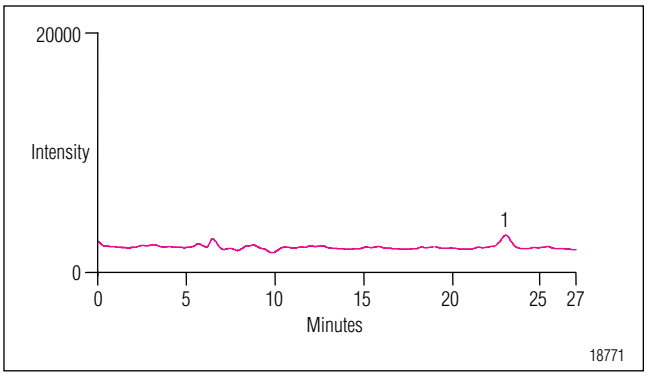

Figure 4. Chromatogram of a crisp bread sample with low acrylamide content. Chromatographic conditions are listed under "Conditions". MS detection SIM mode. Peak: 1. Acrylamide 0.08 mg/Kg.

Analysis of Acrylamide Using IC/MS

A single-stage quadrupole mass spectrometric detector (Thermo Election MSQ—Dionex, Sunnyvale, CA) was installed in series with the UV detector. The MS was operated in the positive electrospray (ESI+) ionization mode. Figure 2 shows a mass spectrum of acrylamide at a cone voltage of 50 V. The protonated molecular ion $[M+H]^+$ of acrylamide is detected at a mass-to-charge ratio *(m/z)* 72. In addition, a fragment ion is observed at *m/z* 55. This lower-intensity fragment ion can be used for confirmation while a more sensitive detection is achieved at *m/z* 72. Calibrations were performed using external standards in the range 0.01–1 mg/L. The corresponding calibration plots for both UV and MS detection show good linearity in the range 0.01–10 mg/L ($r^2 = 0.996$).

CONCLUSION

The ASE method provides a fast and efficient extraction of acrylamide from various food samples. The extracted samples were analyzed directly using IC with UV or MS detection. Although UV detection is sufficient for most of the analyzed samples, MS detection offers a higher specificity and sensitivity, as shown in Figures 3–6. Results are summarized in Table 1. The required limits of determination of 50 µg/kg acrylamide in food can be achieved with this method. This method is robust, selective, and relatively easy to perform.

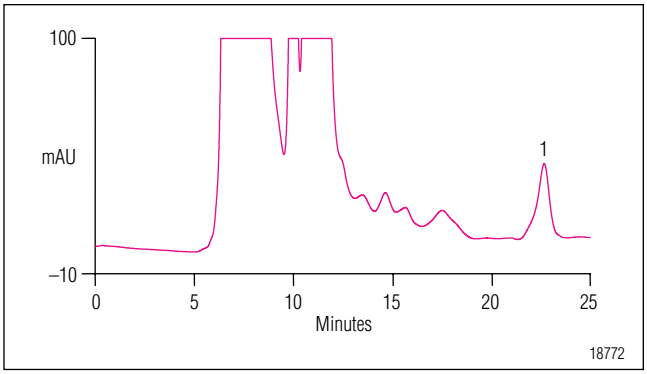

Figure 5. Chromatogram of a potato chips sample with high acrylamide content. Chromatographic conditions are listed under "Conditions". UV detection. Peak: 1. Acrylamide 1.56 mg/Kg.

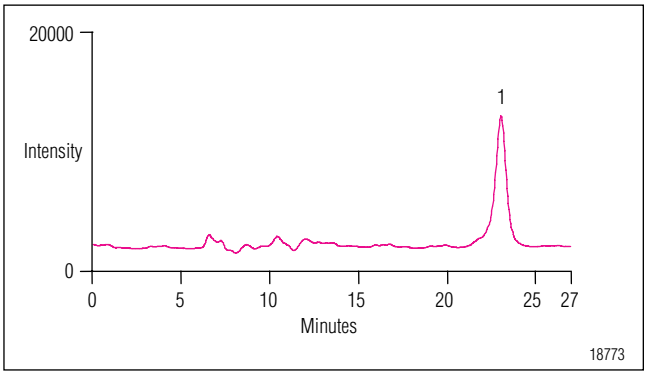

Figure 6. Chromatogram of a potato chips sample with high acrylamide content. Chromatographic conditions are listed under "Conditions". MS detection SIM mode. Peak: 1. Acrylamide 1.06 mg/Kg.

Table 1. Acrylamide Contents of Food Samples

Sample	Acrylamide UV* mg/kg	Acrylamide MS** mg/kg
Chips bacon flavored	n.d.	<0.05
Crisp bread	n.d.	0.08
Potato chips	1.57	1.06
Tortilla chips	n.d.	n.d.
French fries	0.11	0.10
French fries spiked	0.70[a]	0.69[b]

* 202 nm; ** SIM 72 m/z; [a] recovery 96.4%; [b] recovery 95.1%

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4. Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. *Anal. Chem.* **1996,** *68,* 1033.

Extraction and Cleanup of Acrylamide in Complex Matrices Using Accelerated Solvent Extraction (ASE®) Followed by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

INTRODUCTION

Acrylamide is formed during the cooking process of certain plant-based foods which are rich in carbohydrates and low in protein. Specifically, it forms when asparagine reacts with sugars such as glucose at high temperatures. Acrylamide was detected in fried foods by the Swedish National Food Authority in 2002. Since then, many food laboratories have successfully performed determinations for this compound on a variety of different food matrices. Acrylamide is a known carcinogen in animals.

ASE is an excellent technique for extraction of acrylamide from various fried food products; until recently, however, extraction of this compound from matrices such as coffee and chocolate has proven difficult. Traditional extraction techniques are time consuming, and may cause bottlenecks in sample preparation. This Application Note describes a new ASE method that combines the extraction of low-levels of acrylamide from coffee and chocolate with an in-cell, solid-phase cleanup step.

EQUIPMENT

Dionex ASE 200 with 33-mL stainless steel extraction cells (P/N 048763)

Dionex cellulose filters (P/N 049458)

Dionex collection vials 60 mL (P/N 048784)

Dionex SE 500 Solvent Evaporator (P/N 063221, 120 v) (P/N 063218, 240 v)

Standard laboratory tissue homogenizer

Standard laboratory centrifuge (rated to 10,000 rpm or greater)

Centrifuge tubes (40–50 mL)

CHEMICALS AND REAGENTS

Acrylamide, purity 99% (Sigma-Aldrich)

d3-Acrylamide (2,3,3-*d3*-2-propenamide) (Cambridge, Isotope Laboratories USA)

Florisil, 60–100 mesh (Sigma-Aldrich)

Potassium hexacyanoferrate (II) trihydrate (Carrez I) (Sigma-Aldrich)

Zinc sulfate heptahydrate (Carrez II) (Sigma-Aldrich)

Dionex ASE Prep DE (P/N 062819)

Termamyl® 120 L (Type L thermostable amyloglucosidase enzyme) (Novozymes, Denmark)

Ethyl acetate (Fisher Scientific, HPLC Grade)

Dichloromethane (Fisher Scientific, HPLC Grade)

Methanol (Fisher Scientific, HPLC Grade)

FOOD SAMPLES

The coffee and chocolate samples were purchased from a local grocery store and stored at room temperature.

REAGENT SOLUTIONS

0.68 M potassium hexacyanoferrate (II) trihydrate (Carrez I) solution

Dissolve 28.722 g of $K_4Fe(CN)6\ 3H_2O$ in 100 mL of water.

2 M zinc sulfate heptahydrate (Carrez II) solution

Dissolve 57.512 g of $ZnSO_4\ 7H_2O$ in 100 mL of water.

STANDARD SOLUTIONS

Prepare aqueous stock solutions of acrylamide and *d3*-acrylamide at concentrations of 50 and 5 µg/mL, respectively.

To make 50 µg/mL acrylamide, add 5 mg to 100 mL water.

To make 5 µg/mL *d3*-acrylamide, add 0.5 mg to 100 mL water.

Dilute the acrylamide solutions in water to obtain the following matrix-equivalent levels: 0, 10, 50, 200, 500, and 2500 µg/kg. The matrix-equivalent concentration of *d3*-acrylamide should be 250 µg/kg.

SAMPLE PREPARATION

Hydrolysis

Weigh 2.0 g of sample into a centrifuge tube and add 10 mL of water, (heated to 60 °C) then add 50 µL of Termamyl. Place the tube in a water bath at 90°C for 45 min. Homogenize the mixture for 1 min. To precipitate the proteins, add 1 mL of the 0.68 M potassium hexacyanoferrate (II) trihydrate solution and 1 mL of the 2 M zinc sulfate heptahydrate solution to the centrifuge tube, swirling constantly for 1–2 min. Add 5 mL dichloromethane and swirl for an additional minute. Centrifuge for 15 min at 10,000 rpm.

Preparing the ASE Cell

Prepare the 33-mL extraction cell by successively inserting: (1) a cellulose filter, (2) 6 g Florisil deactivated with 3% deionized water, (3) a second cellulose filter, and (4) at least 8 g ASE Prep DE so that there is approximately 0.5 cm of empty space at the top of the cell.

Transfer 6 mL of the supernatant from the centrifuge tube and drip onto the ASE Prep DE layer in the prepared extraction cell. Fill the extraction cell to the top with additional ASE Prep DE and cover with a third cellulose filter. Carefully place the extraction cell cap on the cell and tighten by hand.

ASE CONDITIONS

Solvent:	Ethyl acetate (100%)
Temperature:	Ambient*
Pressure:	2000 psi
Static Time:	3 min
Static Cycles:	3
Flush:	100%
Purge:	60 s

** Although the authors of this Application Note used ambient temperature for the extraction, this is not a typical extraction temperature for ASE. Normal extraction temperatures range from 40–200 °C. If recoveries are lower than expected using ambient temperature, increasing temperature may improve results.*

EXTRACTION

Place the prepared extraction cells onto the ASE carousel. Enter the ASE conditions into the *Method Editor* screen, and save this method with the desired number. Begin the extraction by pushing *Start*. The method can also be set up as a Schedule in the *Schedule Editor* screen. Running the ASE system under *Schedule* control enables the system to track any errors that may occur throughout an extraction run. This is especially helpful if the system is set up to run unattended overnight. Any problems are logged in the *Error Log* for the user to view the next morning.

When the extraction is complete, evaporate the extracts under vacuum (40 °C, 200 mbar) until only a few droplets remain. Evaporate the residual ethyl acetate under a gentle stream of nitrogen, or transfer the vials to the Dionex SE 500 Solvent Evaporation System (P/N 063221~120v; 063218~240v) and evaporate to dryness using standard conditions. Redissolve the extract with 500 µL water.

If necessary, the extract can be filtered through a 0.2-µm cellulose filter. Decant approximately 180 µL of the extract and mix with 90 µL of methanol prior to analysis by LC-MS/MS.

RESULTS AND DISCUSSION

The ASE method automates the extraction and cleanup steps of extraction of acrylamide from cocoa and coffee. Compared to the manual method, ASE greatly reduces the time and the amount of sample handling required (Table 1). The addition of Florisil to the extraction cell eliminates the need for an additional cleanup step of the extract. Figure 1 shows coffee extracted with various amounts of Florisil, followed by filtration through SPE cartridges. Method optimization determined that 6 g of Florisil were sufficient to obtain a clear extract, however some samples may require additional filtering before analysis.

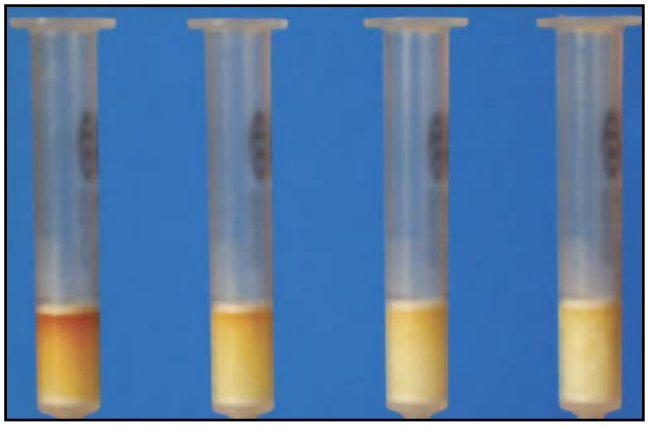

Figure 1. Residual coextractables from coffee extracts, trapped on an Isolute Multimode SPE cartridge. The four extraction cells contained from 0 to 6 g of Florisil. From left to right: (1) no Florisil, (2) 2 g Florisil, (3) 4 g Florisil, and (4) 6 g Florisil.

Table 1. Comparison of the Sample Preparation Steps for the Manual Method and ASE Method for Extraction of Acrylamide from Coffee and Chocolate

Manual Method	**ASE Method**
1. Weigh 2 g of sample, add 100 µL of d3-acrylamide solution (5 µg/mL) and 10 mL of water into a centrifuge tube. Homogenize for 1 min.	1. Weigh 2 g of sample, add 100 µL of *d3*-acrylamide solution (5 µg/mL) and 10 mL of water into a centrifuge tube. Homogenize for 1 min.
2. Add 1 mL Carrez I, swirl, add 1 mL Carrez II, swirl. Add 5 mL dichloromethane, shake vigorously for 1 min.	2. Add 1 mL Carrez I, swirl, add 1 mL Carrez II, swirl. Add 5 mL dichloromethane, shake vigorously for 1 min.
3. Centrifuge at 3–5 °C, 10,000 rpm for 15 min.	3. Centrifuge at 3–5 °C, 10,000 rpm for 15 min.
4. Transfer 6 mL of the supernatant into a centrifuge tube containing 1.8 g of NaCl, swirl to dissolve.	4. Prepare ASE cell by successively adding 1 cellulose filter, 6 g of Florisil (deactivated with 3% water), a second cellulose filter and 8 g ASE Prep DE.
5. Add 13 mL ethyl acetate and shake vigorously (1 min).	5. Transfer 6 mL of the extract on the ASE Prep DE, fill the rest of the column with ASE Prep DE, add a third cellulose filter and close the cell.
6. Centrifuge at 3–5 °C, 13800 g for 15 min.	6. Perform the ASE extraction step.
7. Transfer the organic phase into an amber vial containing 2 mL water. Shake vigorously for 1 min.	7. Evaporate the organic fraction under vacuum (40 °C, 200 mbar) to about 500 µL and finish the evaporation with a gentle stream of N_2.
8. Evaporate the organic phase with N_2 at 40 °C.	8. Redissolve the extract in 500 µl water.
Repeat the ethyl acetate extraction (2×), steps 5-7.	**Add 90 µL of methanol to 180 µL of extract. Proceed with LC-MS/MS analysis (60 µL injected)**
9. Condition the SPE cartridge with 3 mL methanol, then twice with 3 mL distilled water.	
10. Load the aqueous extract onto the cartridge, elute and rinse with 1 mL water. collect both fractions.	
11. Reduce the extract volume to approximately 500 µL (N_2, 40 °C).	
12. Add 90 µL of methanol to 180 µL of extract. Proceed with LC-MS/MS analysis (60 µL injected).	

Table 2 compares the results of manual extraction to ASE extraction of blank samples spiked with acrylamide standard.

Table 2. Comparison of Manual Extraction versus ASE for Quantification of Acrylamide Spiked Samples in Soluble Chocolate Powder (n = 6)

	Manual Extraction		ASE Extraction	
Spiking Levels	**Recovery %**	**%RSD**	**Recovery %**	**%RSD**
12.7 µg/kg	103.7	17.2	94.6	4.3
304.7 µg/kg	108.0	6.3	102.2	7.0
2504 µg/kg	104.3	5.3	101.5	2.4

Table 3 shows the results of using ASE for the extraction of acrylamide from various difficult matrices.

Table 3. ASE of Roast Ground Coffee, Soluble Coffee, Coffee Surrogate, and Cocoa

	Acrylamide Level (µg/kg)			
		Spiked at 150 µg/kg		
Materials	**Incurred[a]**	**Expected[b]**	**Measured[a]**	**CV%**
R&G coffee	136	286	298	3.1
Soluble coffee powder	299	449	435	2.9
Coffee surrogate	632	782	782	1.0
Cocoa powder	192	342	343	1.1

[a]Mean of two independent determinations. [b]Mean incurred level + spike level.

CONCLUSIONS

ASE has consistently proven to be an excellent alternative to the traditional labor-intensive extraction methods used for determination of acrylamide in food. ASE allows extraction and cleanup to be performed simultaneously, eliminating the need for a post-extraction cleanup step. Automation of ASE allows for unattended extraction, and can be set up to run over night to provide the user with filtered extracts that are ready for analysis in the morning. The advantages of speed and decreased sample handling as compared to manual extraction techniques are clear. Acrylamide recoveries obtained with the ASE method were comparable to traditional methods, proving that ASE is an effective tool for the extraction of polar compounds from complex matrices.

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4. Hoefler F., Maurer R., Cavalli S.; Schnelle Analyse von Acrylamid in Lebensmitteln mit ASE und LC/MS, *GIT Labor-Fachzeitschrift*, **2002**, *9*, 968–970.

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, www.sigmaaldrich.com

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Determination of Inorganic Oxyhalide Disinfection Byproduct Anions and Bromide in Drinking Water Using Ion Chromatography with the Addition of a Postcolumn Reagent for Trace Bromate Analysis

INTRODUCTION

The chlorination of drinking water can produce trihalomethanes and other suspected carcinogenic disinfection byproducts (DBPs) that endanger human health.[1] Unfortunately, common alternatives to chlorination also can produce harmful DBPs. The use of chlorine dioxide for the disinfection of drinking water generates the inorganic oxyhalide DBPs chlorite and chlorate, and the presence of chlorate has been reported in waters treated with hypochlorite.[2] Ozonation, an increasingly prevalent and effective disinfection technique, produces bromate as a DBP anion if the source water contains naturally occurring bromide.[3] Bromate has been judged by both the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) as a potential carcinogen, even at very low µg/L levels. The U.S. EPA has estimated a potential cancer risk equivalent to 1 in 10^4 for a lifetime exposure to drinking water containing bromate at 5 µg/L.[4]

The U.S. EPA has recently issued new rules that require public water supplies to control previously unregulated microbes (e.g., cryptosporidium and giardia) and cancer-causing DBPs in finished drinking water. The Stage 1 D/DBP Rule specifies a Maximum Contaminant Level (MCL) for bromate of 10 µg/L and an MCL for chlorite of 1000 µg/L.[5] The EPA intends to convene Stage 2 of the D/DBP Rule in the near future, while both Germany and Japan are considering regulatory limits for inorganic DBPs.[6]

The recent efforts by global regulatory agencies to monitor levels and establish regulatory limits has generated considerable interest in the development of improved analytical methods for the determination of trace level inorganic oxyhalide DBPs. The determination of bromate and other inorganic DBPs traditionally has been accomplished by ion chromatography (IC) using an IonPac® AS9-SC anion-exchange column with a carbonate/bicarbonate eluent and suppressed conductivity detection, as described in U.S. EPA Method 300.0 (B).[7] EPA Method 300.1 was published as an update to Method 300.0 in 1997. Method 300.1 specifies the use of an IonPac AS9-HC column and suppressed conductivity detection for the determination of bromate, bromide, chlorite, and chlorate at low µg/L levels by direct injection.[8] The detection limit for bromate determined by IC with suppressed conductivity detection can be further reduced to 1 µg/L by using preconcentration after appropriate sample cleanup.[1]

Postcolumn derivatization can also be used to improve detection limits when using IC for inorganic DBP analysis. The use of IC with dual postcolumn addition of hydrochloric acid and then chlorpromazine can achieve a method detection limit (MDL) for bromate of 0.49 µg/L.[9] Iodate, chlorite, and bromate have been detected by using a postcolumn reaction with excess bromide under acidic conditions. The tribromide ion formed can be detected spectrophotometrically at 267 nm, allowing an MDL of less than 0.5 µg/L for bromate

with a large-volume injection.[10] Sub-µg/L MDLs for bromate have also been reported by workers using other postcolumn reagents, such as fuchsin or excess iodide under acidic conditions.[11,12] In addition to postcolumn reaction (PCR) methods, electrospray tandem mass spectrometry (MS-MS) and inductively coupled plasma mass spectrometry (ICP-MS) have been used as specific detection techniques for the ion chromatographic analysis of bromate. The use of electrospray MS-MS detection can achieve an MDL for bromate of approximately 0.1 µg/L; the use of ICP-MS detection has been reported to permit an MDL for bromate of 0.8 µg/L.[13,14]

This Application Note describes an improved IC method to quantify low levels of oxyhalide DBP anions and bromide in reagent water, bottled water, and finished drinking water. The method uses an IonPac AS9-HC column and suppressed conductivity detection, followed by postcolumn addition of *o*-dianisidine (ODA) to enhance visible absorbance detection of the bromate ion. This method allows quantification of all the key oxyhalide anions and bromide at low µg/L levels by using conductivity detection, and the postcolumn addition of ODA followed by visible detection allows quantification of bromate down to 0.5 µg/L. This method requires only a single postcolumn reagent delivered pneumatically with conventional postcolumn instrumentation.[2] The approach described in this Application Note is technically equivalent to that described in U.S. EPA Method 317.0 titled "Determination of Inorganic Oxyhalide Disinfection By-Products in Drinking Water Using Ion Chromatography with the Addition of a Postcolumn Reagent for Trace Bromate Analysis".[15]

EQUIPMENT

Dionex DX-500 ion chromatographic system consisting of:
- GP50 Gradient Pump with vacuum degas option
- ED40 Conductivity Detector with DS3 Detector Cell
- AD20 UV/Vis Absorbance Detector with 10-mm cell
- AS50 Autosampler

PC10 Pneumatic Postcolumn Delivery Module (P/N 50601)

PCH-2 Postcolumn Reaction Heater (P/N 39348)

Knitted Reaction Coil, 500 µL, potted (for PCH-2) (P/N 39349)

Two 4-L plastic bottle assemblies (for external water mode suppression)

PeakNet® 5.1 Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better

0.5 M Carbonate Anion Eluent Concentrate (Dionex P/N 37162)

o-Dianisidine, dihydrochloride salt (ODA; Sigma D-3252)

Iron (II) sulfate heptahydrate ($Fe_2SO_4 \cdot 7H_2O$; Aldrich 21,542-2)

Ethylenediamine (EDA; Sigma E-1521)

Nitric acid, (70%; J.T. Baker INSTRA-ANALYZED 9598-00)

Methanol (spectrophotometric grade; Sigma M-3641)

Potassium bromide (KBr; J.T. Baker 2998)

Sodium bromide (NaBr; Aldrich 31,050-6)

Sodium bromate ($NaBrO_3$; EM SX 03785-1)

Sodium chlorate ($NaClO_3$; Fluka 71370)

Sodium chlorite ($NaClO_2$; Fluka 71388, ~80% pure)

Bromate standard, 1000 mg/L, $NaBrO_3$ in H_2O (SPEX CertiPrep AS-BRO39-2Y)

Bromide standard, 1000 mg/L, NaBr in H_2O (SPEX CertiPrep AS-BR9-2Y)

Chlorate standard, 1000 mg/L, $NaClO_3$ in H_2O (SPEX CertiPrep AS-CLO39-2Y)

Chlorite standard, 1000 mg/L, $NaClO_2$ in H_2O (SPEX CertiPrep AS-CLO29-2Y)

CONDITIONS

Columns:	Dionex AG9-HC, 50 × 4 mm ID guard column (P/N 51791) Dionex AS9-HC, 250 × 4 mm ID analytical column (P/N 51786)
Eluent:	9.0 mM Sodium carbonate (Na_2CO_3)
Flow Rate:	1.3 mL/min
Temperature:	Ambient
Sample Volume:	225 µL
Detection:	Suppressed conductivity: ASRS®-ULTRA (P/N 53946), AutoSuppression® external water mode, 100 mA current, DS3 Cell (P/N 44130), 35 °C, 1.7%/°C.
Background Conductance:	~24 µS
System Backpressure:	~2300 psi
Run Time:	25 min

PCR

Detection: Absorbance at 450 nm (tungsten lamp)
Postcolumn Reagent Flow: 0.7 mL/min
Postcolumn Heater Temp.: 60 °C

PREPARATION OF SOLUTIONS AND REAGENTS

Reagent Water

Distilled or deionized water, 18 MΩ-cm or better, free of the anions of interest and filtered through a 0.2-µm filter.

Eluent Solution (9 mM Sodium Carbonate)

Dilute 18 mL of 0.5 M sodium carbonate concentrate to 1 L with deionized water. Unless the in-line degas option is being used, sparge eluent prior to use with helium or sonicate under vacuum for 10 min.

Postcolumn Reagent

Add 40 mL of 70% nitric acid to about 300 mL reagent water in a 500-mL volumetric flask. Add 2.5 g KBr and stir to dissolve. Dissolve 250 mg of *o*-dianisidine • 2 HCl in 100 mL methanol and add to the nitric acid/KBr solution. Bring to volume with reagent water. Prepare in advance, set aside overnight until the slight champagne color fades, and filter through a 0.45-µm filter. Discard any PCR reagent that is not colorless or nearly colorless after sitting overnight. The reagent is stable for one month when stored at room temperature.

Stock Standard Solutions

Purchase certified solutions or prepare stock standard solutions by dissolving the corresponding mass of the salt for each of the anions of interest (see Table 1) in reagent water and dilute to 100 mL.

Table 1 Masses of Compounds Used to Prepare 100 mL of 1000 mg/L Anion Standards

Anion	Compound	Mass (g)
BrO_3^-	Sodium bromate ($NaBrO_3$)	0.1180
Br^-	Sodium bromide (NaBr)	0.1288
ClO_3^-	Sodium chlorate ($NaClO_3$)	0.1275
ClO_2^-	Sodium chlorite ($NaClO_2$)	0.1680*

Because sodium chlorite is usually available only as an 80% technical grade salt, the 80% purity is accounted for in the 0.1680 g mass cited above. If an alternate purity is used, make an appropriate adjustment in the mass of salt used after determining the exact percentage of $NaClO_2$, which can be done using an iodometric titration procedure.[16]

Prepare a mixed anion calibration stock standard at 20 mg/L by combining 2 mL of each of the bromide, chlorite, and chlorate stock standards in a 100-mL volumetric flask. Mix and bring to volume with reagent water. These standards are stable for at least 1 month when stored at < 6 ºC.

Because bromate decomposes in the presence of chlorite, prepare a bromate-only calibration stock standard at 5 mg/L by adding 0.5 mL of the bromate stock standard to a 100-mL volumetric flask and bringing to volume with reagent water. This standard is stable for 2 weeks when stored at < 6 °C.

Working Standard Solutions

Use reagent water to prepare appropriate dilutions of the calibration stock standards as needed.

Ethylenediamine (EDA) Preservative Solution

Dilute 2.8 mL of ethylenediamine (99%) to 25 mL with reagent water. Prepare fresh monthly.

Ferrous Iron Solution [1000 mg/L Fe (II)]

Add 6 µL concentrated nitric acid to about 15 mL reagent water in a 25 mL volumetric flask. Add 0.124 g ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), dissolve, and bring to volume with reagent water (final pH ~ 2). Prepare fresh every 2 days.

Sulfuric Acid Solution (0.5 N)

Dilute 1.4 mL of concentrated sulfuric acid to 100 mL with reagent water.

SAMPLE PREPARATION

When taking a sample from a treatment plant that uses chlorine dioxide or ozone, the sample must be sparged immediately with an inert gas (e.g., nitrogen, argon, or helium) for 5 min. Add 1.00 mL of EDA Preservative Solution per 1.0 L of sample to prevent conversion of residual hypochlorite or hypobromite to chlorate or bromate. This also prevents metal-catalyzed conversion of chlorite to chlorate. The samples preserved in this manner are stable for at least 14 days when stored in amber glass bottles at 4 °C.[17]

After appropriate preservation, most samples can be filtered through a 0.45-µm filter and directly injected onto the ion chromatograph. However, each sample that contains excess chlorite must be treated to remove chlorite and then reanalyzed for bromate, because elevated levels of chlorite can interfere with the quantification of bromate by PCR.

The treatment procedure to remove chlorite requires two portions of sample. Place two 10-mL aliquots of the sample into separate 20-mL beakers. Fortify one aliquot with bromate at a level approximating the native concentration of bromate in the untreated sample. This laboratory fortified matrix (LFM) will indicate correct performance of the chlorite removal step. Acidify both aliquots with 33 µL of sulfuric acid reagent and confirm the final pH (5–6) with pH test strips. Add 40 µL of ferrous iron solution, mix, and allow to react for 10 min. Filter the treated samples through a 0.45-µm nylon filter to remove precipitated ferric hydroxide, and then pass the solution through a hydronium form cation-exchange cartridge (Dionex OnGuard®-H, P/N 39596) to remove excess soluble iron. The treated samples must be analyzed within 30 h.

SYSTEM PREPARATION AND SET-UP

Configure the IC with the PCR system as depicted in Figure 1. Determine the PCR flow rate by collecting the combined effluent from the IC pump and the PCR module in a 10-mL graduated cylinder for 1 min. The PCR flow rate is the difference between the total flow rate and that of the IC pump. Adjust the air pressure of the postcolumn delivery module (PC10) and remeasure the flow rate until the correct flow rate of 0.7 mL/min is established. Confirm this flow rate on a weekly basis or whenever detector response for a calibration check standard deviates beyond quality control acceptance criteria.

To determine target anions at trace concentrations, it is essential to have low baseline noise. Minimize baseline noise by taking the following steps during system set-up. Install the ASRS-ULTRA in the external water mode rather than the recycle mode. Prior to sample analysis, determine a system blank by analyzing 225 µL of deionized water using the method described above. An equilibrated system has a background conductance of ~ 24 µS, peak-to-peak noise of ~ 5 nS per minute, and no peaks eluting at the same retention time as the anions of interest.

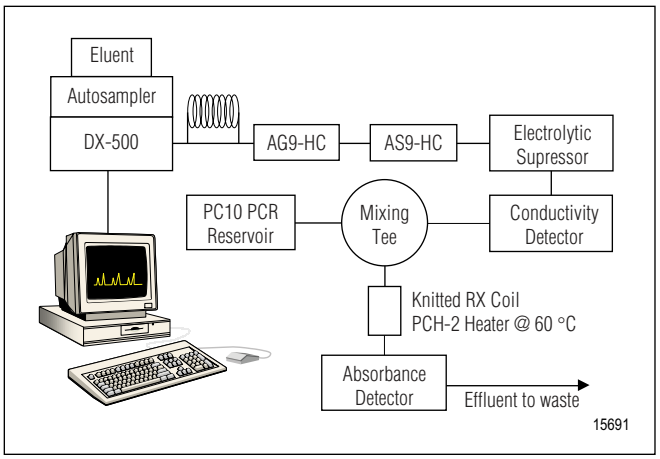

Figure 1. IC system configuration for EPA Method 317.0.

RESULTS AND DISCUSSION

Figure 2 shows the chromatograms of a mixed anion standard containing 10 µg/L bromate and 15 µg/L each of chlorite, bromide, and chlorate obtained by using dual A) suppressed conductivity and B) UV/Vis absorbance after postcolumn reaction with ODA. The bromate peak is baseline-resolved from chlorite on both detector channels; however, it shows a significantly enhanced response on the absorbance detector after PCR with ODA compared to the response obtained on the conductivity detector.

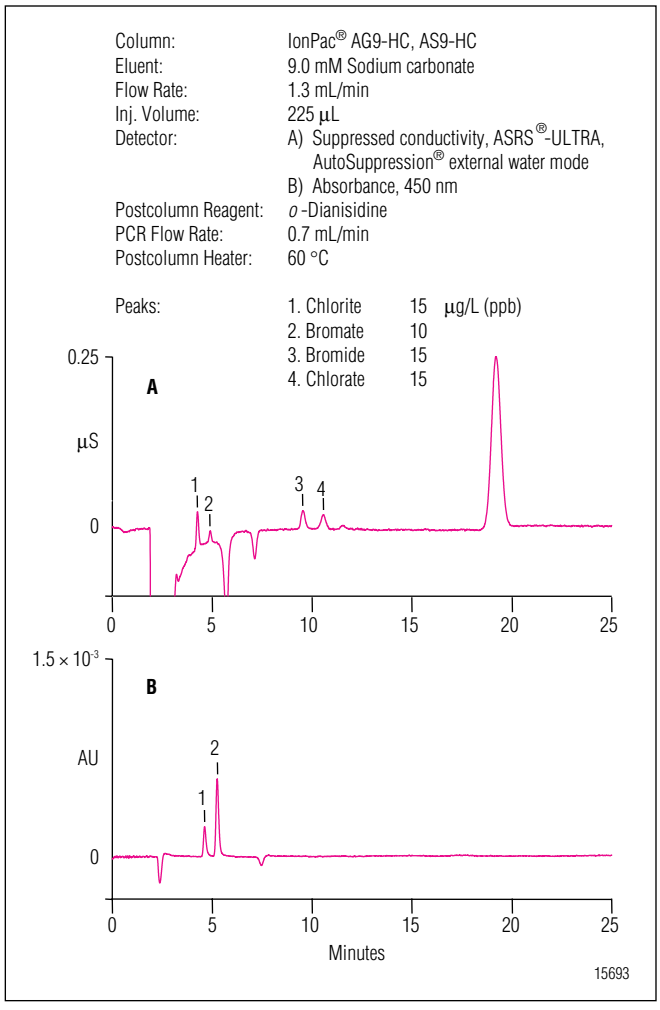

Figure 2. Separation of a low-ppb DBP anion standard using an IonPac AS9-HC column: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o-dianisidine.

Table 2 summarizes the calibration data and method detection limits (MDLs) obtained for the oxyhalide DBP anions and bromide using dual conductivity and PCR detection. The MDL for each analyte was established by making seven replicate injections of a reagent water blank fortified at a concentration of 3 to 5 times the estimated instrument detection limit.[2] The use of PCR addition and UV/Vis detection allows quantification of bromate down to 0.5 µg/L without compromising detection limits obtained with suppressed conductivity detection for the other anions of interest.[6] Note that the use of electronic smoothing (Olympic, 25 points, 5 sec, 1 iteration) of the UV/Vis signal improves the calculated MDL for bromate.[2] Figure 3 demonstrates the effect of smoothing on the performance of the PCR detection for a 1.0 µg/L bromate standard. No significant loss of peak response is observed after smoothing, although baseline noise is reduced by a factor of approximately 2×, which results in a similar improvement in the detection limit (Table 2).

Table 2 Linear Ranges and MDLs for Oxyhalides and Bromide

	Range (µg/L)	r^2	MDL Standard (µg/L)	Calculated MDL* (µg/L)
Chlorite	5.0–500	0.9999	5.0	1.80
Bromate–conductivity	5.0–50	0.9986	5.0	1.22
Bromide	5.0–500	0.9999	5.0	1.90
Chlorate	5.0–500	0.9999	5.0	1.85
Bromate–UV/Vis (smoothed)	0.5–15	0.9986	1.0	0.09
Bromate–UV/Vis (no smoothing)	0.5–15	0.9986	1.0	0.19

*MDL = (t) x (S) Where t = student's t value for a 99% confidence level and a standard deviation estimate with n - 1 degrees of freedom [t = 3.14 for seven replicates of the MDL standard], and S = standard deviation of the replicate analysis.

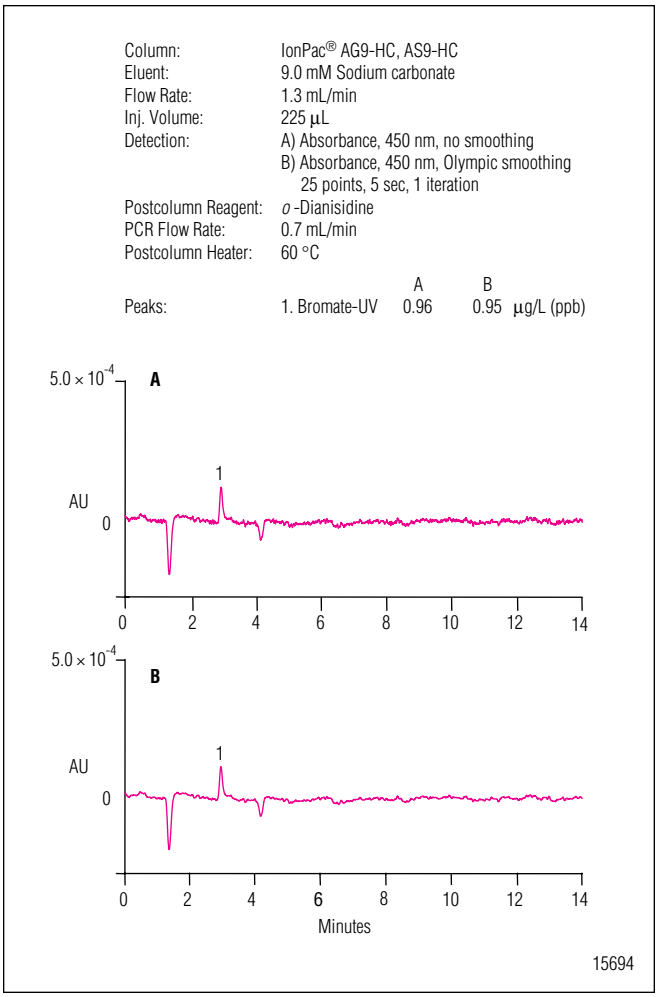

Figure 3. Effect of smoothing on bromate determination: A) unsmoothed data and B) smoothed data (Olympic, 25 points, 5 sec, 1 iteration).

Figures 4–7 illustrate the performance of the method for the determination of inorganic oxyhalide DBP anions and bromide in drinking and bottled water samples. Figure 4 shows the chromatograms from a direct injection of drinking water (from Sunnyvale, California) obtained by using dual A) suppressed conductivity and B) UV/Vis absorbance after postcolumn reaction with ODA. Neither chlorite nor bromate are observed in the drinking water sample; however, bromide and chlorate (frequently observed as a disinfection byproduct from the use of hypochlorite) are well resolved from the sample matrix.

Figure 5 shows the chromatograms of the same drinking water sample spiked with chlorite, bromate, bromide, and chlorate at levels of 108, 11.3, 36, and 72 µg/L, respectively. The chromatograms were obtained using, in series, dual A) suppressed conductivity and B) UV/Vis absorbance after postcolumn reaction with ODA. Quantitative recoveries were obtained for all anions, as shown in Table 3. The benefits of PCR with UV/Vis detection for bromate determination can clearly be seen in Figure 5B: bromate peak response is significantly enhanced compared to the response on the conductivity detector and no response is observed for the large peak from about 20 µg/L chloride that elutes immediately after bromate. The use of PCR with UV/Vis detection allows the quantification of bromate down to 0.5 µg/L in the presence of 200 mg/L chloride (a 400,000-fold excess) with no sample pretreatment.

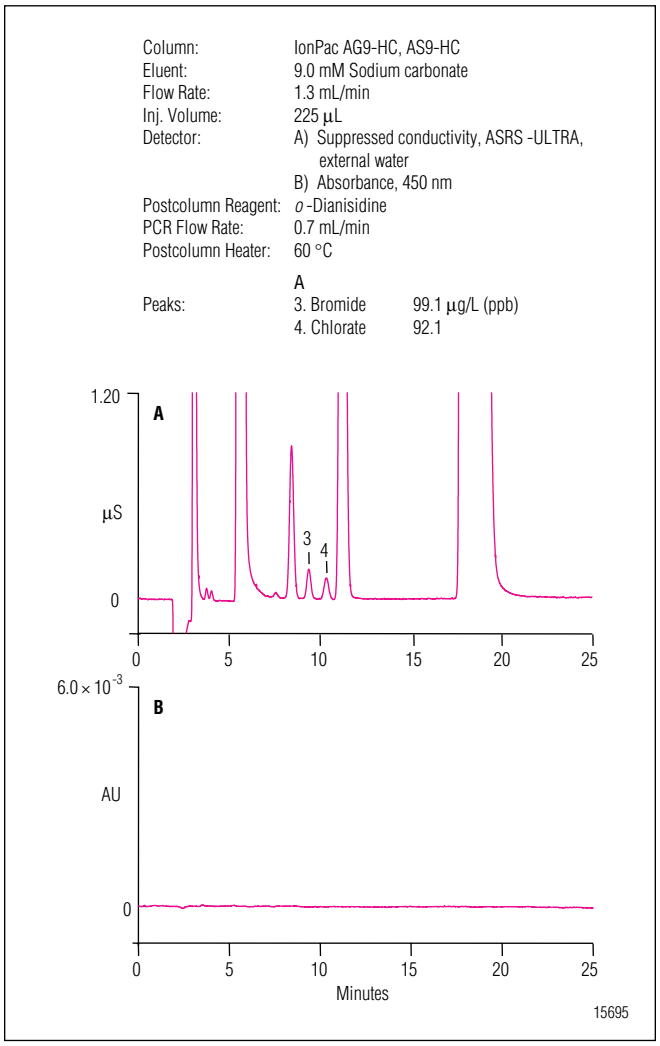

Figure 4. Determination of DBP anions in tap water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

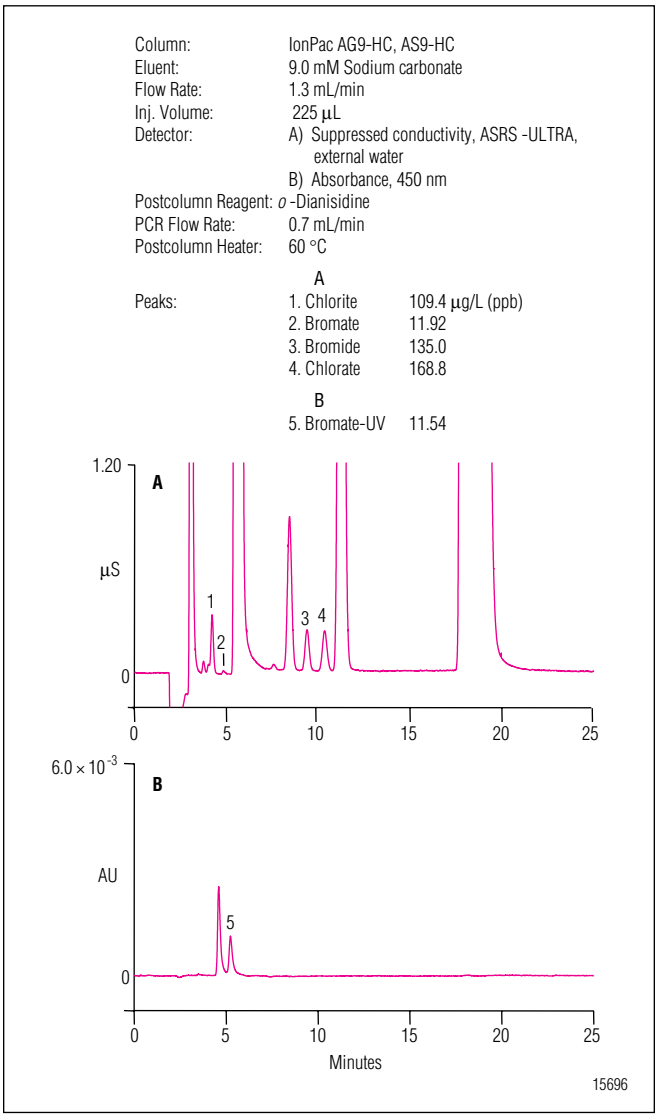

Figure 5. Determination of DBP anions in spiked tap water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

Figure 6 shows the chromatograms from a direct injection of bottled spring water obtained using, in series, dual A) suppressed conductivity and B) UV/Vis absorbance after postcolumn reaction with ODA. In this instance, both bromate and bromide are observed in the bottled water sample. Bromate, which is formed during ozonation of source water containing bromide, is present at about 2 µg/L and can clearly be seen in the UV/Vis chromatogram, although no peak is evident on the conductivity detector. Figure 7 shows the chromatograms of the same bottled water sample spiked with chlorite, bromate, bromide, and chlorate at levels of 126, 13.2, 42, and 84 µg/L, respectively. These chromatograms were obtained by using, in series, dual A) suppressed conductivity and B) UV/Vis absorbance after postcolumn reaction with ODA. Table 3 shows that quantitative recoveries were again obtained for all anions. Table 3 also shows the recoveries obtained for bromate spiked into the same drinking and bottled water samples at a lower concentration of 2.2 µg/L when using UV/Vis absorbance after postcolumn reaction with ODA. This method permits quantitative recoveries (80–120%) for bromate at levels down to 1 µg/L when using PCR and UV/Vis detection.

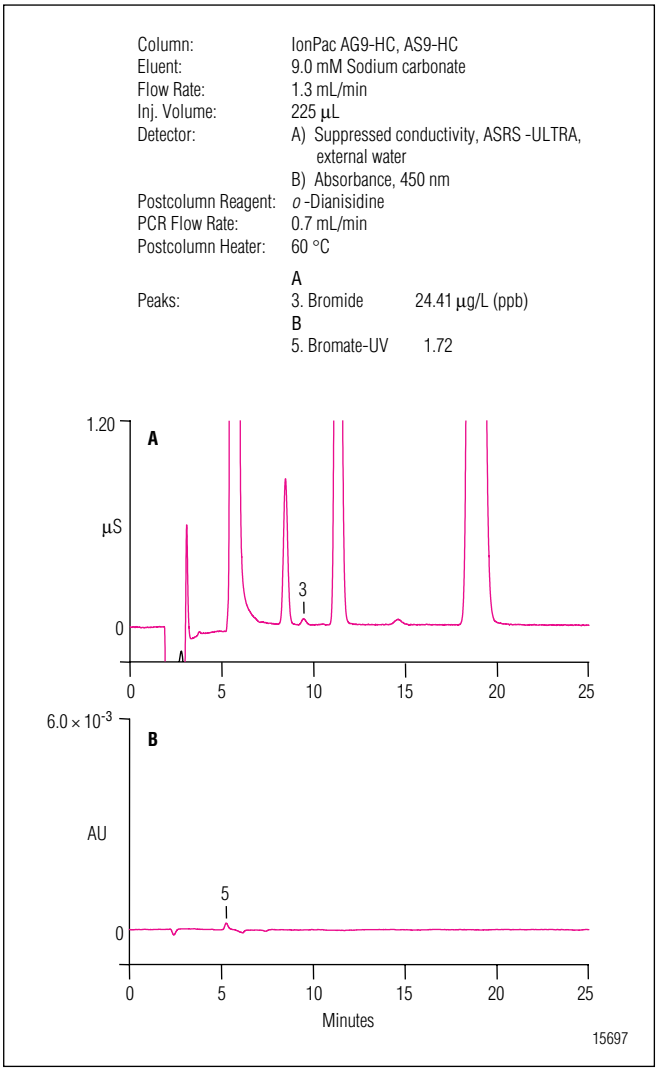

Figure 6. Determination of DBP anions in bottled water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o-dianisidine.

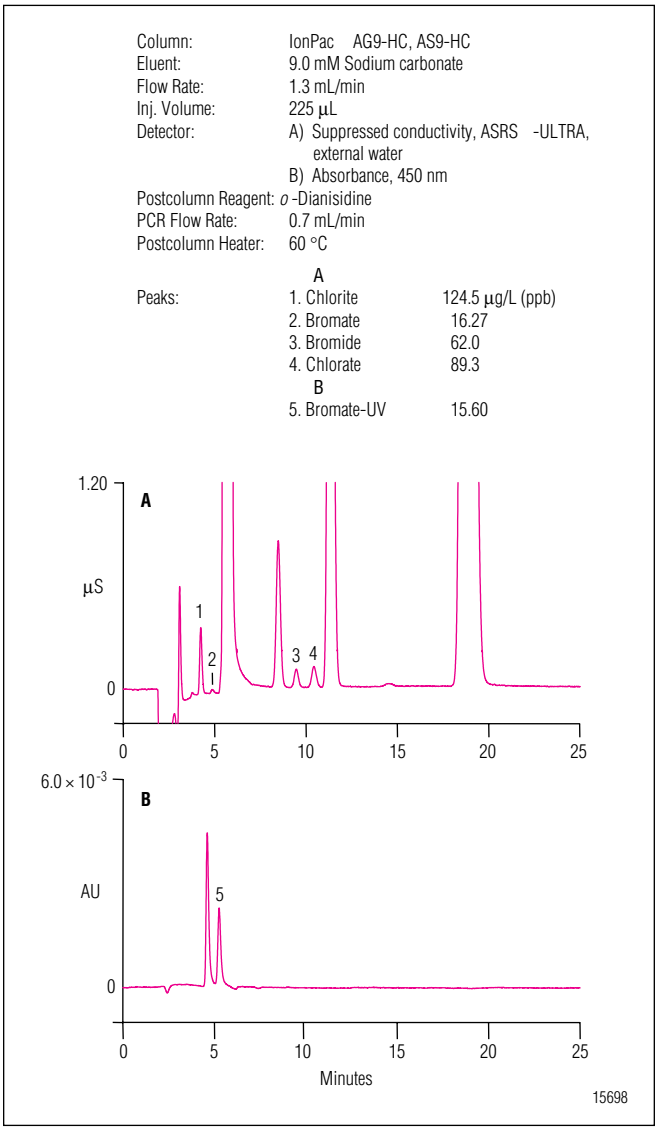

Figure 7. Determination of DBP anions in spiked bottled water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o-dianisidine.

Table 3 Anion Recoveries for Spiked Water Samples

	Tap Water		Bottled Water	
Anion*	**Amount Added (µg/L)**	**Recovery**	**Amount Added (µg/L)**	**Recovery**
Chlorite	108	104%	126	104%
Bromate–conductivity	11.3	105%	13.2	105%
Bromide	36.0	100%	42	100%
Chlorate	72	107%	84	107%
Bromate–UV/Vis	11.3	102%	13.2	102%
Bromate–UV/Vis**	2.2	91%	2.2	96%

*Data were obtained from multi-analyte spikes into tap and bottled water samples.

**Bromate only (2.2 µg/L) was added to tap and bottled water samples to determine low level recovery for this anion using UV/Vis detection.

Table 4 Bromate Recovery from Simulated Chlorine Dioxide Treated Waters (STW)*

	Spiked STW Fe (II)-Treated		Laboratory Fortified Matrix Fe (II)-Treated	
	Amount Added (µg/L)	**Recovery**	**Amount Added (µg/L)**	**Recovery**
STW	0	ND	2.0	90%
STW-1	1.75	74%	2.0	78%
STW-2	2.15	80%	4.0	75%
STW-3	4.61	76%	6.0	82%
STW-4	5.14	80%	8.0	75%

* Chlorite present at 100 µg/L

REMOVAL OF CHLORITE INTERFERENCE

When chlorine dioxide is used to disinfect drinking water, the DBP anion chlorite is found in the finished drinking water. Chlorite, like bromate, reacts with *o*-dianisidine to form a complex that absorbs at 450 nm. High chlorite levels can interfere with quantification of low-level bromate.[2] One approach to minimize the interference from chlorite is to remove the chlorite by reduction with ferrous sulfate, as described in the "Sample Preparation" section. This treatment was evaluated by applying it to a series of simulated chlorine dioxide-treated tap waters, which had been spiked with varying levels of bromate, and the corresponding LFMs. The results, summarized in Table 4, show that acceptable recoveries of bromate are obtained after such treatment. This treatment approach is recommended when analysis of low-level bromate is required in chlorine dioxide-treated drinking waters.

SUMMARY

The IC method described in this Application Note, which uses an IonPac AS9-HC column and suppressed conductivity detection, followed by postcolumn addition of *o*-dianisidine with UV/Vis detection specifically to enhance bromate response, allows the determination of all the key oxyhalide anions and bromide at low µg/L levels in drinking and bottled waters. The use of postcolumn addition and UV/Vis detection allows the quantification of bromate in the range of 0.5-15 µg/L without compromising the suppressed conductivity detection of chlorite, bromide, and chlorate. Conductivity detection is recommended for the quantification of bromate in the range of 15–50 µg/L.

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SUPPLIERS

Aldrich Chemical Co., P.O. Box 2060, Milwaukee, WI 53201, USA. Tel: 800-558-9160. www.sigma-aldrich.com.

Fluka, P.O. Box 2060, Milwaukee, WI 53201, USA. Tel: 800-558-9160. www.sigma-aldrich.com.

Pierce Chemical Co., 3747 North Meridian Road, P.O. Box 117, Rockford, IL 61105, USA. Tel: 800-874-3723. www.piercenet.com.

Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178, USA. Tel: 800-325-3010. www.sigma-aldrich.com.

SPEX CertiPrep, Inc., 203 Norcross Ave., Metuchen, NJ 08840, USA. Tel.:800-522-7739. www.spexcsp.com.

VWR Scientific Products, 3745 Bayshore Blvd., Brisbane, CA 94005, USA. Tel: 800-932-5000. www.vwrsp.com.

Determination of Bromate in Bottled Mineral Water Using the CRD 300 Carbonate Removal Device

INTRODUCTION

Drinking and bottled waters are commonly disinfected with ozone. Ozone is highly effective and, unlike many other disinfectants, does not remain in the water or change its taste. Unfortunately, when bromide is present in water, it is converted to bromate by the ozone treatment. Bromate is recognized as a potential human carcinogen, which has led to the regulation of its concentration in drinking and bottled water. Major regulatory bodies worldwide (e.g., U.S. EPA and the European Commission) have set a maximum allowable bromate concentration in drinking water of 10 µg/L.[1] In Europe, the limit was lowered to 3 µg/L for bottled natural mineral and spring waters disinfected by ozonation.[2]

Over the past two decades, Dionex has led the effort in developing sensitive and robust ion chromatography (IC) methods for determining bromate and other oxyhalides (e.g., chlorite and chlorate). U.S. EPA Method 300.0 (B) and 300.1 (B) used the IonPac® AS9-SC and IonPac AS9-HC columns, respectively, along with suppressed conductivity detection for bromate, chlorite, and chlorate determinations in drinking water. In 1997, Dionex introduced the AS9-HC column to allow the direct injection of 250 µL of drinking water to easily meet the 10 µg/L regulatory requirement. This method was documented in Dionex Application Note 81 (AN 81).[3] Since then, Dionex has developed a number of products and techniques, and worked with regulatory agencies and international standards organizations to improve the sensitivity and ruggedness of bromate determinations as well as the types of samples that can be directly injected.

Dionex products were instrumental in the development of the postcolumn derivatization techniques in U.S. EPA methods 317.0 and 326.0. These methods used the AS9-HC and Dionex suppression technology for conductivity detection of oxyhalides combined with postcolumn addition and absorbance detection for enhanced determination of bromate. EPA Methods 317.0 and 326.0 are documented in AN 136 and AN 149.[4,5] To improve the sensitivity for bromate using direct injection, Dionex developed the IonPac AS19 column. This column was designed for use with hydroxide eluents rather than the carbonate eluents used with the AS9-HC. Hydroxide eluents offer improved sensitivity for suppressed conductivity detection as compared to carbonate eluents. This improved sensitivity was documented in AN 167.[6] Hydroxide eluents are also advantageous because they can be generated easily using an eluent generator as part of a Reagent-Free™ IC (RFIC™) system. RFIC systems improve reproducibility and simplify analysis. The AS19 separation can also replace the AS9-HC separation in EPA Methods 317.0 and 326.0, which is documented in AN 168 and AN 171.[7,8] The AS19 was also used with an isocratic hydroxide eluent rather than the typical gradient for analysis of drinking water for bromate.[9] This method, presented in Application Update 154 (AU 154), cannot determine all the common inorganic anions in a single injection like the gradient method in AN 167. For determination of sub-µg/L concentrations of bromate

in drinking water and higher ionic strength matrices without postcolumn derivatization, Dionex developed a two-dimensional IC technique (AN 187) that uses an AS19 column in the first dimension, and an AS24 column, developed specifically for determining haloacetic acids and bromate by IC-MS and IC-MS/MS, in the second dimension.[10]

Dionex AN 184 showed that the AS19 method in AN 167 could be used to meet the 3 µg/L European limit for bromate in natural mineral and spring waters disinfected by ozonation.[11] The same application note compared the AS19 chromatography to chromatography with the AS23, a column that uses carbonate eluents and was designed to replace the AS9-HC. The AS23 has a higher capacity than the AS9-HC, and a different selectivity for the carbonate ion so that it is less likely to interfere with bromate determinations. AN 184 showed that poorer sensitivity associated with using carbonate eluents when compared to hydroxide eluents made the AS23 performance inferior to that of the AS19.

The present application note describes the use of a carbonate removal device, the CRD 300, to remove the majority of carbonate from the eluent and allow hydroxide-like performance and detection sensitivity. This device was used with the IonPac AS23 to determine bromate in a bottled mineral water samples. Detection sensitivity when using the CRD 300 was improved compared to chromatography without the CRD 300. Scientists responsible for water analysis can choose the column and eluent chemistry that best meets their needs to reliably determine bromate at concentrations below the common 10 µg/L regulatory limit.

EQUIPMENT

Dionex ICS-2000 Reagent-Free Ion Chromatography System* equipped with the following for carbonate/bicarbonate eluent generation:

EluGen® EGC II K_2CO_3 cartridge (P/N 058904)

EPM Electrolytic pH Modifier (P/N 063175)

EGC Carbonate Mixer (P/N 079943)

CRD 300 Carbonate Removal Device (4 mm) with VC Vacuum Pump (P/N 068474)

Chromeleon® 6.8 Chromatography Management Software

**This application can be run on any Dionex system equipped for carbonate/bicarbonate eluent generation. Alternately, this application can be run with a manually prepared carbonate/bicarbonate eluent.*

REAGENTS AND STANDARDS

Deionized water, type I reagent grade, 18 MΩ-cm resistivity or better

Sodium chlorite, 80% ($NaClO_2$, Fluka)

Potassium bromate ($KBrO_3$, Fluka)

Sodium chlorate ($NaClO_3$, Fluka)

Individual stock standards of fluoride, chloride, and sulfate, 1000 mg/L each (Merck)

PREPARATION OF SOLUTIONS AND REAGENTS

Carbonate Eluent Generation

The Eluent Generator (EG) produces the eluent using the EluGen EGC II K_2CO_3 cartridge, Electrolytic pH Modifier, EGC Carbonate Mixer, and deionized water supplied by the pump. The eluent concentration is controlled by the Chromeleon software. Backpressure tubing must be added to achieve 2300–2500 psi backpressure that will allow the EG degasser to function properly. See the ICS-2000 Operator's Manual Section 2.4.4, "Eluent Generator" for instructions on adding backpressure.

To set up the EGC II K2CO3, see the EGC II K2CO3 cartridge, Electrolytic pH Modifier, and EGC Carbonate Mixer Product Manual (Doc. No. 065075) for more information.

Manual Eluent Preparation

From Eluent Concentrate

Prepare 1 L of eluent by adding 10 mL of the Dionex IonPac AS23 Eluent Concentrate (P/N 064161) to a 1 L volumetric flask. Bring to volume with DI water and mix thoroughly.

From Manually Prepared Stock Solutions

Stock Carbonate/Bicarbonate Eluent Preparation

1.0 M Na_2CO_3 and 1.0 M $NaHCO_3$

Weigh 10.596 g sodium carbonate and 8.400 g sodium bicarbonate into separate 100 mL volumetric flasks. Bring each to volume with DI water.

IonPac AS23 Eluent (4.5 mM Na_2CO_3 /0.8 mM $NaHCO_3$)

For 1L, prepare by adding 4.5 mL of 1.0 M Na_2CO_3 and 0.8 mL of 1.0 M $NaHCO_3$ to a 1L volumetric flask, bring to volume with DI water, and mix thoroughly.

Stock Standard Solutions

Prepare 1000 mg/L stock standard solutions of fluoride, chloride, sulfate, chlorite, bromate, and chlorate by weighing 0.221 g, 0.165 g, 0.148 g, 0.168 g, 0.131 g, and 0.128 g, respectively, into separate 100 mL volumetric flasks. Bring each to volume with DI water.

Secondary Standards

The stock standards are used to prepare the 1000 µg/L secondary standards of chlorite, bromate, and chlorate. Take a defined volume of the stock standard and dilute it 1 to 1000 with DI water (e.g., dilute 100 µL to 100 mL in a 100 mL volumetric flask). Use these standards to prepare the working standards and to spike the bottled mineral water sample.

Working Standards

Prepare the standards for calibration and MDL studies by mixing defined volumes of the 1000 mg/L stock stadard solutions of fluoride, chloride, and sulfate and the 1000 µg/L secondary standards of chlorite, bromate, and chlorate. For example, to prepare the working standard containing 0.5 mg/L fluoride, 50 mg/L chloride, 100 mg/L sulfate, and 40 µg/L of each of the oxyhalides, add 0.05 mL of the fluoride stock standard, 5 mL of the chloride stock standard, 10 mL of the sulfate stock standard, and 4 mL of each oxyhalide secondary standard to a 100 mL volumetric flask and bring to volume.

Sample

The bottled mineral water sample was purchased from a local market in Bangkok, Thailand and was bottled at its source in the mountains of Thailand. The label reported the presence of fluoride, chloride, sulfate, and bicarbonate, but not their concentrations.

CRD 300 IN VACUUM MODE SETUP

The CRD 300 in vacuum mode uses a vacuum pump to evacuate the regenerant chamber of the CRD 300 so that CO_2 gas is literally sucked out of the eluent. A bleed tube feeds a trickle of fresh air into the regenerant chamber to constantly sweep out the CO_2 gas. To operate the CRD 300 in vacuum mode, mount the CRD 300 directly on top of the suppressor and plumb the eluent from the Eluent Out of the suppressor to the Eluent In of the CRD 300. The Eluent Out of the CRD 300 is connected to the conductivity cell In and conductivity cell Out goes to waste if the system is running in external water mode. If the system is operated in recycle mode, connect conductivity cell Out to the suppressor Regen In. Connect the vacuum tubing to the vacuum port of the vacuum pump and to the ballast bottle. Connect a length of 1/8" Teflon® tubing from the ballast bottle to the Regen Out of the CRD 300. Make sure the third port on the ballast bottle is closed and air tight. Connect 15 cm of red (0.005" i.d.) PEEK™ tubing to the Regen In of the CRD 300; this is the air bleed assembly. Begin eluent flow before beginning vacuum operation. When eluent flow is established, turn on the vacuum pump. The background conductivity should drop almost immediately. When the eluent pump is turned off, immediately turn off the vacuum pump. Avoid operating the vacuum pump while eluent flow is stopped. A TTL can be wired to automate stopping the vacuum pump.

CONDITIONS

Condition A (Eluent Generation and CRD 300)

Column:	IonPac AS23 (4 × 250 mm) (P/N 064149)
	IonPac AG23 (4 × 50 mm) (P/N 064147)
Eluent:	EGC II K_2CO_3 (P/N 058904)
	EPM (P/N 063175)
	4.5 mM K_2CO_3/0.8 mM $KHCO_3$
Flow Rate:	1.0 mL/min
Inj. Volume:	250 µL
Temperature:	30 °C
Suppressor:	Suppressed conductivity, ASRS® 300, 4 mm (P/N 064554), external water mode, 25 mA
	CRD 300, 4 mm, (P/N 064637) vacuum mode
Background:	< 1.5 µS
Noise:	~ 0.3 nS
Back Pressure:	~2200 psi

Condition B (Manual Eluent Preparation and no CRD 300)

Column:	IonPac AS23 (4 × 250 mm) (P/N 064149)
	IonPac AG23 (4 × 50 mm) (P/N 064147)
Eluent:	4.5 mM Na_2CO_3/0.8 mM $NaHCO_3$
Flow Rate:	1.0 mL/min
Inj. Volume:	250 µL
Column Temp:	30 °C
Suppressor:	Suppressed conductivity, ASRS 300, 4 mm (P/N 064554), external water mode, 25 mA
Background:	17-19 µS
Noise:	~ 3.0 nS
Back Pressure:	~1800 psi

RESULTS AND DISCUSSION

Chromatography

Bromate, chlorite, and chlorate were resolved from seven common inorganic anions using an IonPac AS23 column under its recommended eluent conditions (4.5 mM Na_2CO_3/0.8 mM $NaHCO_3$). Chromatogram B in Figure 1 shows this separation. The background conductivity after suppression using the carbonate eluent is between 18 and 19 µS. The higher the background, the higher the noise, and this results in a lower signal-to-noise ratio (i.e., lower sensitivity). The background of the suppressed hydroxide eluent used for the IonPac AS19 column is < 1 µS. In order for the carbonate eluent system of the AS23 to approach the detection limits delivered by the hydroxide eluent system of the AS19, the background must be reduced. The CRD 300 was designed to remove carbonate from the eluent (after suppression) and thereby reduce the background to improve detection limits. Chromatogram A shows the same AS23 separation as B using a CRD 300. Note that the background has been reduced to about 1 µS, the injection dip at about 2 min is greatly reduced in size, and there is a noticeable improvement in analyte sensitivity. Throughout this application note, we compare the determination of bromate, chlorite, and chlorate with the AS23 and suppressed conductivity, both with and without the CRD 300.

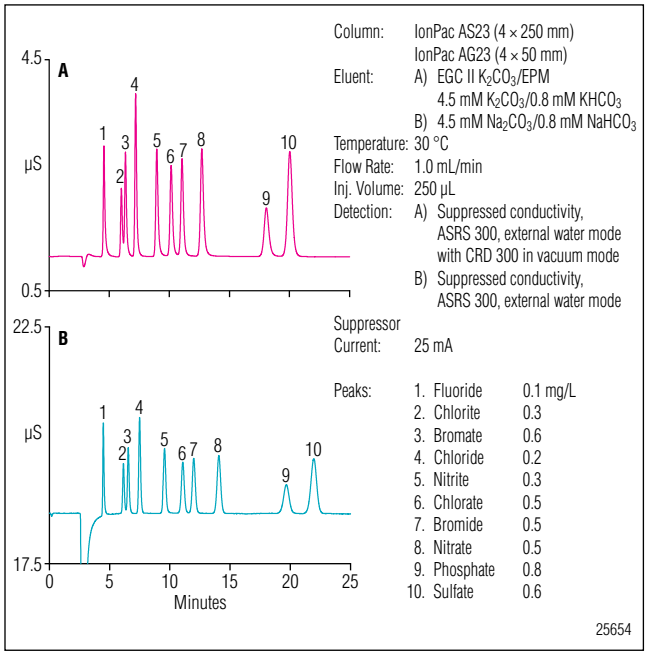

Figure 1. Chromatography of a mixed anion standard A) with a CRD 300 and electrolytically prepared eluent, and B) without a CRD 300 and with manually prepared eluent.

Figure 2 shows single injections from the MDL determinations of bromate, chlorite, and chlorate with and without the CRD 300. Fluoride, (0.5 mg/L), chloride (50 mg/L), and sulfate (100 mg/L) were added to the MDL standards to simulate the ionic strength of bottled water samples. Due to the higher background and noise of the system without the CRD 300 (Chromatogram B, Figure 2), higher analyte concentrations were used for the MDL test compared to the system with the CRD 300. Table 1 shows the results of the MDL determination. For all three oxyhalide analytes, the MDL is lower for the system with the CRD 300. The MDL values without the CRD 300 are similar to those determined with the AS23 in AN 184. The values when using the CRD 300, though lower than without, are not as low as those determined with the AS19 and hydroxide eluent in AN 184.

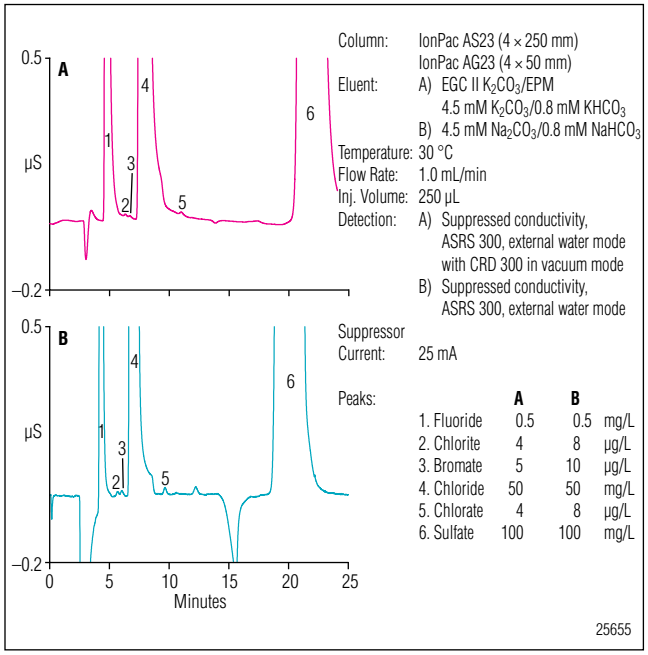

Figure 2. Example chromatograms from the MDL determination A) with a CRD 300, and B) without a CRD 300.

Table 1. MDL Determinations of Chlorite, Bromate, and Chlorate with and without a CRD 300

	Height (µS)					
	With CRD 300			Without CRD 300		
	Chlorite	Bromate	Chlorate	Chlorite	Bromate	Chlorate
Injection No.	4 µg/L	5 µg/L	4 µg/L	8 µg/L	10 µg/L	8 µg/L
1	0.0057	0.0041	0.0076	0.0099	0.0121	0.0189
2	0.0051	0.0042	0.0071	0.0114	0.0128	0.0199
3	0.0053	0.0042	0.0065	0.0093	0.0115	0.0204
4	0.056	0.0043	0.0074	0.0105	0.0132	0.0215
5	0.059	0.0047	0.0074	0.0111	0.0133	0.0205
6	0.0061	0.0045	0.0077	0.0103	0.0125	0.0201
7	0.0057	0.0042	0.0076	0.0114	0.0111	0.0199
Average	**0.0056**	**0.0043**	**0.0073**	**0.0105**	**0.0124**	**0.0202**
RSD	**5.97**	**5.04**	**5.42**	**7.41**	**6.67**	**3.94**
MDL (µg/L)	**0.75**	**0.79**	**0.68**	**1.86**	**2.10**	**0.99**

Another calibration was performed for both systems using consistent concentrations of fluoride, chloride, and sulfate (0.5 mg/L, 2 mg/L, and 10 mg/L, respectively) in standards with three levels of chlorite, bromate, and chlorate concentrations; 10, 20, and 40 µg/L. Overlays of three calibration standards are shown in Figure 3 and the results are in Table 2. The calibration data are equivalent.

Both systems were used to analyze a bottled mineral water sample from the mountains of Thailand. Figure 4 shows the analysis of this sample and Table 3 reports the results of the analysis. The sample had just over 10 µg/L bromate and 1–2 µg/L chlorate, suggesting a second disinfection process besides ozonation was used. Due to the noise of the system without the CRD 300, the chlorate peak could not be identified with confidence. To evaluate accuracy, known amounts of bromate, chlorite, and chlorate were spiked into the bottled mineral water sample. Figure 5 shows the chromatography from this study and Table 4 shows that all analytes were recovered at >85%. In this experiment, the recovery was better for the system with the CRD 300.

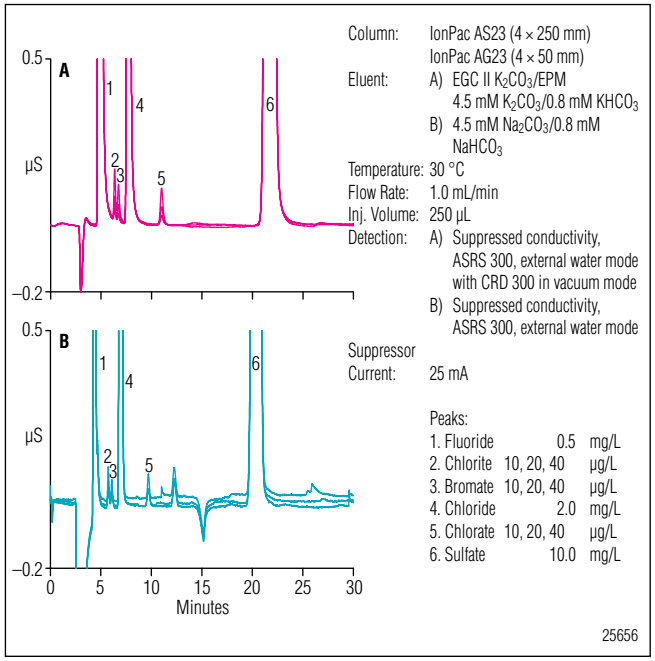

Figure 3. Overlay of chromatograms of three concentration levels of chlorite, bromate, and chlorate in a mixed anion standard A) with a CRD 300, and B) without a CRD 300.

Table 2. Chromeleon Calibration Report for Chlorite, Bromate, and Chlorate with and without a CRD 300

Peak Name	Points	R^2 (%)	
		With CRD 300	Without CRD 300
Chlorite	3	99.9961	99.9748
Bromate	3	100.0000	99.9986
Chlorate	3	99.9995	99.9637

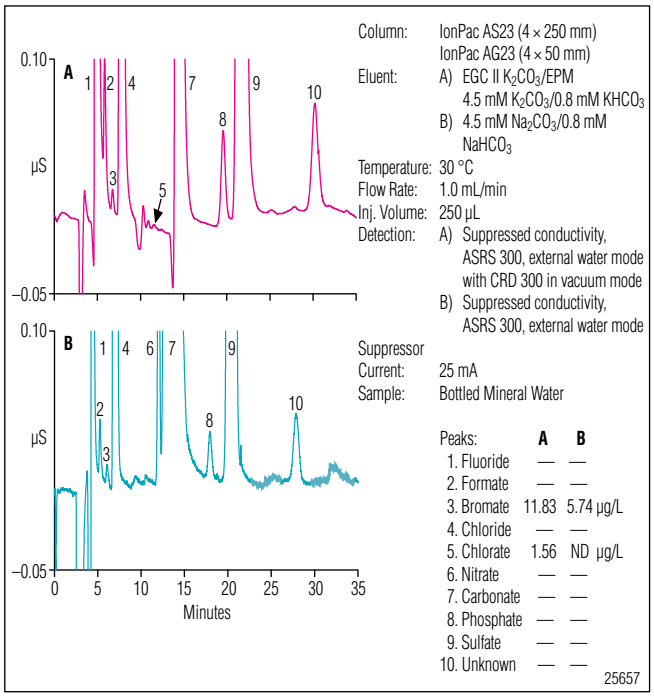

Figure 4. Chromatography of a bottled mineral water sample A) with a CRD 300, and B) without a CRD 300.

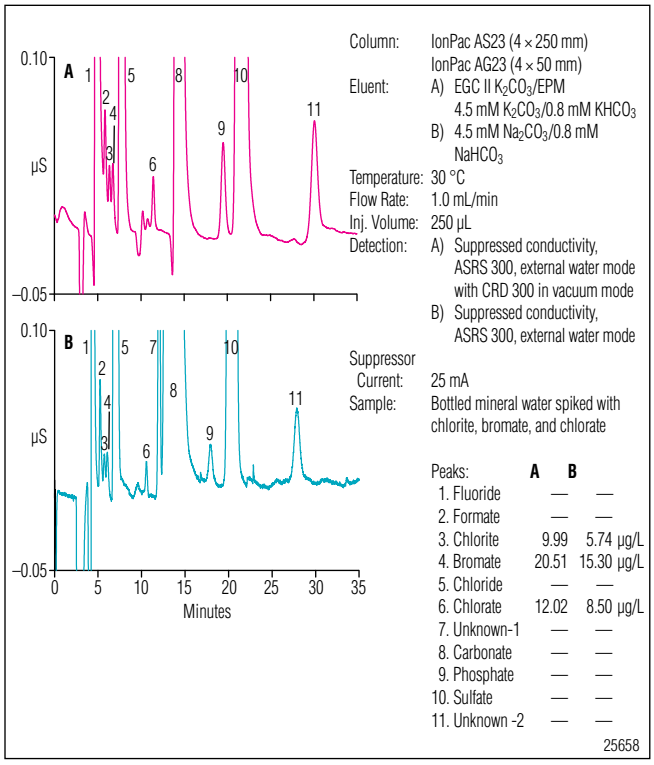

Figure 5. Chromatography of a bottled mineral water sample spiked with chlorite, bromate, and chlorate (10 µg/L each) A) with a CRD 300, and B) without a CRD 300.

Table 3. Determination of Bromate and Chlorate in a Bottled Mineral Water Sample with and without a CRD 300

Injection No.	With CRD 300 (µg/L)		Without CRD 300 (µg/L)	
	Bromate	Chlorate	Bromate	Chlorate
1	11.0	1.52	5.33	ND
2	10.9	1.55	6.23	ND
3	10.9	1.35	5.02	ND
4	10.1	1.91	6.25	ND
5	11.3	1.48	5.89	ND
Average	10.8	1.56	5.74	—
RSD	4.34	13.42	9.61	—

Table 4. MDL Determinations of Chlorite, Bromate, and Chlorate with and without a CRD 300

	With CRD 300			Without CRD 300		
	Chlorite	Bromate	Chlorate	Chlorite	Bromate	Chlorate
Sample	ND[a]	10.83	1.56	ND[a]	5.74	ND[a]
Spike	10	10	10	10	10	10
Measured[b] Amount	9.88	20.51	12.02	8.58	15.30	8.50
RSD	2.39	1.60	2.45	2.39	1.60	2.45
Recovery(%)	98.8	98.5	104	85.8	97.2	85.0

[a] ND = Not Detected; [b] The average of five injections

SUMMARY

This application note shows that using the CRD 300 with the IonPac AS23, bromate can be determined in bottled mineral water at concentrations < 5 µg/L. The method sensitivity for bromate and other oxyhalides approaches that of the hydroxide eluent system featured in Dionex Application Note 184.

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Determination of Trace Concentrations of Chlorite, Bromate, and Chlorate in Bottled Natural Mineral Waters

INTRODUCTION

Bottled water has been one of the fastest growing beverage markets in the last five to ten years. Global consumption approached 41 billion gallons in 2004, an increase of 6.5% from 2003.[1] The bottled water industry markets to health conscious consumers as an alternative not only to tap water, but also to carbonated soft drinks and juice drinks.[1] Regardless of whether the water is delivered from a local municipality or is prepackaged in a bottle, the consumption of safe and reliable drinking water is essential to maintain a healthy lifestyle.

Bottled water must be disinfected to remove pathogenic microorganisms and ensure it is safe for human consumption. Water companies prefer ozone as a disinfectant because it is one of the most effective treatments available, it does not leave a taste, and there is no residual disinfectant in the bottled water.[2,3] Some bottlers, however, use ultraviolet light or chlorine dioxide as alternative treatment methods.[2] Reactions between disinfectants and natural organic and inorganic matter in the source water can result in the production of undesirable disinfection byproducts (DBPs), such as chlorite, bromate, and trihalomethanes, that are potentially harmful to humans.[4] Bromate, for example, can be formed by ozonation of water containing naturally occurring bromide, or may be present as an impurity in sodium hypochlorite used for treatment.[5]

Results from toxicological studies led the International Agency for Research on Cancer to conclude that bromate is a potential human carcinogen, even at low µg/L (ppb) concentrations.[6] The World Health Organization (WHO) estimated excess lifetime cancer risks of 10^{-4}, 10^{-5}, and 10^{-6} for drinking water containing bromate at 20, 2, and 0.2 µg/L, respectively.[9] The U.S. EPA,[7] European Commission,[8] and the WHO[9] set a maximum permissible limit of 10 µg/L bromate in tap water. The U.S. FDA[10] adopted the same regulatory limit for bottled water. In Europe, natural mineral waters and spring waters treated by ozonation have a maximum permissible limit of 3 µg/L bromate.[11]

Traditionally, ion chromatography (IC) with suppressed conductivity detection has been used for determination of bromate and other DBPs in drinking water, as described in EPA Method 300.1.[12] This method describes the use of a high-capacity IonPac AS9-HC column with a carbonate eluent and large loop injection to achieve a method detection limit (MDL) of 1.4 µg/L bromate. In early 2006, the U.S. EPA enacted stage 2 of the disinfectants/disinfection byproducts (D/DBP) rule, maintaining the maximum permissible limit for bromate but adding three additional analytical methods to further improve the selectivity and sensitivity for bromate.[13] U.S. EPA Methods 317.0 and 326.0 combine suppressed conductivity detection and absorbance detection after postcolumn addition to achieve bromate MDLs less than 0.2 µg/L.[14,15] IC coupled to inductively coupled plasma mass spectrometry has also been demonstrated for the determination of low concentrations of bromate in environmental waters, permitting a bromate MDL of 0.3 µg/L.[16]

A high-capacity IonPac® AS19 column with an electrolytically generated hydroxide eluent, large loop injection, and suppressed conductivity detection can achieve a calculated bromate MDL of 0.34 µg/L.[17] Absorbance detection after postcolumn addition can reduce this MDL to less than 0.2 µg/L, using EPA Methods 317.0 and 326.0.[18,19] In this application note, we compare the IonPac AS19 using an electrolytically generated hydroxide eluent to the IonPac AS23 column using an electrolytically generated carbonate/bicarbonate eluent for the determination of chlorite, bromate, and chlorate in natural mineral waters. We compare the linearity, method detection limits, precisions, and recovery for three mineral waters obtained from three European countries to determine whether these columns have the sensitivity required to meet current EPA and EU requirements.

EQUIPMENT

A Dionex ICS-2000 Reagent-Free™ Ion Chromatography (RFIC™) system was used in this work. The ICS-2000 is an integrated ion chromatograph and consists of:

Eluent generator

Pump with in-line vacuum degas

Column heater

Hydroxide system:
EluGen® EGC II KOH cartridge (Dionex P/N 058900)

CR-ATC (Dionex P/N 060477)

Carbonate system:
EluGen EGC II K_2CO_3 cartridge (Dionex P/N 058904)

EPM Electrolytic pH Modifier to generate the carbonate/bicarbonate eluent (Dionex P/N 063175)

EGC Carbonate Mixer (Dionex P/N 079943)

Two 4-L plastic bottle assemblies (for external water mode of suppression)

AS Autosampler

Chromeleon® Chromatography Management Software

REAGENTS AND STANDARDS

Deionized water, type I reagent grade, 18 MΩ-cm resistivity or better

Sodium chlorite ($NaClO_2$, Fluka 71388, 80% pure)

Sodium bromate ($NaBrO_3$, EM SX 03785-1)

Sodium chlorate ($NaClO_3$, Fluka 71370)

CONDITIONS

Columns:	(A) IonPac AS19 Analytical, 4 × 250 mm (Dionex P/N 062885) IonPac AG19 Guard, 4 × 50 mm (Dionex P/N 062887)
	(B) IonPac AS23 Analytical, 4 × 250 mm (Dionex P/N 064149) IonPac AG23 Guard, 4 × 50 mm (Dionex P/N 064147)
Eluent:	(A) 10 mM KOH from 0–10 min, 10–45 mM from 10–25 min, 45 mM from 25–30 min*
	(B) 4.5 mM K_2CO_3/0.8 mM $KHCO_3$
Eluent Source:	(A) EGC II KOH with CR-ATC
	(B) EGC II K_2CO_3 with EPM
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection:	250 µL
Detection:	(A) Suppressed conductivity, ASRS® ULTRA II, 4 mm (Dionex P/N 061561) AutoSuppression® recycle mode 130 mA current
	(B) Suppressed conductivity, ASRS ULTRA II, 4 mm AutoSuppression external water mode 25 mA current
CRD:	(A) 4-mm format (P/N 062983)
Background Conductance:	(A) <1 µS
	(B) 18–20 µS
System Backpressure:	~2200 psi
Run Time:	30 min

*Method returns to 10 mM KOH for 3 min prior to injection.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution for the AS23 Column

4.5 mM Carbonate/0.8 mM Bicarbonate

Generate the carbonate/bicarbonate eluent on-line by pumping high quality deionized water (18 MΩ-cm resistivity or better) through the EluGen EGC II K_2CO_3 Cartridge and EPM. Chromeleon will track the amount of eluent used and calculate the remaining lifetime.

Alternatively, prepare the eluent solution by adding 10 mL of the AS23 Eluent Concentrate (Dionex

P/N 064161) to a 1-L volumetric flask containing approximately 700 mL of degassed deionized water. Bring to volume and mix thoroughly. The 0.45 M sodium carbonate/0.08 M sodium bicarbonate concentrate can also be prepared from the salts by combining 47.7 g sodium carbonate (MW=106 g/mole) and 6.72 g sodium bicarbonate (MW=84 g/mole) in a 1-L volumetric flask containing approximately 700 mL of degassed deionized water. Bring to volume and mix thoroughly.

Stock Standard Solutions

Prepare 1000 mg/L stock standard solutions of chlorite, bromate, and chlorate by dissolving 0.1676 g, 0.1180 g, and 0.1275 g, respectively, of the corresponding sodium salts in separate 100 mL volumetric flasks of DI water.

Calibration Standard Solutions

Prepare a secondary stock solution containing 1 mg/L each of chlorite and chlorate and a separate secondary stock solution containing 1 mg/L bromate by performing the appropriate dilutions of the 1000 mg/L stock standards. Calibration standards can then be prepared from the secondary solutions using the appropriate dilutions. Dilute working standards should be prepared monthly, except those that contain chlorite, which must be prepared every two weeks, or sooner if evidence of degradation is indicated by repeated QC failures. Concentration ranges used in this application note are shown in Table 1.

SAMPLE PREPARATION

For the present analysis, mineral waters B and C were degassed for 10–15 min under vacuum due to an excess amount of bicarbonate in the samples. Increased amounts of bicarbonate in the sample can produce shifts in retention times as shown in Figure 1 traces A and B. In addition, due to the presence of significantly high concentrations of sulfate in mineral water C, the sample was diluted 1:5 with DI water prior to analysis.

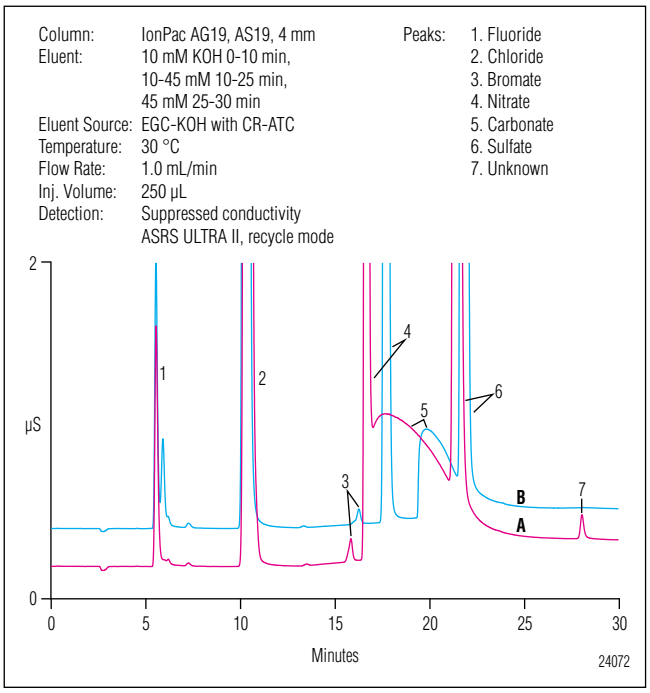

Figure 1. Comparison of mineral water B A) before vacuum degas and B) after vacuum degas.

Table 1. Calibration Data, Retention Time Precisions, Peak Area Precisions, and Method Detection Limits For DBP Anions

	IonPac AS19 Column					
Analyte	**Range (µg/L**	**Linearity r^2**	**Retention Time* RSD (%)**	**Peak Area RSD (%)**	**MDL Standard (µg/L)**	**Calculated MDL (µg/L)**
Chlorite	2–50	0.9999	0.04	1.20	1.0	0.18
Bromate	1–25	0.9995	0.03	1.40	2.0	0.31
Chlorate	2–50	0.9999	0.01	0.54	1.0	0.28
	IonPac AS23 Column					
Chlorite	10–50	0.9999	0.07	2.20	5.0	1.02
Bromate	5–25	0.9998	0.07	2.63	5.0	1.63
Chlorate	10–50	0.9998	0.11	2.48	9.0	2.05

[a]RSD= relative standard deviation, n = 10 for a standard consisting of 10 ppb bromate and 20 ppb each of chlorite and chlorate.

RESULTS AND DISCUSSION

The IonPac AS23 is a high-capacity anion-exchange column specifically designed to be used with carbonate /bicarbonate eluent for the determination of the trace DBPs, chlorite, bromate, and chlorate, together with common inorganic anions, including bromide (precursor to bromate), in drinking waters. To simplify the method and avoid manual eluent preparation, this column can be used with electrolytically generated potassium carbonate that is modified by an Electrolytic pH Modifier (EPM) to automatically generate the carbonate/bicarbonate eluent that is required for analyte separation. The IonPac AS23 column was developed using a unique polymer technology to achieve a capacity of 320 µeq/column, higher than the IonPac AS9-HC column (190 µeq/column) described in EPA Method 300.1. The combination of an optimized selectivity for DBP anions, high anion exchange capacity, and improved selectivity of carbonate from inorganic anions and oxyhalides, makes this column an ideal replacement for the AS9-HC column.

In this application, we compare the IonPac AS23 column to the hydroxide-selective IonPac AS19 column for the determination of trace DBP anions in natural mineral waters. Figure 2 compares the separation for chlorite, bromate, and chlorate on the IonPac AS19 and AS23 columns. As shown, both columns provide good selectivity for the target DBP anions.

The linear calibration ranges, MDLs, and quality control standard (QCS) performances were evaluated for the hydroxide and carbonate eluent systems. The hydroxide eluent system was calibrated using four increasing concentrations of chlorite and chlorate (2-50 µg/L) and five increasing concentrations of bromate (1-25 µg/L). For the carbonate-based system, chlorite and chlorate were calibrated from 10-50 µg/L whereas bromate was calibrated from 5-25 µg/L using three different concentrations. Each system produced a linear response in its respective range with a correlation coefficient greater than 0.999. The improved sensitivity of the hydroxide eluent system, however, allowed a lower minimum reporting limit (MRL) than the carbonate-based system. The MDLs for the target DBPs were determined for each system by performing seven replicate injections of reagent water fortified with the calibration standards at concentrations of three to five times the estimated instrument detection limits.

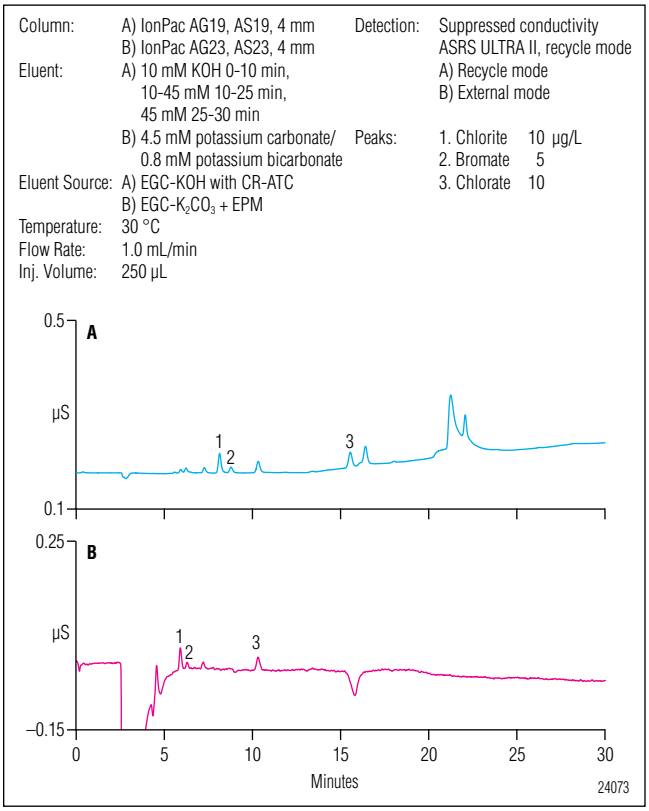

Figure 2. Separation of disinfection byproducts using the A) IonPac AS19 column and B) IonPac AS23 column.

Table 1 compares the calibration data, retention time and peak area precisions for a QCS, and MDLs for the IonPac AS19 with an electrolytically generated hydroxide eluent to the IonPac AS23 with an electrolytically generated carbonate/bicarbonate eluent. The calculated MDL of bromate with the IonPac AS19 column was 0.31 µg/L compared to 1.63 µg/L using the IonPac AS23 column. This demonstrates that hydroxide eluents improve the sensitivity for bromate compared to carbonate-based eluents and are therefore more suitable to meet the current European regulatory requirement of 3 µg/L bromate in natural mineral waters. Either the AS19 or AS23 based IC systems are capable of measuring the 10 µg/L requirement of bromate for tap water or U.S. bottled water according to the regulations established by the U.S. EPA, U.S. FDA, WHO, and European Commission.

In the U.S., mineral water is defined as water that contains no less than 250 ppm total dissolved solids (TDS) and that originates from a geologically and physically protected underground water source. Mineral

content must be maintained at a constant level and no minerals may be added to the water.[18] In Europe, mineral water is defined as microbiologically wholesome water, originating from an underground water table or deposit and emerging from a spring tapped at one or more natural or bored exits. It can contain less than 50 ppm TDS.[19] The total mineral content of the waters can vary significantly, with higher mineral concentrations generally appearing in Russia, the Baltic States, and Germany. The differences between regions are most likely a result of differences in the overall compositions of the waters and the geological locations.[20]

In this application, three natural mineral waters from different European countries with TDSs that varied significantly from 136 to 2359 ppm were evaluated. The properties of the investigated water samples are summarized in Table 2. As shown, the ionic strength of mineral water C is significantly higher than observed in typical drinking waters. The absence of bromate in the bottled mineral waters analyzed indicated that ozonation was not used for disinfection.

Tables 3 and 4 summarize typical recoveries for single-operator data obtained using the IonPac AS19 and AS23 columns, respectively, for trace concentrations of DBP anions in three European natural mineral water samples. As shown, chlorite and bromate were notdetected in any of the samples analyzed,whereas only a trace concentration of chlorate was detected in mineral water A. To determine the accuracy of the method, the samples were spiked with 5 µg/L bromate and 10 µg/L each of chlorite and chlorate. Calculated recoveries for the spiked mineral water samples were in the range of 86–97% and 84–111% using the IonPac AS19 and AS23 columns, respectively. The analyte recoveries using either a hydroxide or carbonate/bicarbonate eluent were within the acceptable range of 75–125% according to the criteria described in EPA Method 300.1. Figure 3 compares chromatograms of mineral water A

Table 2. Concentrations in mg/L of Cations and Anions in the Investigated Mineral Water Samples

Mineral Water	Na^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_3^-	HCO_3^-	SO_4^{2-}
A	11.8	6.2	8	11.5	–[a]	13.5	6.3	71	8.1
B	4.5	0.5	8	32.0	–[a]	5.0	< 2	133	7.0
C	4.2	–[a]	117	510	1.8	3.0	< 0.1	278	1445

[a]Not specified

Table 3. Recoveries of Disinfection Byproduct Anions in Natural Mineral Waters Using the IonPac AS19 Column

Mineral water	Analyte	Amount found (µg/L)	Amount added (µg/L)	Recovery (%)
A	Chlorite	<MDL	10	87.7
	Bromate	<MDL	5.0	96.0
	Chlorate	4.4	10	91.1
B	Chlorite	<MDL	10	86.4
	Bromate	<MDL	5.0	97.4
	Chlorate	<MDL	10	90.7
C	Chlorite	<MDL	10	87.6
	Bromate	<MDL	5.0	94.7
	Chlorate	<MDL	10	92.8

Table 4. Recoveries of Disinfection Byproduct Anions in Natural Mineral Waters Using the IonPac AS23 Column

Mineral water	Analyte	Amount found (µg/L)	Amount added (µg/L)	Recovery (%)
A	Chlorite	<MDL	10	107.6
	Bromate	<MDL	5.0	91.2
	Chlorate	4.6	10	99.3
B	Chlorite	<MDL	10	110.6
	Bromate	<MDL	5.0	93.5
	Chlorate	<MDL	10	92.9
C	Chlorite	<MDL	10	104.3
	Bromate	<MDL	5.0	83.9
	Chlorate	<MDL	10	102.6

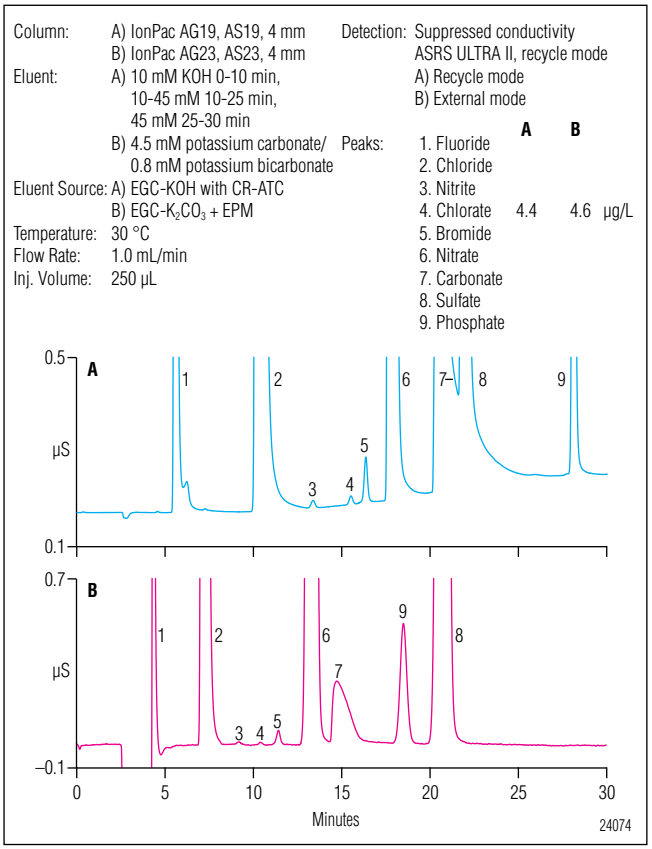

Figure 3. Comparison of the A) IonPac AS19 and B) IonPac AS23 columns for the separation of DPB anions in mineral water A.

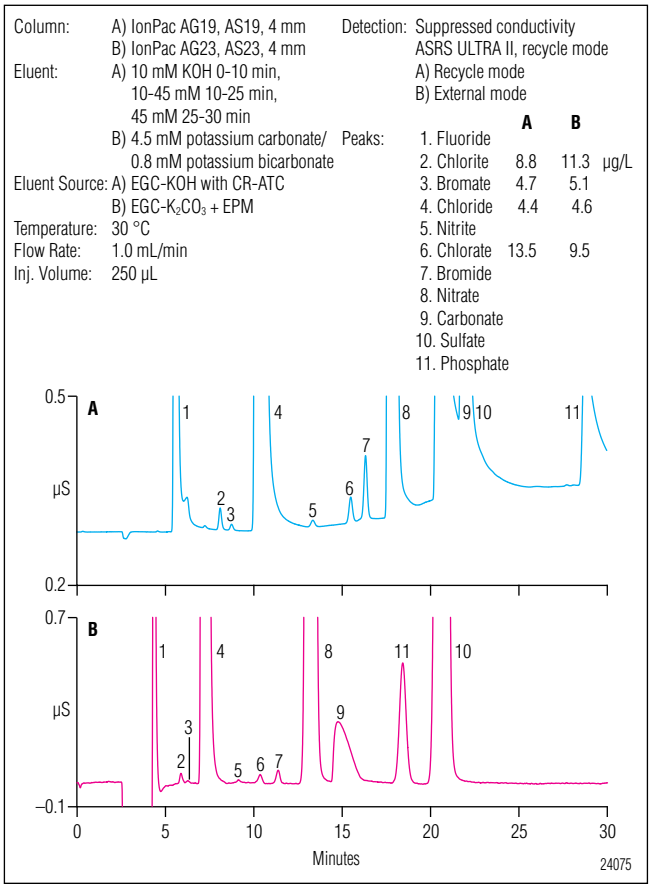

Figure 4. Comparison of the A) IonPac AS19 and B) IonPac AS23 columns for the separation of trace concentrations of common anions and DPB anions spiked in mineral water A.

using the IonPac AS19 and AS23 columns. Figure 4 shows the same chromatograms spiked with 5 µg/L bromate and 10 µg/L each of chlorite and chlorate, which resulted in good recoveries for both eluents. Although bromide was not quantified in this study, the estimated concentrations were approximately 16 µg/L in mineral waters A and B and 2 µg/L in mineral water C. Therefore, ozonation of mineral waters A and B could potentially produce bromate. To demonstrate the applicability of detecting bromate at concentrations significantly less than the 3 µg/L European regulatory limit for ozonated mineral waters, mineral water A was spiked with 0.5 µg/L bromate (Figure 5). As shown, bromate can be observed easily at this concentration, with good peak-to-peak baseline noise of 0.3–0.5 nS.

CONCLUSION

The IonPac AS19 column using an electrolytically generated hydroxide eluent was compared to the AS23 column using an electrolytically generated carbonate/ bicarbonate eluent for the determination of trace concentrations of DBP anions in natural mineral waters. The improved sensitivity using a hydroxide eluent allowed the detection of lower concentrations of bromate, a potential human carcinogen, in drinking waters. Therefore, the IonPac AS19 with an electrolytically generated hydroxide eluent is recommended for laboratories that must comply with EU Directive 2003/40/EC, which permits a maximum of 3 µg/L bromate in mineral waters treated with ozone. The use of either the IonPac AS19 column with a hydroxide eluent or

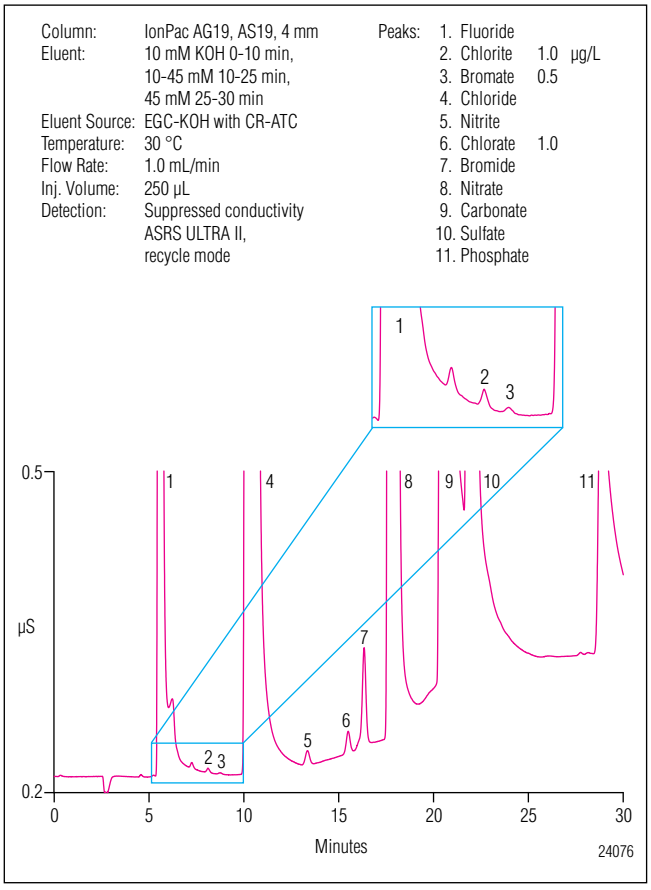

Figure 5. Chromatogram of mineral water A spiked with 1 µg/L each chlorite and chlorate and 0.5 µg/L bromate.

IonPac AS23 column with a carbonate/bicarbonate eluent provides the required sensitivity to meet the maximum permissible limit of 10 µg/L bromate currently required by most regulatory agencies. Both columns demonstrated good resolution between bromate and chloride and comparable recovery for mineral water samples spiked with known concentrations of chlorite, bromate, and chlorate. In addition, hydroxide or carbonate/bicarbonate eluents can be generated on-line from deionized water, freeing the operator from manually preparing eluents. This increases the automation, ease-of-use, and reproducibility between analysts and laboratories.

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Determination of Trace Concentrations of Oxyhalides and Bromide in Municipal and Bottled Waters Using a Hydroxide-Selective Column with a Reagent-Free™ Ion Chromatography System

INTRODUCTION

All drinking water municipalities share the same goal of providing their communities with a reliable source of safe drinking water. To achieve this goal, most water systems must treat their water. The type of treatment used varies depending on the size, source, and water quality.[1] Disinfection protects public water systems (PWSs) from potentially dangerous microbes. The most common chemical disinfectants are chlorine, chlorine dioxide, chloramine, and ozone.[1,2] These chemical disinfectants can react with natural organic and inorganic matter in the source water to produce disinfection by-products (DBPs) that are potentially harmful to humans. For example, chlorination of drinking water can produce trihalomethanes, haloacetic acids, and chlorate. While chlorine dioxide treatment generates the inorganic oxyhalide DBPs chlorite and chlorate, and the presence of chloramine has also been known to generate chlorate.[2] Ozone reacts with naturally occurring bromide to produce bromate. The International Agency for Research on Cancer has identified bromate as an animal carcinogen and potential human carcinogen.[3] The World Health Organization (WHO) has estimated[4] an excess lifetime cancer risk of 10^{-5} for drinking water containing bromate at 3 µg/L.*

** Probable increase in deaths due to a cancer, 10^{-5} = 1 in 100,000 people*

From July 1997 to December 1998, the U.S. Environmental Protection Agency (EPA) documented the occurrence of bromate and other DBPs through a comprehensive collection of sampling data mandated by the Information Collection Rule (ICR).[5] The ICR required that PWSs serving 100,000 or more connections report the concentration of target microorganisms present, the removal process used, and the concentration of DBPs present in their drinking water. In 1998, the EPA set the maximum contaminant level (MCL) for bromate at 10 µg/L and chlorite at 1000 µg/L under the Disinfectants/Disinfection By-Products (D/DBP) Stage 1 Rule.[6] This rule resulted in the promulgation of EPA Method 300.1 as an update to Method 300.0. Method 300.1 reduced the detection limit for bromate from 20 to 1.4 µg/L to allow the PWSs' laboratories to meet the MCL requirement set by the EPA.[7] The European Union (EU Directive 98/83/EC) also proposed the same regulatory value of 10 µg/L bromate (previously at 50 µg/L) in drinking water.[8]

The U.S. EPA reconvened in 2003 to establish the Stage 2 Rule of the D/DBP. Based on a thorough evaluation, the EPA could not estimate the additional benefits of reducing the MCL for bromate. Therefore, this rule resulted in no changes to the current MCL for either chlorite or bromate. However, additional methods for determining low µg/L bromate were promulgated

under the Stage 2 Rule and included ion chromatography (IC) with postcolumn reaction (EPA Methods 317.0 and 326.0) and IC/ICP-MS (EPA Method 321.8). The addition of these methods resulted in improved sensitivity and selectivity for bromate.[9] Recently, the WHO reduced their bromate guideline value from 25 µg/L to a provisional value of 10 µg/L bromate.[4] This change resulted from the availability of improved analytical methods capable of determining low-µg/L concentrations of bromate in environmental waters.

Unlike tap water, bottled water is treated as a food product in the U.S. and therefore regulated by the U.S. Food and Drug Administration (FDA). Bottled water is an increasingly popular product in the U.S. From 1997 to 2002, bottled water sales increased from roughly 6% to 13% per year of total beverage sales, according to the Beverage Marketing Corporation.[10] Because some bottled water companies use ozone or other disinfection treatments, the FDA adopted the EPA's MCLs for chlorite and bromate and the analytical methods used to monitor these contaminants in public drinking water.[11] The FDA also requires that bottled water manufacturers monitor their finished product for these contaminants at least once each year under current good manufacturing practice as stated in part 129 of the Code of Federal Regulations (21 CFR part 129).

Previous methods developed for determining low-µg/L concentrations of bromate by direct injection have focused primarily on using columns specifically designed for carbonate eluents.[12,13] Columns designed for use with hydroxide eluents have not been widely used for the determination of trace bromate in environmental waters due to their lack of appropriate column selectivity and the difficulty in preparing contaminant-free hydroxide eluents. The introduction of electrolytic eluent generation has not only eliminated the difficulty in preparing hydroxide eluents, but has simplified the development of optimized methods. In this application note, we use the IonPac® AS19, a column specifically designed for use with hydroxide eluents and developed with an optimized selectivity for the determination of trace DBPs and bromide in environmental waters. We describe the linearity, method detection limits, and the recovery and precision of spiked municipal and bottled waters.

EQUIPMENT

A Dionex ICS-2000 Reagent-Free Ion Chromatography (RFIC™)* System was used in this work. The ICS-2000 is an integrated ion chromatograph and consists of:

- Eluent Generator
- Column Heater
- Pump with Degasser
- EluGen® EGC II KOH Cartridge (Dionex P/N 058900)
- CR-ATC (Dionex P/N 060477)

AS50 Autosampler

Chromeleon® Chromatography Workstation

*This application note is also applicable to other RFIC systems.

REAGENTS AND STANDARDS

Deionized water, Type I reagent-grade, 18 MΩ-cm resistivity or better

Sodium and Potassium salts, ACS reagent-grade or better, for preparing anion standards (VWR or other)

Fluoride standard 1000 mg/L, 100 mL (Dionex P/N 037158)

Chloride standard 1000 mg/L, 100 mL (Dionex P/N 037159)

Sulfate standard 1000 mg/L, 100 mL (Dionex P/N 037160)

Bromide standard 1000 mg/L, 100 mL (Ultra Scientific, VWR P/N ICC-001)

Sodium Chlorite, 80% (Fluka Chemical Co.)

Sodium Bromate (EM Science, VWR P/N EM SX0385-1)

Ethylenediamine, 99% (Sigma-Aldrich)

CONDITIONS

Columns:	IonPac AS19 Analytical, 4 × 250 mm (Dionex P/N 062885) IonPac AG19 Guard, 4 × 50 mm (Dionex P/N 062887)
Eluent:	10 mM KOH from 0 to 10 min, 10–45 mM from 10 to 25 min*
Eluent Source:	ICS-2000 EG with CR-ATC
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection:	250 µL

Detection: Suppressed conductivity, ASRS® ULTRA II, 4 mm (Dionex P/N 061561) AutoSuppression® Recycle Mode 130 mA current

Background Conductance: <1 µS

System Backpressure: ~2200 psi

Run Time: 30 min

*Method returns to 10 mM KOH for 3 min prior to injection

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Standard Solutions

For several of the anions of interest, 1000-mg/L standard solutions can be purchased from Dionex or other commercial sources. When commercial standards are not available, 1000-mg/L standards can be prepared by dissolving the appropriate amounts of the required analytes in 100 mL of deionized water according to Table 1. Stock standards for most anions are stable for at least 6 months when stored at 4 °C. The chlorite standard is only stable for two weeks when stored protected from light at 4 °C. The nitrite and phosphate standards are only stable for one month when stored at 4 °C.

Working Standard Solutions

Dilute working standard solutions were prepared using the 1000-mg/L stock standards. Working standards containing less than 100 µg/L anions should be prepared fresh daily. Seven levels of calibration standards were used in this study for chlorite, chlorate, and bromide to cover the expected concentration range found in typical environmental samples. The bromate calibration curve was prepared using eight calibration standards. Additional anions listed in Table 1 were used to prepare a simulated drinking water sample containing 1 ppm fluoride, 50 ppm chloride, 0.1 ppm nitrite, 10 ppm nitrate, 100 ppm carbonate, 50 ppm sulfate, and 0.1 ppm phosphate.

Preservation Solution

Dilute 2.8 mL of 99% ethylenediamine (EDA) to 25 mL with deionized water according to section 7.4 in EPA Method 300.1 to prepare a 100-mg/mL solution of EDA. Use 50 µL of this solution per 100 mL of standard or sample so that the final concentration is 50 mg/L.

Table 1. Masses of Compounds Used to Prepare 100 mL of 1000-mg/L Ion Standards

Analyte	Compound	Amount (g)
Fluoride	Sodium fluoride (NaF)	0.2210
Chlorite	Sodium chlorite ($NaClO_2$), 80%	0.1676
Bromate	Sodium bromate ($NaBrO_3$)	0.1180
Chloride	Sodium chloride (NaCl)	0.1649
Nitrite	Sodium nitrite ($NaNO_2$)	0.1500
Chlorate	Sodium chlorate ($NaClO_3$)	0.1275
Bromide	Sodium bromide (NaBr)	0.1288
Nitrate	Sodium nitrate ($NaNO_3$)	0.1371
Sulfate	Sodium sulfate (Na_2SO_4)	0.1479
Phosphate	Potassium phosphate, monobasic (KH_2PO_4)	0.1433

Sample Preparation

Filter samples, as necessary, through a 0.45-µm syringe filter, discarding the first 300 µL of the effluent. To prevent degradation of chlorite or the formation of bromate from hypobromous acid/hypobromite, preserve the samples by adding 50 µL of EDA preservation solution per 100 mL of sample.

RESULTS AND DISCUSSION

EPA Method 300.1 Part B currently specifies an IonPac AS9-HC column using a carbonate eluent and suppressed conductivity detection for the determination of trace DBP anions and bromide in environmental waters, such as drinking water, surface water, and groundwater.[7] The use of the IonPac AS9-HC column in EPA Method 300.1 (B) significantly improved the determination for trace bromate compared to the AS9-SC specified in Method 300.0, Part B.[14] The AS9-HC allowed for detection limits to 1.4 µg/L bromate with a 200-µL injection volume, even in the presence of excess chloride. However, the use of a hydroxide eluent for the determination of trace bromate is more appealing than carbonate eluents. Hydroxide eluent has significantly lower suppressed background conductivity, lower noise, and therefore lower detection limits compared to carbonate eluents. Previously, we described the advantages of hydroxide over carbonate eluents for the determination of common anions.[15] Therefore, similar advantages for bromate should be expected using a column with an appropriate selectivity combined with a hydroxide eluent.

The IonPac AS19 is a high-capacity, hydroxide-selective column specifically designed for the determination of trace bromate and other oxyhalides using a large-volume injection. The novel polymer chemistry of the AS19 yields a higher capacity of 240 µequiv/column compared to the AS9-HC (190 µeq/column). The AS19 stationary phase is based on a new hyper-branched anion-exchange condensation polymer that is electrostatically attached to the surface of a wide-pore polymeric substrate. The AS19 selectivity and capacity are optimized to achieve good resolution between bromate and chloride. Unlike previous IonPac columns, the anion-exchange resin of the AS19 contains alternating treatments of epoxy and amines to produce a coating that grows directly off the surface-sulfonated substrate. The number of alternating coating cycles results in a carefully controlled ion-exchange capacity with an extremely hydrophilic polymer. Therefore, the column has excellent selectivity for hydroxide eluents, allowing lower concentrations of hydroxide to be used.[16] Figure 1 shows a separation of common anions and disinfection by-product anions separated within 30 min using the AS19 column with a hydroxide gradient. As this figure shows, the AS19 achieves excellent resolution between bromate and chloride, making it ideal for determining low concentrations of bromate in municipal and bottled water samples.

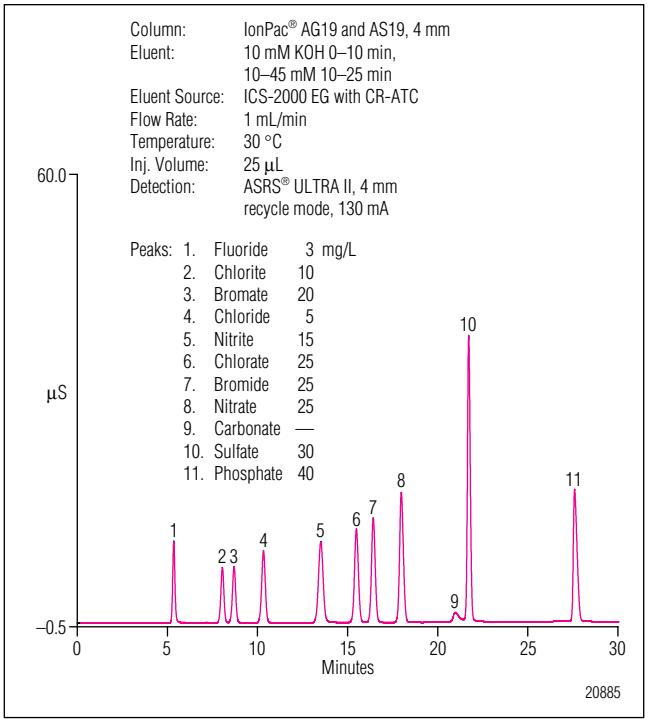

Figure 1. Separation of common anions and disinfection by-product anions on the IonPac AS19 column.

Table 2. Method Detection Limits for Oxyhalides and Bromide in Reagent Water and Simulated Drinking Water Using the IonPac AS19 Column[a]

Analyte	MDL Standard (µg/L)	Calculated MDL[b] in Reagent Water (µg/L)	Calculated MDL[b] in Simulated Drinking Water (µg/L)
Chlorite	1.0	0.23	0.26
Bromate	1.5	0.34	0.42
Chlorate	1.3	0.32	0.30
Bromide	2.0	0.54	0.52

a 250-µL injection volume

b MDL = $\sigma\tau_{s,99}$ where $\tau_{s,99}$ = 3.14 for n = 7

Linearity and Method Detection Limits

Before conducting any sample analyses, the linear calibration range, MDLs, and acceptable performance of a quality control sample (QCS) should be demonstrated. Initially, a seven-point calibration range was used for chlorite, chlorate, and bromide, whereas eight calibration points were used for bromate. MDLs for each anion listed in EPA Method 300.1, Part B were determined by performing seven replicate injections of reagent water fortified at a concentration of three to five times the estimated instrument detection limits. In addition, the MDLs were also determined by fortifying the same concentration of anions in a simulated drinking water sample. Table 2 shows typical calculated MDLs in reagent water and simulated drinking water using the IonPac AS19 column combined with an electrolytic eluent generator and a 250-µL injection. In comparing the detection limits in the two matrices, the results showed no significant difference. The only exception was the calculated MDL for bromate in simulated drinking water was only slightly greater, as expected, because increasing concentrations of chloride will affect the determination of low concentrations of bromate. The calculated MDL for bromate using this method was 0.34 µg/L, approximately 70% lower than previously reported with the AS9-HC column at comparable injection volumes.[12] The lower detection limit results from the excellent peak efficiencies

of the AS19 combined with low noise and exceptionally low suppressed background conductivities obtained by using an electrolytically generated hydroxide eluent. These results demonstrate the significant advantages of using an RFIC system for the determination of trace bromate. Figure 2 shows a separation of an MDL standard prepared in reagent water. As shown, bromate concentrations as low as 1.5 µg/L are easily detected by this method.

Table 3 shows the linear concentration ranges investigated, the coefficients of determination (r^2), and the retention time and peak area precisions of a QCS based on 10 replicate injections. The excellent retention time stability and peak area precisions are consistent with results typically encountered when using an electrolytically generated high-purity potassium hydroxide eluent. The data presented in Table 3 demonstrate the advantages of using a hydroxide-selective column for routine applications, such as the determination of oxyhalides and bromide in environmental waters. The advantages of using IC with a hydroxide eluent are improved linearity, lower background conductivity, and improved method detection limits when compared with "conventional" IC columns that use carbonate eluents, such as the IonPac AS9-HC. The use of an electrolytically generated potassium hydroxide eluent further simplifies the method by eliminating the time required to manually prepare eluents and by reducing the time required for method development.

Effect of Column Overloading

The effect of sample overload on the IonPac AS19 column was evaluated as part of this study. One of the many challenges encountered when determining trace concentrations of bromate is the potential presence of a high sample chloride concentration. In addition to chloride, a high concentration of other anions can together reduce the amount of bromate recovered from a sample. For most environmental samples, chloride, sulfate, and carbonate are generally present at the greatest amounts with respect to other common anions. For this study, we chose a 250-µL sample injection for the analyses because this volume provided us the sensitivity necessary to achieve low-ppb detection of bromate and reduced the likelihood of overloading the column when analyzing high-ionic-strength samples.

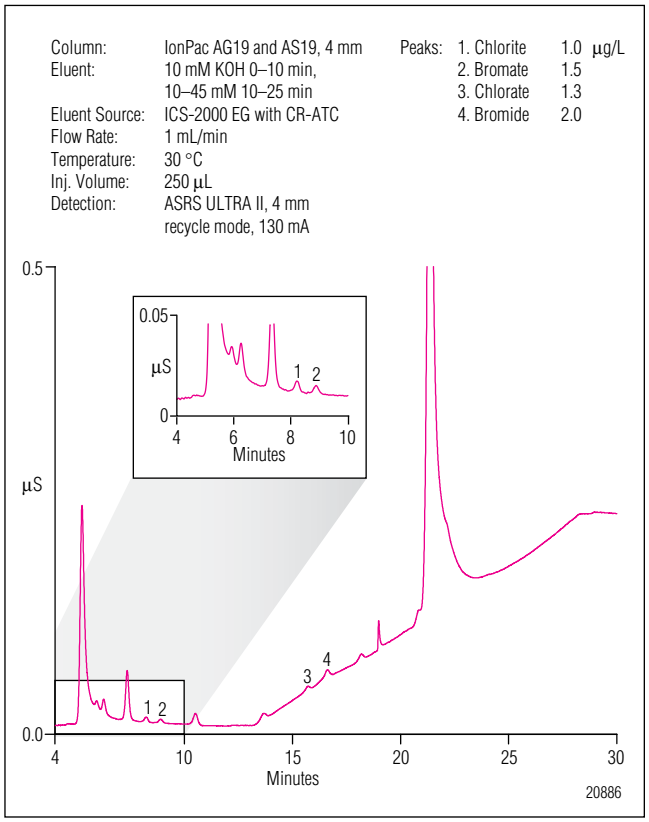

Figure 2. Separation of DBP anions and bromide method detection limit standard.

Table 3. Linearity and Retention Time and Peak Area Precisions Obtained Using the IonPac AS19 Column[a]

Analyte	Range (µg/L)	Linearity (r^2)	Retention Time Precision (%RSD[b])	Peak Area Precision (%RSD)
Chlorite	20–500	0.9997	<0.03	0.44
Bromate	1–40	0.9995	<0.03	1.09
Chlorate	20–500	0.9996	<0.03	0.12
Bromide	20–500	0.9997	<0.03	0.11

[a] Dionex ICS-2000 Reagent-Free IC system with a 250-mL injection volume

[b] RSD = relative standard deviation, n = 10

To determine the effect of chloride on bromate recovery, a series of increasing concentrations of chloride was added to Sunnyvale drinking water. Figure 3 illustrates the effect of increasing concentrations of chloride on the recovery of 5 µg/L bromate. As shown, the recovery of bromate is acceptable in the presence of ~150 ppm chloride. Above this concentration, the bromate significantly decreases to an unacceptable recovery (e.g., <75%). Based on this analysis, the IonPac AS19 can tolerate up to ~150 ppm chloride, resulting in a bromate-to-chloride ratio of 1:30,000, comparable to the AS9-HC column.[12] A similar experiment was performed by increasing the sulfate concentration without any additional chloride added. This experiment demonstrated very little change in the bromate recovery for up to 200 ppm sulfate (results not shown). However, high concentrations of chloride and sulfate can combine to have a greater impact on reducing the bromate recovery. Equal concentrations of chloride and sulfate (up to 120 ppm each) were added to Sunnyvale drinking water, resulting in a 75% bromate recovery. However, most drinking water samples contain significantly less chloride and sulfate than the concentrations included in this study. For example, 18 of the samples examined contained chloride concentrations ranging from <0.1 to 70 ppm and sulfate from <0.1 to 60 ppm. Therefore, almost all samples can be easily analyzed using a 250-µL injection volume, while the column can tolerate 500-µL injections of low- to moderate-ionic-strength samples. Figure 4 shows a chromatogram of a 500-µL injection of Sunnyvale drinking water spiked with oxyhalides and bromide. As shown, bromate was well resolved from chloride with bromate recovered at nearly 100%. Figure 5 compares 250- to 500-µL injection volumes for a simulated drinking water sample. The 500-µL injection volume caused some column overloading and therefore a lower bromate recovery of ~74%. However, a 250-µL injection of the same sample significantly improved the recovery of bromate to 92%. Therefore, a 250-µL injection is recommended for most sample analyses. The effect of column overloading is most prevalent on early-eluting peaks, observed by increased peak broadening and lower recoveries, as demonstrated in this example.

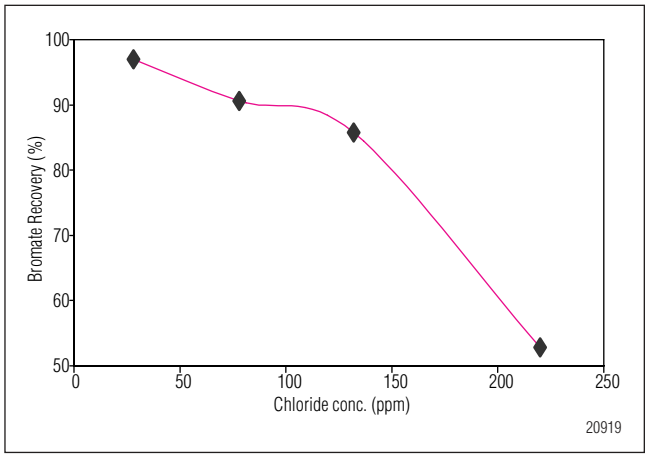

Figure 3. Effect of increasing the chloride concentration on the recovery of 5 µg/L bromide.

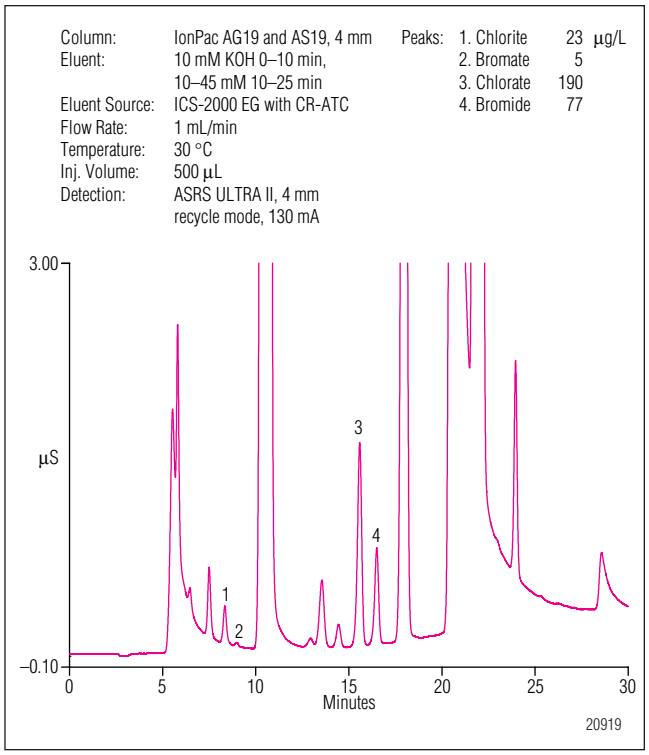

Figure 4. Determination of DBP anions and bromide spiked in drinking water A using a 500-µL injection volume.

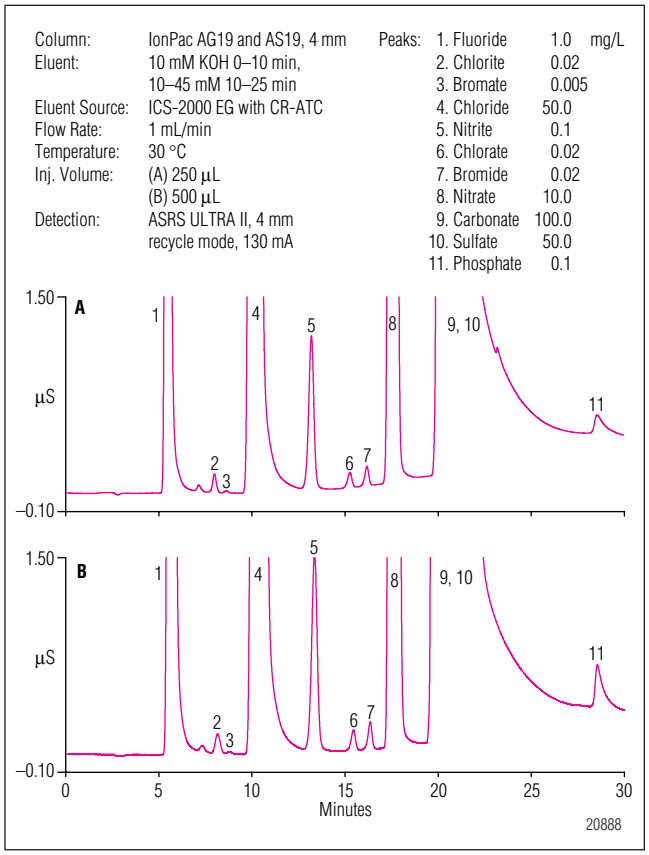

Figure 5. Comparison of simulated drinking water using (A) 250-µL injection and (B) 500-µL injection.

Accuracy and Precision

The performance of the IonPac AS19 was also evaluated through a single-operator precision and bias study using spiked municipal and bottled water samples. Table 4 shows typical recoveries for single-operator data obtained using the IonPac AS19 column for trace concentrations of DBPs and bromide in environmental waters. Most anions demonstrated acceptable recoveries (i.e., 75–125%) according to the criteria outlined in EPA Method 300.1. However, drinking water E resulted in an exceptionally lower recovery for bromide, regardless of the amount of bromide spiked in the sample. Section 9.4.1.5 of EPA Method 300.1 states, "If the recovery of any analyte falls outside the LFM [Laboratory Fortified Matrix] recovery range and the laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related." Therefore, the sample was labeled as "suspect/matrix" to indicate that the poor recovery of bromide was sample related and not system related.

Table 4. Recoveries of Trace Oxyhalides and Bromide Spiked into Environmental Waters

Analyte	Drinking Water A			Drinking Water B			Drinking Water C			Drinking Water D		
	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Chlorite	8.8	10.0	95.3	<MDL	21.0	105.6	11.6	10.0	95.7	<MDL	20.0	108.0
Bromate	<MDL	5.0	92.2	<MDL	5.1	95.6	<MDL	5.0	96.8	1.3	4.9	93.9
Chlorate	81.9	106.0	96.9	120	144.0	104.4	85.3	90.7	97.6	73.6	79.4	98.2
Bromide	26.3	30.0	99.6	202	200.0	99.8	1.2	25.0	94.2	9.7	10.0	107.4
Analyte	**Drinking Water E**			**Surface Water**			**Shallow Well Water[b]**			**Well Water[b]**		
	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found[c] (µg/L)	Amount Added (µg/L)	Recovery (%)
Chlorite	4.6	14.0	93.4	<MDL	20.0	95.7	<MDL	21.0	103.1	<MDL	20.0	101.4
Bromate	<MDL	5.0	100.5	<MDL	5.0	94.7	16.0	9.8	101.1	<MDL	5.0	86.5
Chlorate	136.0	151.0	99.9	<MDL	20.0	96.8	<MDL	30.0	96.8	10.6	20.0	93.0
Bromide	<MDL	20.0	24.8[a]	<MDL	20.0	103.3	381.0	200.0	104.0	452.0	230.0	100.7

[a] Suspect/matrix
[b] Sample diluted 1:1
[c] Calculated amounts

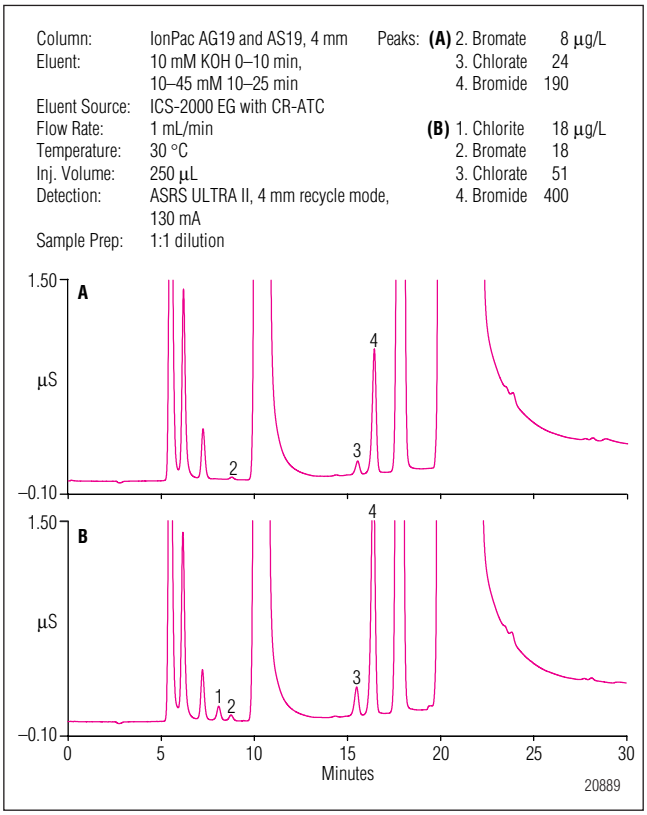

Figure 6. Determination of DBP anions and bromide in (A) shallow well water and (B) spiked shallow well water using the IonPac AS19 column.

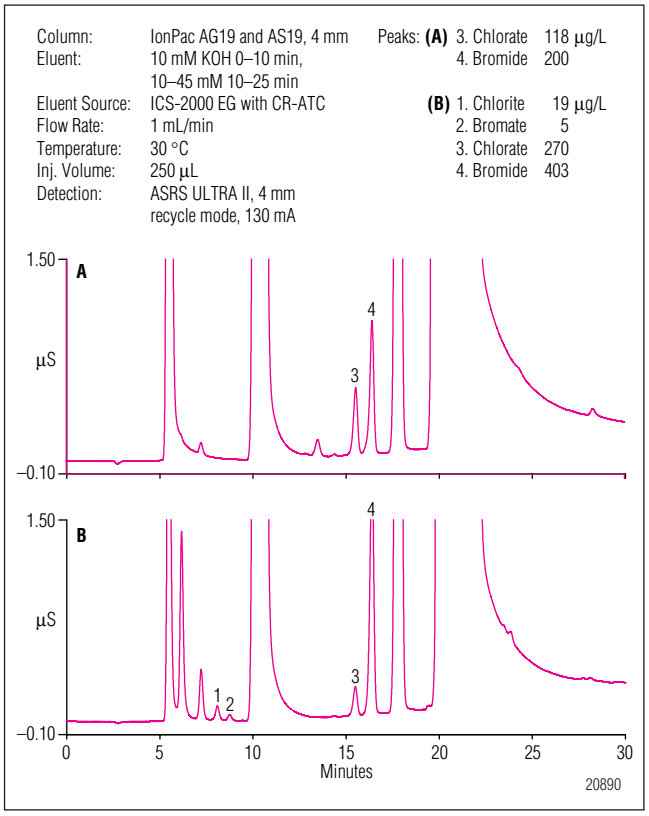

Figure 7. Determination of DBP anions and bromide in (A) drinking water B and (B) spiked drinking water B using the IonPac AS19 column.

Due to the high ionic strength of the well water samples, both were diluted 1:1 to avoid column overloading. The estimated chloride and sulfate concentrations were 160 and 270 ppm, respectively, for the shallow well water, and 150 and 170 ppm, respectively, for the well water prior to dilution. These concentrations exceed the limits determined for this column during the sample overload study. Section 4.1.2 in EPA Method 300.1 states that "sample dilution will alter your Minimum Reporting Limit (MRL) by a proportion equivalent to that of the dilution." In this study, dilution of the well water samples increased the bromate MRL from 1 to 2 µg/L. However, the adjusted MRL was still sufficient to report the 8 µg/L bromate detected in the diluted sample. Because this well water sample is not known to be treated, the presence of bromate was unexpected. The detection of bromate in the well water may result from contamination by a nearby site that originally contained a high concentration of the anion. Figure 6A shows a chromatogram of diluted shallow well water. Figure 6B shows the same well water sample spiked with 10–20 µg/L of DBP anions and 200 µg/L of bromide. As shown, bromate was well resolved from the high concentration of chloride, resulting in a recovery of 101.1%.

Figure 7 shows chromatograms of an unspiked and spiked drinking water B. This sample also demonstrates the excellent resolution and accuracy of analysis for the determination of trace DBP anions and bromide using an RFIC system. The calculated recoveries of the target analytes ranged from 96 to 106%.

This study also included the analysis of a variety of bottled water samples randomly obtained from a local supermarket. A previous study conducted from 1997–1998 in Canada found many bottled waters contained bromate, some at concentrations greater than 25 µg/L.[17] These results in combination with the increasing popularity of bottled water, led us to examine the presence of bromate in several different brands of bottled waters. More than half of the bottled waters tested in this study reported using ozonation as a form of treatment according to the bottle's label or company's web site (Table 5).

Table 5. Treatments Used for Different Bottled Waters	
Bottled Water	**Treatment**
1	Natural spring water (no treatment)
2	UV light, RO[a], ozonation
3	Ozonation
4	Natural mineral water (no treatment)
5	RO
6	Microfiltration, UV light, ozonation
7	Filtration
8	Microfiltration, ozonation
9	Natural spring water (no treatment)
10	Microfiltration, ozonation
11	Microfiltration, RO, DI[b], ozonation
12	Ozonation

[a] RO = reverse osmosis

[b] DI = deionization

Table 6 shows the amount found and the recoveries obtained using the AS19 column for trace concentrations of DBP anions and bromide spiked in the bottled waters. All target analytes demonstrated acceptable recoveries according to U.S. EPA Method 300.1. Only four bottles tested contained some amount of bromate, with two of these near or slightly above the bromate MCL of 10 µg/L. No correlation was observed between the concentrations of bromide in the samples versus the amount of bromate detected. For example, bottled water #10 contained approximately 4 µg/L bromate, but no bromide was detected in the sample. However, the conversion of bromide to bromate upon ozonation is affected by several factors, such as the presence of natural organic matter, pH, temperature, and other variables.[2] As expected, most bottled waters analyzed contained appreciably less chloride and sulfate than tap water with estimated maximum concentrations of 8 and 30 ppm, respectively.

Table 6. Recoveries of Trace Oxyhalides and Bromide Spiked into Bottled Waters

Analyte	Bottled Water 1			Bottled Water 2			Bottled Water 3			Bottled Water 4		
	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Chlorite	<MDL	20.0	108.1	<MDL	20.0	102.9	<MDL	20.0	99.8	<MDL	20.0	90.2
Bromate	<MDL	5.0	96.1	<MDL	5.0	100.7	10.2	9.8	104.6	<MDL	5.0	83.5
Chlorate	2.4	20.0	107.7	<MDL	20.0	106.5	<MDL	20.0	102.8	10.2	20.0	103.5
Bromide	7.5	20.0	105.0	<MDL	20.0	106.5	19.4	20.0	92.9	95.5	105.0	97.7
Analyte	**Bottled Water 5**			**Bottled Water 6**			**Bottled Water 7**			**Bottled Water 8**		
	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Chlorite	<MDL	20.0	101.2	<MDL	20.0	101.5	<MDL	20.0	106.7	<MDL	20.0	102.2
Bromate	<MDL	5.0	95.9	9.2	9.8	106.6	<MDL	5.0	92.3	<MDL	5.0	93.7
Chlorate	1.6	20.0	108.6	375.0	150.0	97.3	<MDL	25.0	90.6	<MDL	20.0	105.4
Bromide	1.2	20.0	95.6	2.5	20.0	100.9	31.8	30.0	98.9	18.7	20.0	93.8
Analyte	**Bottled Water 9**			**Bottled Water 10**			**Bottled Water 11**			**Bottled Water 12**		
	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Chlorite	<MDL	20.0	106.1	<MDL	20.0	98.2	<MDL	20.0	104.8	<MDL	20.0	95.2
Bromate	<MDL	5.0	98.4	4.4	5.0	101.1	<MDL	5.0	96.4	0.98	5.0	102.1
Chlorate	<MDL	20.0	105.7	<MDL	20.0	107.7	<MDL	23.0	98.3	4.2	20.0	98.5
Bromide	<MDL	20.0	104.1	<MDL	20.0	105.3	6.3	23.0	94.5	<MDL	20.0	99.2

The low ionic content of most bottled waters allows the use of larger injection volumes (500 µL or more). Figure 8 shows a 250-µL injection of an unspiked and spiked bottled water sample. The disinfection treatment used for this bottled water was UV radiation and ozonation. An unusually high amount of chlorate was detected in the sample, indicating that some form of chlorination may also be used for treatment. Bromate was detected at a slightly lower concentration than the EPA's MCL, possibly indicative of elevated levels of bromide in the source water. The recoveries for oxyhalide DBPs and bromide spiked in the sample ranged from ~97 to 107%, well within EPA Method 300.1 specifications.

The precision of the method using the AS19 column in combination with an electrolytic eluent generation was determined by performing 10 replicate injections of randomly selected samples spiked with trace concentrations of DBPs and bromide. Overall, the calculated peak area precisions varied from 0.21 to 1.78% with retention time precisions <0.04% for most target analytes. For bromate, the worst peak area precision observed was 1.78%. This number represents a deviation of only ±0.09 µg/L based on a sample containing 5 µg/L bromate. The high precision of this method is consistent with results typically found with an RFIC system.

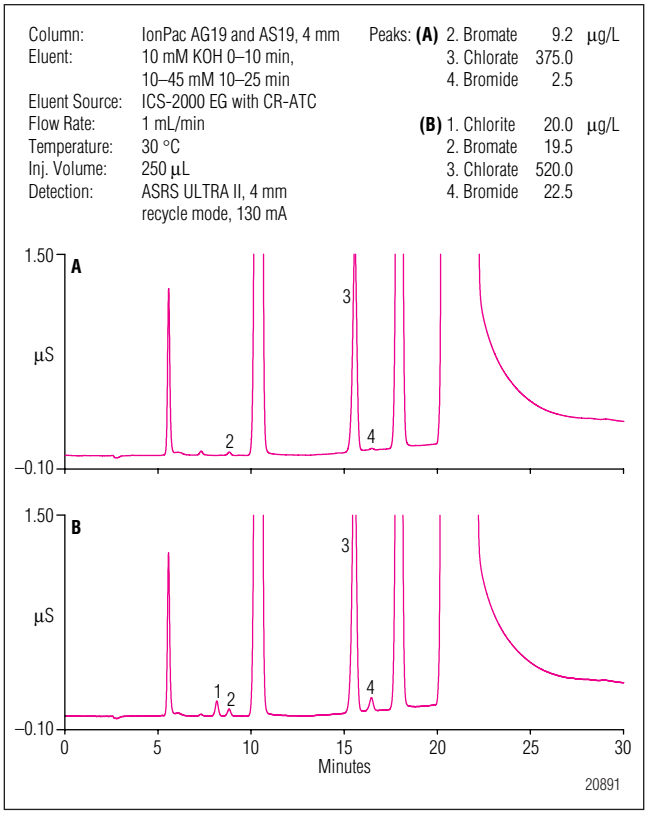

Figure 8. Determination of DBP anions and bromide in (A) bottled water 6 and (B) spiked bottled water 6 using the IonPac AS19 column.

CONCLUSION

IC with a hydroxide-selective IonPac AS19 column and an electrolytic eluent generator is an improved approach for determining trace concentrations of DBP anions and bromide in municipal and bottled water samples. The high-capacity AS19 column can be used with large-volume injections to detect low-ppb concentrations of bromate, a potential human carcinogen, in many municipal and bottled waters. In addition, electrolytic generation of an ultrapure potassium hydroxide eluent, combined with the AS19 column, improves linearity, MDLs, precision, and resolution between bromate and chloride compared to the AS9-HC column described in EPA Method 300.1. This approach also eliminates the need to manually prepare eluents and thereby increases the automation, ease of use, and reproducibility between analysts and laboratories. The U.S. EPA, Office of Water, has determined that the use of hydroxide eluents in EPA Method 300.1 is acceptable for compliance monitoring under the Clean Water Act and Safe Drinking Water Act.[18]

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Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection

INTRODUCTION

Numerous polycyclic aromatic hydrocarbons (PAHs) are carcinogenic, making their presence in foods and the environment a health concern. Regulations around the world limit levels of a variety of PAHs in drinking water, food additives, cosmetics, workplaces, and factory emissions. PAHs also occur in charbroiled and dried foods, and may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. PAHs in foods can also result from petrogenic contamination. The European Commission regulates the amounts of PAHs in foods, and has imposed a limit of 2.0 µg/kg for benzo[a]pyrene (BaP) in edible oils, as BaP was determined to be a good indicator of PAH contamination.[1]

PAHs have traditionally been separated using HPLC and determined using UV,[2] fluorescence,[3,4] electrochemical,[5] and mass spectrometry (using atmospheric-pressure photoionization)[6] detection methods. After an oxygenation reaction, PAHs can also be determined by LC-MS/MS.[7] These methods of determining PAHs in edible oils require multiple manual sample preparation steps. One study of PAHs in over a dozen edible oils used a DMSO extraction followed by three extractions with cyclohexanone and cleanup with a silica column.[8] Another study of six edible oils used solid-phase extraction, but required solvent extraction steps before SPE and evaporation afterward.[9] These manual steps consume solvent, resources, and time.

In recent years, donor-acceptor complex chromatography (DACC) has gained popularity for PAH analysis.[10–13] DACC stationary phases can be used for solid phase extraction (SPE), retaining PAHs while matrix components are flushed to waste. After elution of the analytes, solvent exchange is used to prepare the sample for HPLC analysis. Compared to traditional methods, this cleanup technique uses less solvent, is less labor intensive, and saves considerable time.[11] However, this approach still involves several manual sample-handling steps, and therefore still requires labor and is prone to errors.

In 1996, Van Stijn et al.[12] developed an automated process for oil sample preparation and analysis. The setup consists of coupling a DACC cleanup column with an HPLC analytical column. This solution does not require manual cleanup and solves the previously described challenges. However, adopting the method for routine operation is difficult and requires advanced technical know-how to optimize the system configuration. This optimization can be time consuming. Furthermore, the described solution uses the autosampler software for system control and different software for data collection, instead of using an integrated chromatography data system for system control and monitoring. This leaves room for improvement in ease of operation, process monitoring and documentation, validation, reporting, and automated diagnosis.

Maio et al.[13] adapted Van Stijn's solution to create a method for automated on-line determination of PAHs in edible oils that addresses the remaining challenges. This solution was performed on an HPLC system equipped with a dual-gradient HPLC pump and two switching valves, allowing on-line sample enrichment on a DACC column with HPLC analysis. On-line coupling of sample preparation and analysis eliminates the complex manual pretreatment required by traditional methods. This automation reduces unintentional errors and increases reproducibility. The analysis time per sample is approximately 80 min with the dual-gradient HPLC system, compared to 8–10 h with traditional methods. Moreover, this automated system can run 24 h a day, significantly increasing sample throughput and making this complex analysis routine.

This application note describes the setup and method for determining PAHs in edible oils on-line using a Dionex UltiMate® 3000 ×2 Dual-Gradient HPLC platform, based on the solution described by Maio,[13] and evaluates the method performance (i.e. linearities, detection limits, reproducibilities, recoveries, and carry over of autosampler).

EQUIPMENT

UltiMate 3000 ×2 Dual system consisting of:

- DPG-3600A pump with SRD-3600 Air Solvent Rack
- WPS-3000TSL autosampler
- TCC-3200 thermostatted column compartment with two 2p-6p valves

RF2000 fluorescence detector

Chromeleon® 6.80 SP1 Chromatography Workstation

Device configurations for the online DACC cleanup with analytical HPLC are as shown in Figures 1 to 3. In these figures, the upper valve is the right valve and lower valve is the left valve in the TCC-3200.[13]

REAGENT AND STANDARDS

Deionized water from a Milli-Q® Gradient A10

Acetonitrile (CH_3CN), HPLC grade (Fisher Scientific)

Isopropanol, HPLC grade, (Fisher Scientific)

Charcoal, activated granular (activated carbon), chemical pure grade (Shanghai Chemical Reagent Company)

Mix of PAHs, EPA Sample for Method 610, 200 µg/mL for each component, including phenanthrene, anthracene, fluoranthene, pyrene, benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h] anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene (Restek)

Benzo[b]chrysene, 50 µg/mL, used as an internal standard (I.S.) (AccuStandard)

SAMPLES

Two brands of olive oil (olive oil 1 and 2 from Italy and Spain, respectively), and one brand of sesame oil (from China)

CONDITIONS

Analytical Columns:	Two SUPELCOSIL™ LC-PAH columns, 4.6 × 250 mm (Supelco Cat.# 58229)
On-Line SPE Column:	ChromSpher Pi, 3.0 × 80 mm (Varian P/N: CP28159)
Mobile Phases:	A. Water B. Acetonitrile for both loading and analysis pumps C. Isopropanol for loading pump
Flow Rate:	1 mL/min
Injection Volume:	80 µL (100 µL injection loop)
Column Temperature:	30 °C
Autosampler Temperature:	40 °C
Detection:	Fluorescence (Table 4)

Table 1 shows the gradient for on-line solid-phase extraction (SPE) using the loading pump, and Table 2 shows the gradient for separation using the analysis pump. Table 3 shows the valve switching timing.

Because the maximum fluorescent responses of PAHs occur at different emission wavelengths, it is necessary to change the excitation and emission wavelengths based on individual PAH retention times. Table 4 shows the program for wavelength changes.

PREPARATION OF STANDARDS AND SAMPLES

Purification of Olive Oil Used as Blank and Matrix

Add 1 g activated carbon to 20 g olive oil, heat for 2 h at 60 °C while stirring, then filter through a pleated filter. Pass filtrate through a membrane filter (0.45 µm, PTFE, Millex™-LCR, Millipore) and store the resulting purified oil sample at 4 °C.

Preparation of Olive Oil Containing I.S. Used as Matrix

To prepare a 0.25 µg/mL stock I.S. solution, add 995 µL isopropanol using a 1-mL pipette to a 2-mL vial, and add 5 µL of 50 µg/mL I.S. oil using a 10-µL syringe.

Add 40 µL of the 0.25 µg/mL stock I.S. solution, using a 10-µL syringe, to about 10 g of the purified olive oil used as blank and matrix. The concentration of I.S. in the oil matrix is about 1 µg/kg. In the work presented in this application note, the I.S. working standard was added to 10.0786 g of the purified olive oil sample. The resulting I.S concentration in the matrix was 0.992 µg/kg.

Preparation of Working Standards (Olive Oil as Matrix)

To prepare a 1 µg/mL stock standard solution, add 995 µL isopropanol, using a 1-mL pipette, and 5 µL of the 200 µg/mL standard solution, using a 10-µL pipette, to a 2-mL vial. The stock standard solution is used to prepare working standards as described in Table 5.

Edible Oil Sample Preparation

Prior to injection, filter oil through a 0.45-µm membrane (PTFE, Millex-LCR, Millipore).

Table 1. Gradient Program for On-Line SPE

Time	Flow rate (mL/min)	Solvent A (% vol.)	Solvent B (% vol.)	Solvent C (% vol.)	Curve (%)
0.00	0.35	0	0	100	--
12	0.35	0	0	100	5
12.1	0.35	20	80	0	5
20.9	0.35	20	80	0	5
20.91	0.35	0	100	0	5
50.9	0.35	0	100	0	5
51.5	0.35	0	0	100	5
66.5	0.35	0	0	100	5

Table 2. Gradient Program for Separation

Time	Flow rate (mL/min)	Solvent A (% vol.)	Solvent B (% vol.)	Curve (%)
0.00	0.4	20	80	--
14.6	0.4	20	80	5
16	1	20	80	5
30	1	0	100	6
58	1	0	100	5
58.1	1	20	80	5
65	1	20	80	5
65.5	0.4	20	80	5
70	0.4	20	80	5

Table 3. Valve Switching Programs for the Left and Right Valves

Time (min)	Left Valve	Right Valve
0.00	6-1	1-2
12.1	No Movement	6-1
14.5	1-2	No Movement
17	6-1	No Movement
61.5	No Movement	1-2

Table 4. Wavelength Changes for RF2000 Fluorescence Detector

Time (min)	Excitation Wavelength (nm)	Emission Wavelength (nm)
0.00	256	370
27.05	256	390
29.5	240	420
33.5	270	385
37.5	290	430
51.5	305	480
53.5	290	430

Table 5. Preparation of the Working Standards (Oil as Matrix)				
Vial # (1.5 mL)	**Vial 1**	**Vial 2**		
Volume of 1 µg/mL PAH stock standard solution (µL)	50	100		
Volume of isopropanol (µL)	450	400		
Concentration of PAHs (µg/mL)	0.1	0.2		
Vial # (1.5 mL)	**Vial 3**	**Vial 4**	**Vial 5**	**Vial 6**
Volume of Diluted Standard (Vial 1 or Vial 2) or Stock Standard (µL)	10 µL, Vial 1	10 µL, Vial 2	10 µL, stock standard	20 µL, stock standard
Added weight of the cleaned olive oil used as matrix (containing I.S.) (g)	1.0355	1.0376	1.0389	1.0358
Final concentration of PAHs (µg/kg)	0.956	1.909	9.534	18.943
Final concentration of I.S. (µg/kg)	0.983	0.983	0.983	0.973

RESULTS AND DISCUSSION

Description of the On-Line DACC-HPLC Method

The flow scheme, shown in Figure 1, couples the DACC cleanup directly with the analytical HPLC run, using a second gradient pump and two column-switching valves. Figure 1 shows the valve positions at the time of the injection. The filtered and undiluted oil is injected directly, using isopropanol (IPA) to transfer the sample onto the enrichment column (DACC column). The analytical separation column is equilibrated with the second pump at the same time. After the analytes are bound to the DACC column, the right valve switches to flush out the oils and IPA, in a backflow mode, with acetonitrile/water (Figure 2). When all IPA and oils have been removed, the system switches the enrichment column into the analytical flow path (Figure 3).

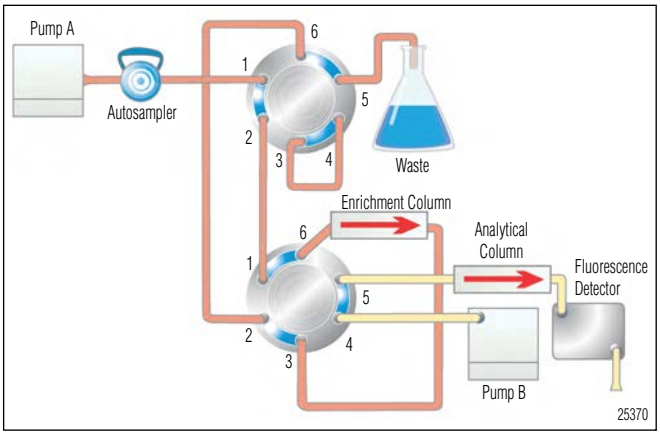

Figure 1. Flow scheme for on-line sample preparation and analysis. The valves are positioned for injection of the sample on the enrichment column.

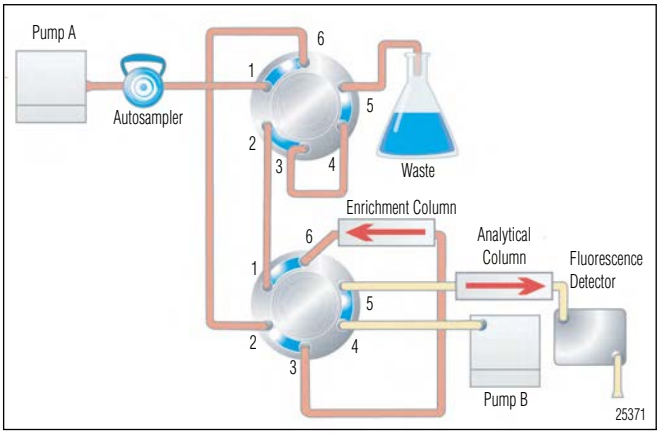

Figure 2. Isopropanol is flushed out of the enrichment column in backflow mode.

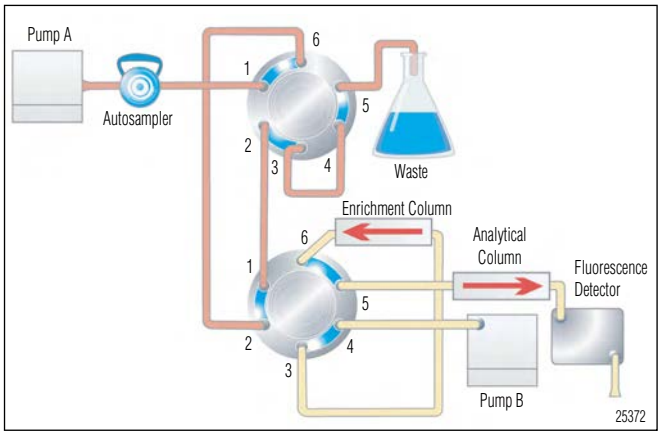

Figure 3. The enrichment column is switched into the analytical flow path, eluting the PAHs onto the analytical column for gradient separation followed by fluorescence detection.

Reproducibility, Detection Limits, and Linearity

Method reproducibility was estimated by making seven replicate injections of olive oil sample 1 spiked with the PAHs standard mix (vial #6 in Table 5) (Figure 4). Table 6 summarizes the retention time and peak area precision data. Calibration linearity for the determination of PAHs was investigated by making five replicate injections of a mixed standard of PAHs prepared at four different concentrations. The internal standard method was used to calculate the calibration curve and for real sample analysis. Table 7 reports the data from this determination as calculated by Chromeleon software. PAH method detection limits (MDLs) are also listed in Table 7.

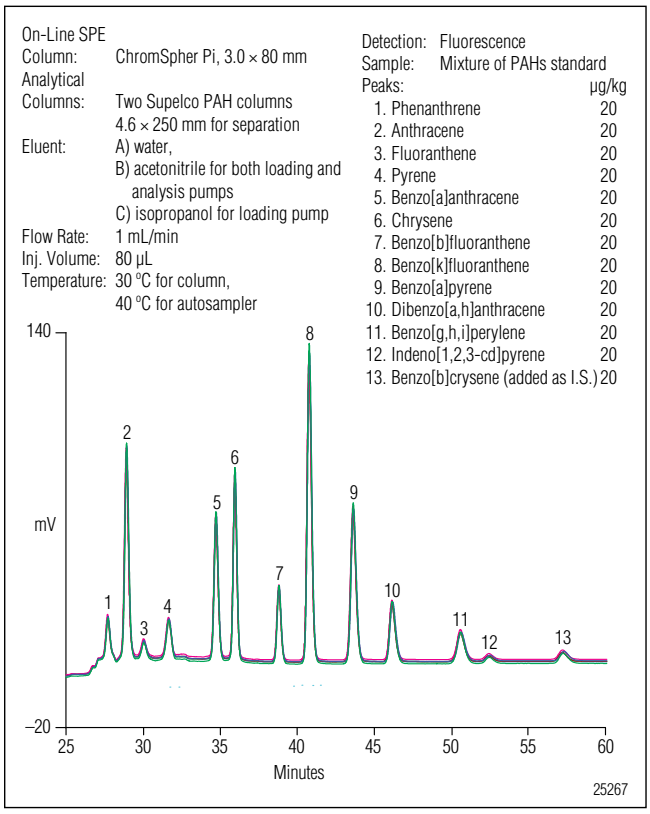

Figure 4. Overlay of chromatograms of seven serial injections of olive oil sample 1 spiked with a PAH standard mixture (20 µg/kg).

Table 6. Reproducibility of Retention Times and Peak Areas[a]

PAH	RT RSD	Area RSD
Phenanthrene	0.064	6.733
Anthracene	0.055	4.350
Fluoranthene	0.072	4.491
Pyrene	0.044	4.965
Benzo[a]anthracene	0.031	4.628
Chrysene	0.026	4.469
Benzo[b]fluoranthene	0.027	4.325
Benzo[k]fluoranthene	0.027	4.173
Benzo[a]pyrene	0.031	4.399
Dibenzo[a,h]anthracene	0.041	4.383
Benzo[g,h,i]perylene	0.042	5.038
Indeno[1,2,3-cd]pyrene	0.048	4.484

[a]Seven injections of olive oil sample 1 spiked with 20 µg/kg mixed PAH standards.

Table 7. Calibration Data for the 12 PAHs

Phenols	Equations	r (%)	MDL (µg/kg)
Phenanthrene	A = 12.0911 C + 7.4235	99.5173	0.42
Anthracene	A = 53.2837 C + 49.1644	99.1062	0.26
Fluoranthene	A = 4.6993 C + 2.8308	98.0798	1.19
Pyrene	A = 11.0580 C + 11.0016	99.0524	0.69
Benzo[a]anthracene	A = 35.6167 C + 68.1072	98.5246	0.68
Chrysene	A = 44.2503 C + 51.2535	98.6398	0.34
Benzo[b]fluoranthene	A = 19.8706 C + 19.8867	99.0712	0.21
Benzo[k]fluoranthene	A = 89.5111 C + 86.5361	99.0725	0.39
Benzo[a]pyrene	A = 53.4937 C + 48.0755	99.1057	0.75
Dibenzo[a,h]anthracene	A = 22.5211 C + 21.6513	99.1431	0.41
Benzo[g,h,i]perylene	A = 14.7151 C + 13.0643	99.1995	0.58
Indeno[1,2,3-cd]pyrene	A = 2.9058 C + 1.8162	99.4115	0.59

The single-sided Student's test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation (SD) of the peak area of seven injections of olive oil sample 1 spiked with 2 µg/kg mixed PAHs standard is multiplied by 3.14 (at n = 7) to yield the MDL.

Carryover Performance

Carryover performance for the WPS 3000TSL autosampler was investigated by serial injections of 500 µg/kg of benzo[b]crysene (I.S.) and a purified olive oil sample prepared as a blank. Figure 5 shows exceptional carryover performance with external needle wash by acetonitrile both before and after the injection. There was no cross contamination observed when using the WPS 3000TSL autosampler for this application.

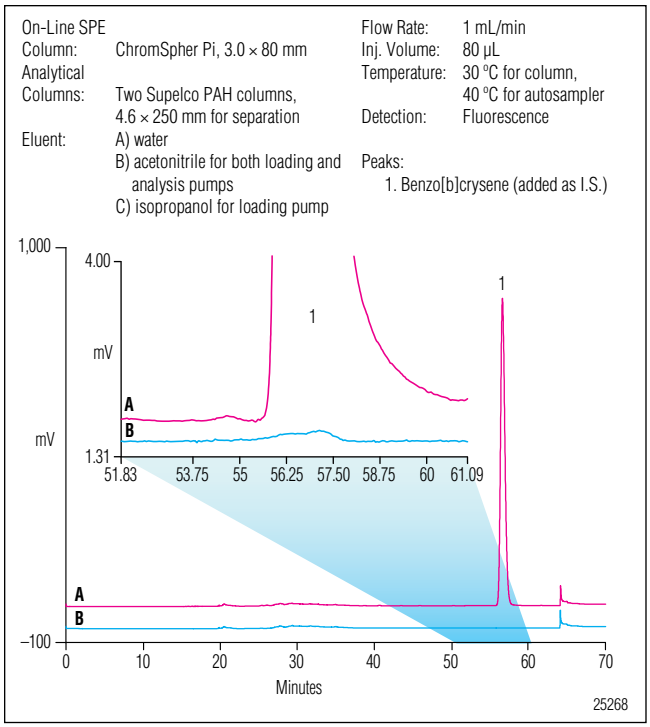

Figure 5. Carryover test on the WPS-3000 autosampler. A) Purified olive oil spiked with 500 µg/kg of benzo[b]crysene (I.S.). B) Purified olive oil prepared as blank, analyzed immediately after A).

Effect of the Purified Olive Oil Used as Blank and Matrix

One brand of olive oil was prepared as a blank and to serve as a matrix according to the procedure specified above. Figure 6 is an overlay of chromatograms of the original and purified olive oils, from which we observe that many ingredients were eliminated from the original olive oil. However, impurities persisted in the prepared olive oil used as a blank and matrix, which might have affected determination of some PAHs. To overcome this effect, the baseline of the purified olive oil blank was subtracted during data processing with Chromeleon software.

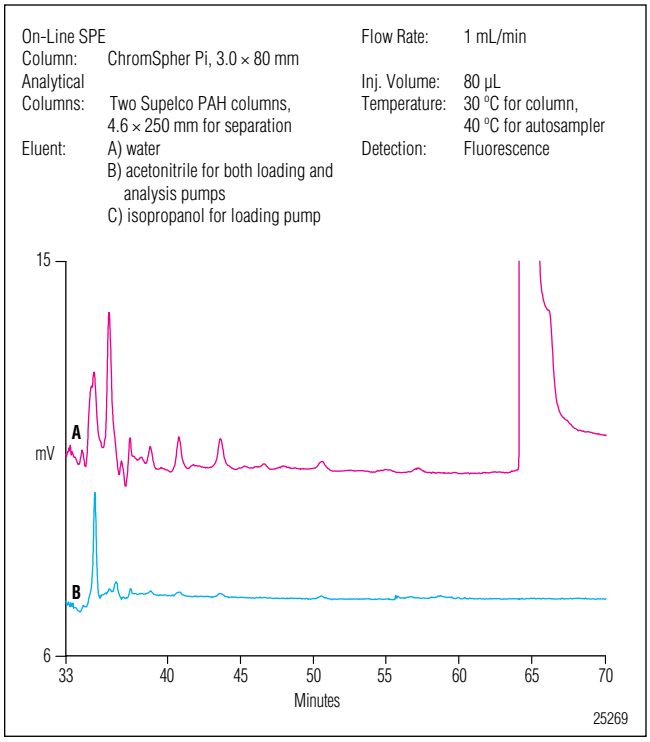

Figure 6. Overlay of chromatograms of A) untreated olive oil, and B) purified olive oil used as a blank.

Sample Analysis

Two olive oil samples and one sesame oil sample were analyzed. The results are summarized in Table 8. Figure 7 shows chromatograms of the oil samples. Spike recoveries for these PAHs were in the ranges from 70% to 131%. Some PAHs were found in the edible oil samples. Five PAHs, phenanthrene, anthracene, benzo[a] anthracene, chrysene and benzo[a]pyrene, existed in all of the three samples and phenanthrene was obviously the most abundant PAH.

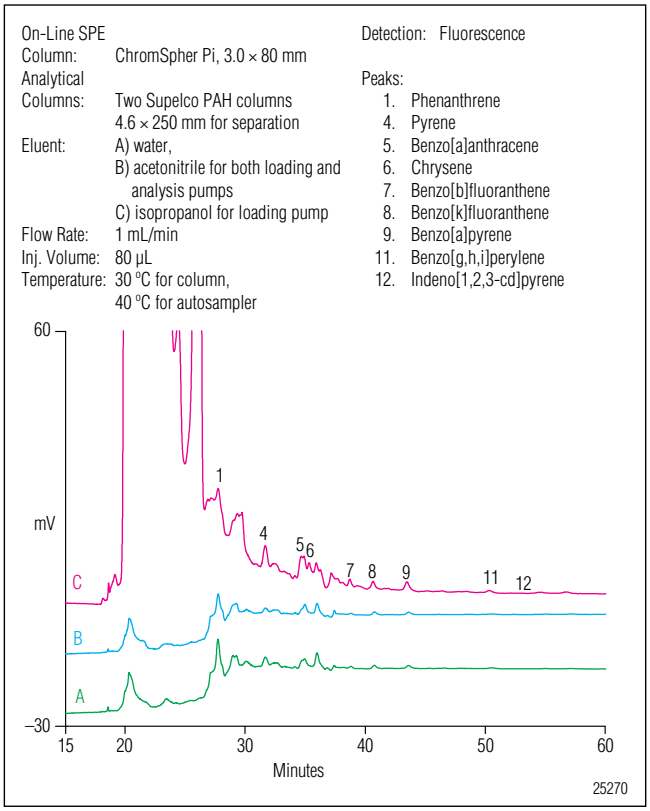

Figure 7. Overlay of chromatograms of A) olive oil 1, B) olive oil 2, and C) sesame oil samples.

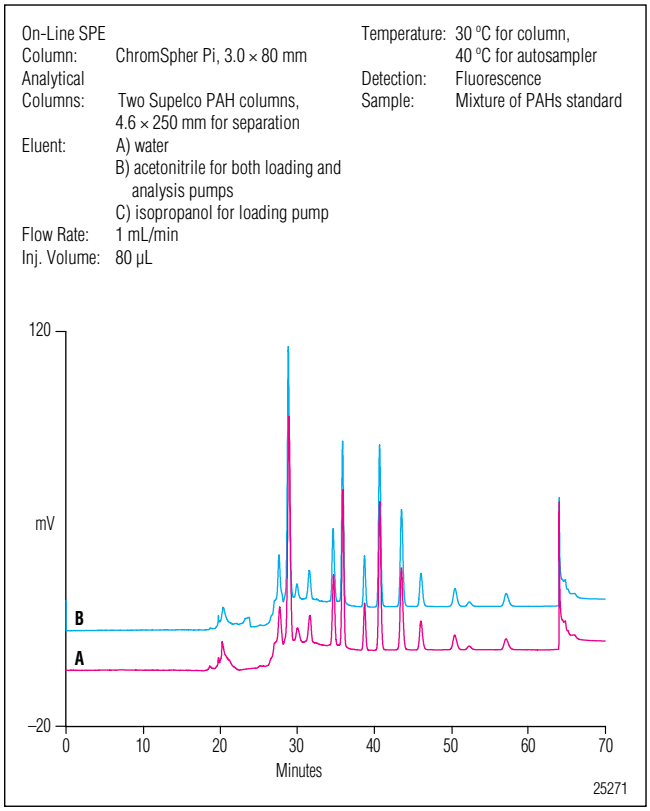

Figure 8. Separation of PAHs in olive oil using different SPE columns. A) SPE column with 600 prior injections; B) new SPE column.

Ruggedness of the SPE Column

The tolerance of the SPE column used in this on-line analysis of PAHs in edible oils was investigated by comparing the separation of PAHs using two different SPE columns. One of these columns already had extracted over 600 injections of an edible oil sample; the other was nearly new. Figure 8 shows an overlay of chromatograms of PAHs using these two SPE columns. Final results of the PAH analyses are very similar, despite the different exposure levels of the two columns.

PRECAUTIONS

Contaminants in solvents, reagents, glassware, and other sample processing hardware may cause method interferences, so glassware must be scrupulously cleaned. Use high-purity reagents and solvents to minimize interference problems.

Table 8. Analytical Results for Olive Oil 1, Olive Oil 2, and Sesame Oil					
PAH	Olive Oil 1			Olive Oil 2	Sesame Oil
	Detected (µg/kg)	Added (µg/kg)	Recovery (%)	Detected (µg/kg)	Detected (µg/kg)
Phenanthrene	37	5	120	13.2	52
Anthracene	4.5	5	109	3.2	6.1
Fluoranthene	1.0	5	112	ND	ND
Pyrene	2.2	5	131	1.3	ND
Benzo[a]anthracene	2.8	5	108	2.1	18
Chrysene	4.4	5	110	3.2	5.3
Benzo[b]fluoranthene	ND	5	90	ND	ND
Benzo[k]fluoranthene	ND	5	84	ND	ND
Benzo[a]pyrene	2.7	5	106	2.5	3.9
Dibenzo[a,h]anthracene	ND	5	84	ND	ND
Benzo[g,h,i]perylene	ND	5	70	ND	1.2
Indeno[1,2,3-cd]pyrene	ND	5	82	ND	ND

One sample and one spiked sample were prepared, and two injections of each were made.

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Robust and Fast Analysis of Tobacco-Specific Nitrosamines by LC-MS/MS

INTRODUCTION

Tobacco-specific nitrosamines (TSNA) are a group of carcinogens found only in tobacco products. They are formed from nicotine and related alkaloids during the production and processing of tobacco and tobacco products.[1] Due to their carcinogenic properties, efforts have been made to reduce TSNA levels in tobacco products. The desired goal of this investigation is to develop a sensitive, high-throughput method to monitor TSNA levels in tobacco and tobacco products. This study focuses on N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

Conventional methods for TSNA analysis are based on gas chromatography with a thermal energy analyzer (GC-TEA)[2,3], or high-performance liquid chromatography (HPLC) with various detection techniques such as UV and mass spectrometry (MS).[4–6]

This application note (AN) describes a robust and fast LC-MS/MS method developed to analyze the four TSNAs in tobacco cigarettes after a simple sample preparation of ammonium acetate liquid extraction and filtration. The chromatographic separation of the four TSNAs was achieved within 3.5 min and these analytes were detected with great sensitivity and selectivity by tandem mass spectrometry using Selected Reaction Monitoring (SRM) detection. This method has been evaluated with respect to linearity, detection limits, precision, and accuracy. The ruggedness of the methodology was proven with more than 1000 replicate injections.

EXPERIMENTAL

Instrumentation

HPLC

P680 dual ternary pump

ASI-100 autosampler

TCC-100 thermostatted column compartment

UVD340U detector

Mass Spectrometer

TSQ Quantum Access™ triple quadrupole mass spectrometer with heated electrospray ionization (HESI) interface.

Software

Xcalibur® 2.0

Dionex DCMS^{Link}™ for Xcalibur (version 2.0)*

* DCMS^{Link} is a Chromeleon®-based software module providing the interface for controlling a wide range of Dionex chromatography instruments from different mass spectrometer software platforms.

CONDITIONS

Chromatographic Conditions

Analytical Column:	Acclaim® RSLC PA2 (5.0 × 2.1 mm, 2.2 µm)
Column Temperature:	60 °C
Injection Volume:	10 µL
Mobile Phase:	10% CH_3CN in buffer (1 mM NH_4OAc, pH adjusted to pH 8.0 by NH_4OH)
Flow Rate:	0.50 mL/min
Detection:	UV at 230 nm
	TSQ Quantum Access SRM

Mass Spectrometric Conditions

Needle Voltage: 2000 V
Vaporizer Temp.: 350 °C
Sheath Gas: 60 (arbitrary unit)
Auxiliary Gas: 50 (arbitrary unit)
Ion Sweep Gas: 2 (arbitrary unit)
Collision Gas Pressure: 1.5 mTorr
Capillary Temp.: 350 °C

SRM Transitions:

NNN	178.1 → 148.1 *m/z*	11 V
	178.1 → 120.1 *m/z*	18 V
NNK	208.1 → 122.1 *m/z*	11 V
	208.1 → 79.2 *m/z*	38 V
NNK-d_4	212.1 → 126.1 *m/z*	11 V
	212.1 → 83.2 m/z	38 V
NAT	190.1 → 160.1 *m/z*	10 V
	190.1 → 79.2 *m/z*	5 V
NAB	192.1 → 162.1 *m/z*	10 V
	192.1 → 133.1 *m/z*	24 V

The 1st SRM transition of each compound is used for quantification and the 2nd SRM transition is used for confirmation.

SAMPLE PREPARATION

Tobacco cuts from five brands of cigarettes were weighed to 0.25 grams into 20 mL glass vials, and extracted with 10 mL of 100 mM ammonium acetate solution. The vials were placed on a swirl table and agitated for 30 min. The extracts were filtered through 0.25 µm membrane syringe filters. A 1.0 mL aliquot of each extract was then spiked with 10 µL internal standard (IStd, NNK-d_4) in preparation for LC-MS/MS analysis.

RESULTS AND DISCUSSION

Chromatography

As shown in Figure 1, the four TSNAs are retained and completely resolved within 3.5 min, with the minimum of the retention factors (K'_{NNN}) greater than 4 and minimum resolution (R_{sNAT}) greater than 2.0. These demonstrated retention factors ensure analytes of interest are chromatographically separated from early eluted compounds that can suppress ionization. The total chromatographic resolution minimizes the possibility of ionization suppression and eliminates cross-contamination of SRM transitions between main analytes.

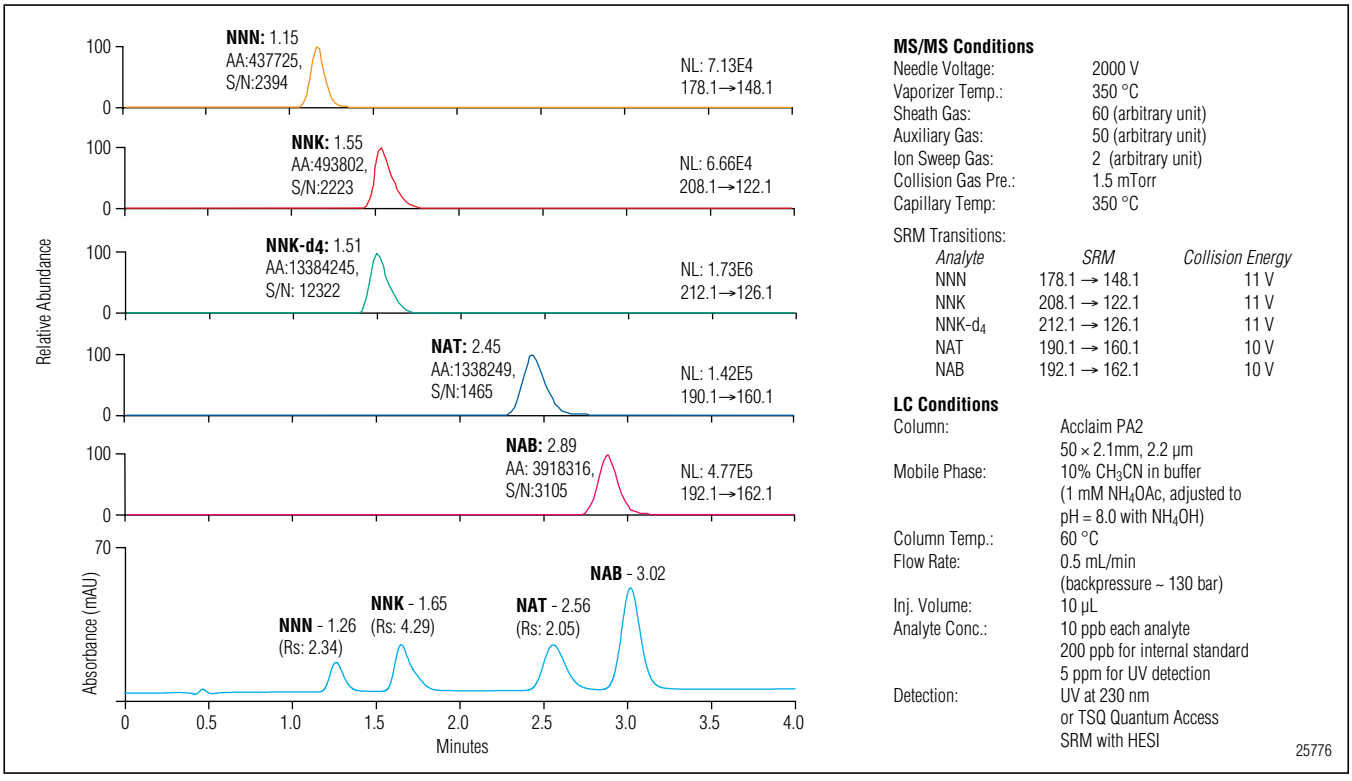

Figure 1. Comparison of SRM and UV chromatograms of TSNAs on the Acclaim RSLC PA2 column.

Mass Spectrometry

Tandem mass spectrometry provides specific and sensitive detection. The SRM chromatograms in Figure 1 show that quantification can be performed with a high level of confidence even at low concentration (10 ng/mL, 0.1 ng injected amount). The use of SRM detection enabled simplification of sample preparation and a significant reduction in total process time.

Method Performance

Internal standard quantification and calibration were performed using isotope labeled NNK-d_4 (IStd). Calibration standards were prepared at 8 levels from 2 ng/mL to 1000 ng/mL. Triplicate injections were performed to generate calibration curves, and a coefficient of determination (r^2) greater than 0.99 was achieved for each analyte. 1/X weighting provided best quantification for low concentration samples. Excellent linearity was achieved for NNK with $r^2 = 0.9998$, suggesting that the use of isotope-labeled analogs as internal standards could provide better quantification accuracy and precision. The calibration curve is shown in Figure 2.

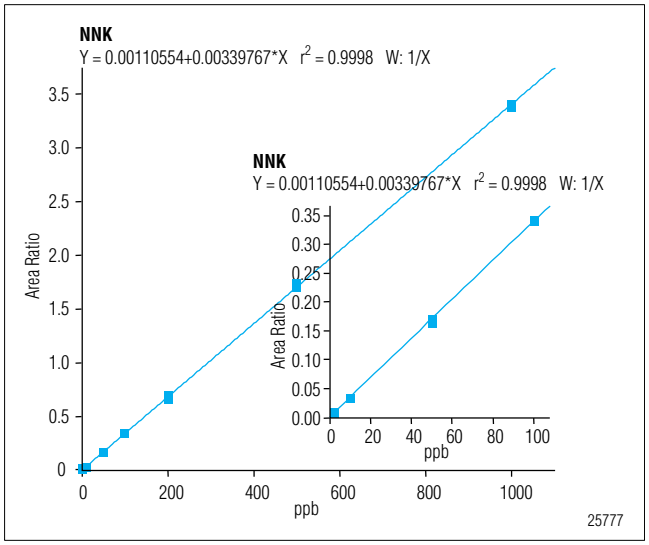

Figure 2. Calibration curve of NNK using NNK-d_4 as internal standard.

Method detection limits (MDLs) were statistically calculated using the equation $MDL = S \times t_{(99\%, n=7)}$ where S is the standard deviation, and t is student's t at 99% confidence interval. Seven replicate injections of a standard solution at 2 ng/mL were performed, and used for MDL calculations. The MDLs for TSNAs range from 0.22 ng/mL (NAB) to 0.38 ng/mL (NNK).

Precision and accuracy were evaluated at 10 ng/mL and 100 ng/mL. Seven replicate injections of calibration standards at both levels were performed, and quantification was calculated based on the calibration curves. The results in Table 1 show that the quantification of NNK was very accurate and precise (< 4 % difference, < 4.5% RSD) at both low (10 ng/mL) and medium levels (100 ng/mL). However, significant inaccuracy was observed for NAB and NAT at the low level, showing 54.2% and 36.6% quantification differences respectively. This observation suggests better quantification accuracy may be achieved using an isotope labeled internal standard for each analyte for low-level TSNA analysis, for example, analyzing TSNAs in biological matrices.

Table 1. Precision and Accuracy

	10 ng/mL		100 ng/mL	
	Accuracy (%)	% RSD	Accuracy (%)	% RSD
NAB	154.2	2.74	103.7	1.49
NAT	136.6	4.69	100.7	1.77
NNK	103.9	4.38	100.9	1.88
NNN	95.3	3.56	94.4	1.56

Method Ruggedness

Since the analytical column was running under the very harsh conditions of high temperature and high pH mobile phase, method ruggedness was evaluated by repeated injections of standard solutions and tobacco extracts. As shown in Figure 3, chromatographic retention and resolution were well-maintained after more than 1000 injections. The column was cleaned with 50/50 CH_3CN/H_2O before injection 250 and a new inlet frit was installed before injection 1003. (The use of an in-line filter is highly recommended to prevent column clogging.)

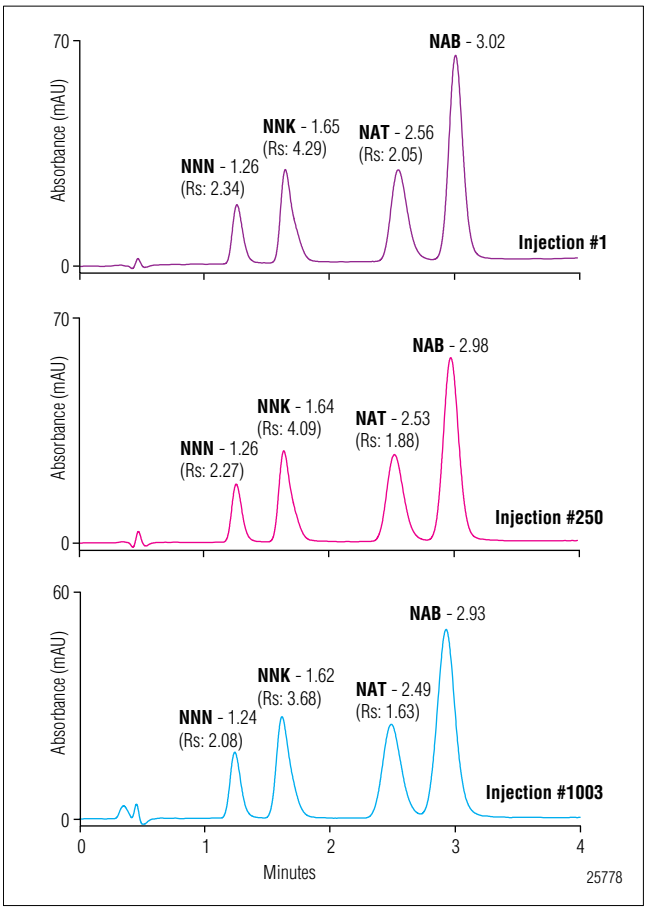

Figure 3. Ruggedness of the Acclaim RSLC PA2 column.

Determination of TSNAs in Tobacco Cigarettes

Five brands of tobacco cigarettes were purchased from a local convenience store. Tobacco cuts from each brand of cigarettes were prepared (n = 3) following the procedure described previously. The results are shown in Table 2. The TSNA contents are significantly different between brands A and B and brands C, D, and E. The differences in TSNA content could be related to differences in the tobacco blended in each brand, such as type, origin, age of the tobacco, curing method, and storage conditions. The comparison of brands A and B (regular and light varieties of a premium US brand of cigarettes) shows no difference for TSNA contents except for a change in the amount of NAB. See Figure 4 for SRM chromatograms of TSNAs from brand A cigarette.

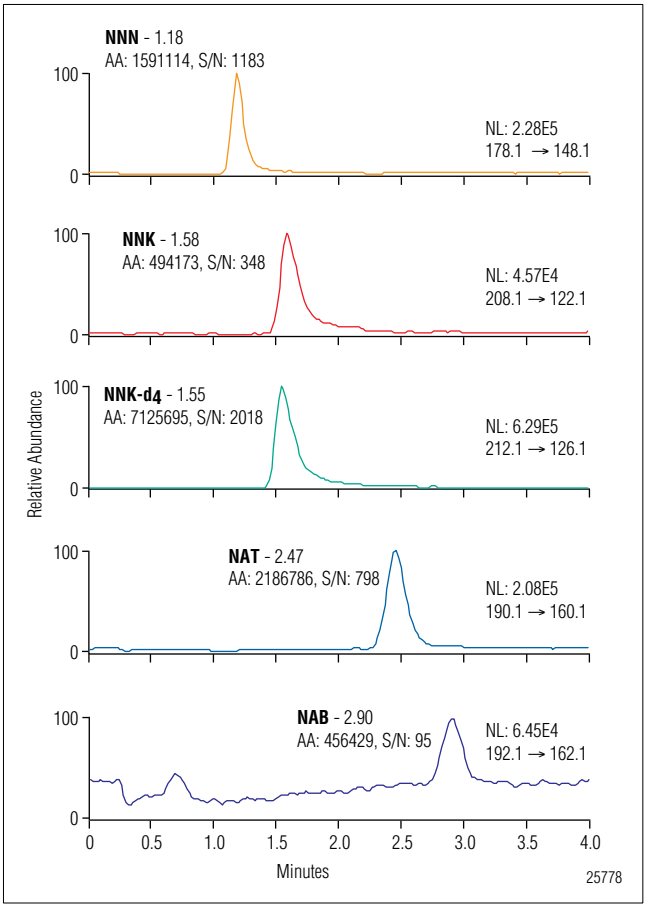

Figure 4. SRM chromatograms of TSNAs from brand A cigarette.

Table 2. TSNAs in Tobacco Cigarettes*					
	NAB	**NAT**	**NNK**	**NNN**	**Note**
Brand A	5.3	1145.1	676.5	2221.1	US brand, regular
Brand B	42.1	1218.1	696.0	2472.5	US brand, lights
Brand C	40.1	160.8	225.2	213.0	US brand, all natural
Brand D	21.2	103.9	70.2	155.4	International brand
Brand E	32.7	78.2	87.8	114.3	Asian brand

*Amounts shown in Table 2 are ng/g tobacco cuts.

CONCLUSION

A rugged and ultrafast method for TSNA analysis was developed using HPLC-MS/MS on an Acclaim RSLC PA2 column. Four TSNAs were chromatographically retained and resolved within 3.5 min. Tandem mass spectrometry ensured selectivity and sensitivity. The use of isotope-labeled intenal standards for each analyte may improve the quantification accuracy, as was observed for NNK in this study. The ruggedness of the method was shown by more than 1000 injections of standards and tobacco extracts. The applicability of the method for the determination of TSNAs in tobacco cuts from five brands of cigarettes was demonstrated.

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Determination of Hydroxymethylfurfural in Honey and Biomass

INTRODUCTION

Hydroxymethylfurfural (HMF), or 5-hydroxymethyl-2-furaldehyde, is a water-soluble heterocyclic organic compound derived from sugars. It is a derivative of furan and has both aldehyde and alcohol functional groups (Figure 1). Very low amounts of this compound are naturally found in fresh sugar-containing foods including milk, honey, fruit juices, spirits, and bread. Additionally, HMF is produced during food pasteurization and cooking as a result of dehydration of sugars such as glucose and fructose[1] and in the initial stages of the Maillard reaction,[2] a reaction between sugars and proteins responsible for changes in color and flavor of food. HMF is also formed during extended food storage under acidic conditions that favor its generation. Therefore, it is an indicator of excessive heat-treatment, spoilage, and of possible adulteration with other sugars or syrups.

Although HMF is not yet considered a harmful substance, the National Institute of Environmental Health Sciences nominated HMF for toxicity testing[3] based on the potential for widespread exposure through consumed foods, and evidence for carcinogenic potential of other members of this class. As a result, many countries impose restrictions on maximum levels of HMF in food and beverages.[4]

Beyond being an indicator of food quality, HMF is a biomass platform chemical because it can be used to synthesize a number of compounds that are currently derived from crude oil, including solvents, fuels, and monomers for polymer production (Figure 1).[5,6] HMF is readily derived from cellulose, either directly or through a two-step process involving hydrolysis and the formation of simple sugars.[7] Thus, it is important to measure HMF in matrices ranging from foods to treated cellulosic biomass.

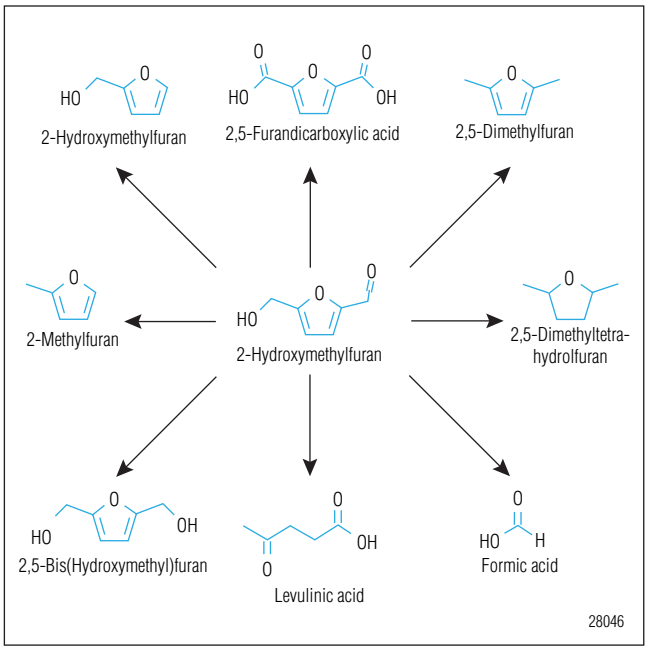

Figure 1. HMF as precursor for a number of commercial chemicals.

There are spectrophotometric and HPLC methods available for HMF determination. One commonly used method is based on spectral absorbance at 284 nm.[8, 9] This direct-absorbance measurement could have interferences from other compounds present in the complex matrices. In the HPLC method, HMF is separated on a reversed-phase column, with water and methanol as the mobile phase, and then detected by UV absorbance.[10]

This work describes a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD)-based method for the determination of HMF in samples ranging from food (honey and pancake syrup) to treated biomass (corn stover and wood hydrolysate). A Dionex ICS-3000 system with a CarboPac® PA1 column, electrolytically generated hydroxide eluent, and electrochemical detection with disposable Au-on-polytetrafluoroethylene (PTFE) working electrodes are used. The CarboPac PA1 is a high-capacity, rugged column suitable for determining mono- and disaccharides, and has high resolution for HMF in a wide variety of matrices.

The testing here demonstrates the linearity, limit of quantitation, limit of detection, precision, and recovery of HMF in diverse matrices ranging from honey to corn stover. It shows that PAD is an appropriate detection technique for HMF, with a broad linear range and low detection limit. Disposable electrodes provide short equilibration time and greater electrode-to-electrode reproducibility, compared to conventional electrodes. Compared to other disposable Au electrodes, the Au-on-PTFE electrodes have longer lifetime and can operate at higher hydroxide concentrations. The described method provides good sensitivity, consistent response, and can be routinely used for HMF analysis in foods and biomass applications, demonstrating the capability of HPAE-PAD for HMF determination in varied matrices.

EQUIPMENT

Dionex ICS-3000 or ICS-5000 system including:

- Gradient or Isocratic Pump, with the vacuum degas option installed
- EG Vacuum Degas Conversion Kit (Dionex P/N 063353)
- DC Detector/Chromatography Module
- 10 µL Injection loop
- Electrochemical Detector (P/N 079830)
- Carbohydrate PTFE Disposable Au Working Electrodes (P/N 066480, package of 6)
- Ag/AgCl Reference Electrode (P/N 061879)
- 3 mil PTFE gaskets (P/N 63537)

AS Autosampler

Chromeleon® Chromatography Data System (CDS) software

Eluent Organizer, including 2 L plastic bottles and pressure regulator

Polypropylene injection vials with caps (0.3 mL vial kit, P/N 055428)

Nalgene® 125 mL HDPE narrow mouth bottles (VWR P/N 16057-062)

Nalgene 250 mL HDPE narrow mouth bottles (VWR P/N 16057-109)

Nalgene 250 mL 0.2 µm nylon filter units (VWR P/N 28199-371)

Nalgene 1000 mL 0.2 µm nylon filter units (VWR P/N 28198-514)

REAGENTS AND STANDARDS

Reagents

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better, filtered through a 0.2 µm filter immediately before use

Standards

HMF (Sigma Aldrich Cat # W501808)

Fructose (Baker Analyzed Cat # M556-05)

Xylose (Aldrich Chemical Company Cat # X-107-5)

Sucrose (Sigma Cat # S-9378)

Glucose (Sigma Cat # G-5250)

Glycerol (JT Baker Cat # M778-07)

Arabinose (Sigma Cat # A3131)

CONDITIONS

Method

Columns:	CarboPac PA1 Analytical, 4 × 250 mm (P/N 035391) CarboPac PA1 Guard, 4 × 50 mm (P/N 43096)
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (full loop)
Temperature:	30 ºC
Back Pressure:	2400 psi
Eluent:	50 mM KOH
Eluent Source:	EGC II KOH with CR-ATC
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrodes
Reference Electrode:	
Mode:	Ag/AgCl mode
Noise:	30 pC

Carbohydrate Waveform

Time (s)	Potential (V)	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

Reference electrode in Ag/AgCl mode

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solutions

Potassium Hydroxide (50 mM)

Generate the potassium hydroxide (KOH) eluent online by pumping high-quality degassed, deionized (DI) water through the EGC II KOH cartridge. Chromeleon software tracks the amount of KOH used and calculates the remaining lifetime. Although electrolytic eluent generation delivers the best performance, manually prepared eluents can be used, if needed. For manually prepared eluent, use NaOH rather than KOH and prepare according to the general instructions for hydroxide eluents in Dionex Technical Note 71.[11] This method requires the installation of the ICS-3000 EG Vacuum Degas Conversion Kit (P/N 063353) to allow sufficient removal of the hydrogen gas formed with the potassium hydroxide eluent.[12]

Stock Standard Solution

Prepare a stock solution of 2 mg/mL HMF by dissolving 10 mg in 5 mL DI water in a plastic volumetric flask. Store aliquots of stock solutions in plastic containers at 4 °C. Stock standards are stable for at least one month.

Working Standard Solutions

Prepare working standards at lower HMF concentrations by diluting appropriate volumes of the 2 mg/mL stock with deionized water. Prepare working standards daily. Store the standard solutions at <6 ºC when not in use.

SAMPLE PREPARATION

Honey and Syrup

Prepare honey and syrup samples by dissolving 1 g in 100 mL of DI water and sonicating for 10 min. Store solutions in plastic containers at < 6 ºC. Further dilute syrup samples twofold with DI water before injection.

Fructose

Prepare a stock solution by dissolving 100 mg in 100 µL DI water. Dilute 500-fold with DI water before injection.

Corn Stover and Wood Hydrolysate

Centrifuge corn stover and wood hydrolysate samples at 14,000 rpm for 10 min and inject with DI water at a dilution of 1/1000 for analysis.

Precautions

Carryover can occur because the honey, syrup, and treated biomass samples have high concentrations of sugars such as glucose, xylose, and sucrose. A syringe flush of 1000 µL is recommended between samples. Column washes at 100 mM KOH are recommended if gradual retention time loss is observed. The application of 100 mM KOH changes the system equilibrium; re-equilibration at 50 mM for ~2 h is recommended to achieve high precision.[13]

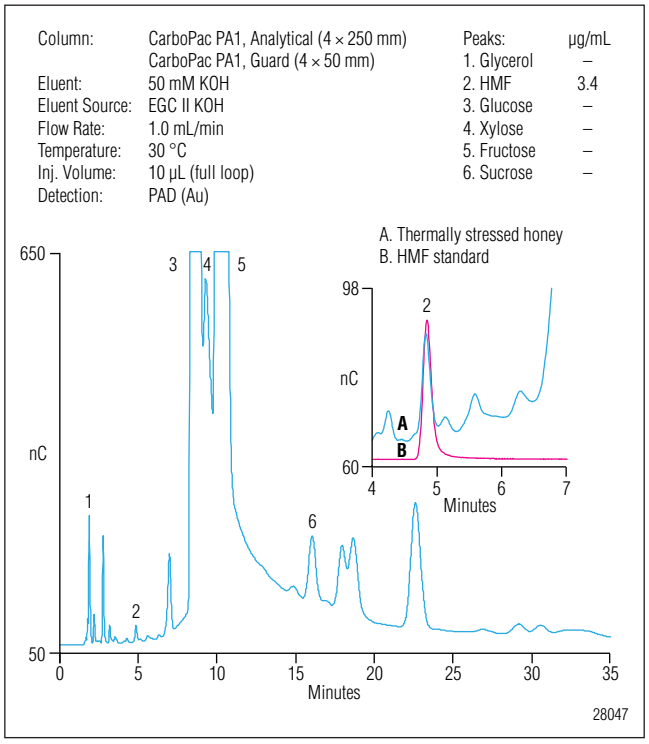

Figure 2. HMF in thermally stressed honey.

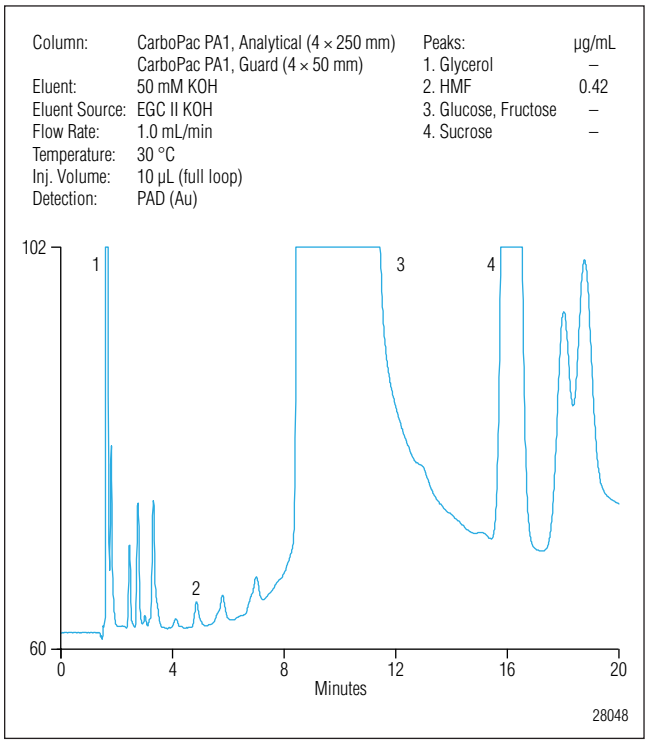

Figure 3. HMF in pancake syrup (high-fructose corn syrup).

Table 1. Intraday and Between-Day Precisions for Honey and Corn Stover Samples

Sample	Amount (µg/mL)	RT Precision (RSD)		Peak Area Precision (RSD)	
		Intraday	Between-Day	Intraday	Between-Day
Fresh honey	0.17	NC			
Thermally stressed honey	3.4	0.09	0.06	1.19	1.17
Corn stover	4.0	0.11	0.67	0.33	3.13

NC: Not Collected

RESULTS AND DISCUSSION

Figure 2 shows HMF in a thermally stressed honey sample. HMF elutes at 4.8 min and can be detected without interference from the other sugars. The HMF content in this sample was determined to be 330 mg/kg of honey. Typically, fresh honey has a low amount of HMF (<15 mg/kg). The HMF concentration increases as honey undergoes heat treatment to reduce viscosity and prevent crystallization to facilitate filling.[14] The EU Directive (110/2001) and the Codex Alimentarius (ALINORM 01/2000) standards limit HMF to 40 mg/kg for honey produced under European conditions and 80 mg/kg for honey coming from tropical countries.[4] In the fresh honey sample, HMF was determined to be 0.17 µg/mL (which amounts to 17 mg HMF/kg of honey, Table 1).

The chromatogram of thermally stressed pancake syrup (Figure 3) shows the separation of HMF from other thermal degradation products. HMF is a product of thermal degradation of fructose, the main constituent of pancake syrup. HMF in high-fructose corn syrup (HFCS) is also a problem for beekeepers because they use HFCS as a source of sugar to feed bees when natural nectar sources are limited. Note that complex matrices like pancake syrup may have later-eluting peaks (e.g., pancake syrup has a peak at ~55 min, not shown), and the long retention time could interfere with subsequent injections if a shorter run time is used.

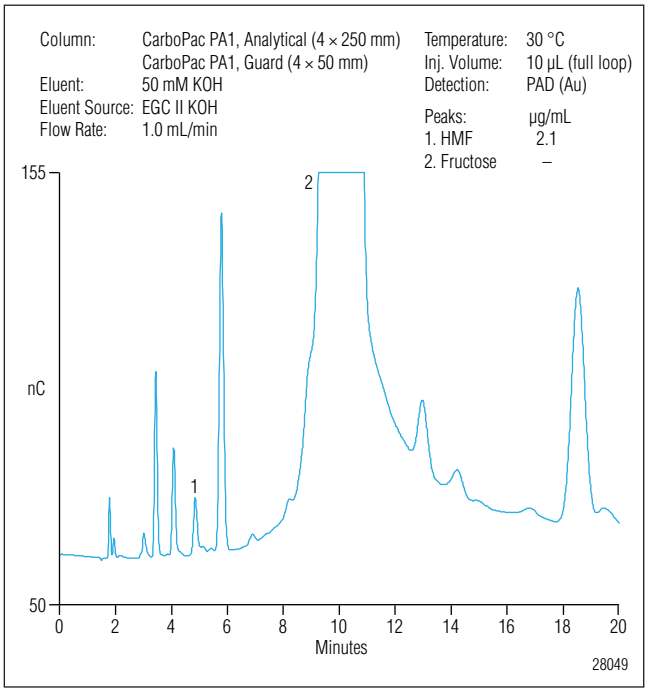

Figure 4. HMF in a thermally stressed fructose solution.

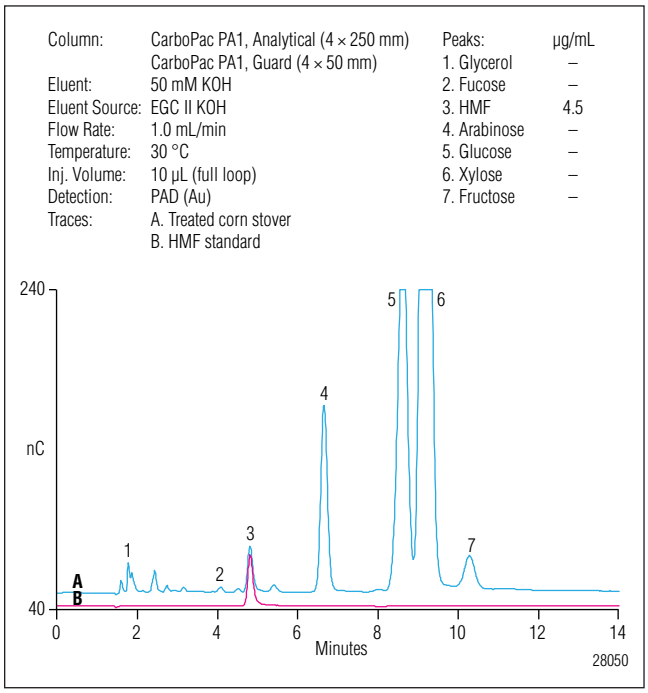

Figure 5. HMF in acid-hydrolyzed corn stover.

The described HPAE-PAD method was applied for HMF detection in fructose. The United States Pharmacopeia (USP)[15] and Food Chemicals Codex (FCC) have monographs[16] for the analysis of HMF in fructose and fructose injections. The USP monograph is based on the Seliwanoff test which depends on the reaction of HMF with resorcinol to form a red-colored compound, and the FCC monograph is a spectrophotometric method based on UV absorbance of HMF at 283 nm. HMF is present as an organic impurity in fructose and must be quantified and meet USP requirements before use as a food substance or as infusion fluids. HMF is formed during sterilization and storage. The chromatogram of thermally stressed fructose solution (Figure 4) shows the separation of HMF from other thermal degradation products. HMF is also formed in aqueous dextrose solutions and a HPAE-PAD-based method has been reported for quantification of HMF in commercial dextrose solutions.[17] Note that the thermally stressed honey, syrup, and fructose solutions were prepared by heating samples at 100±10 ºC for 4 h and cooling to room temperature before dilution and injection.

Figures 5 and 6 show the chromatograms of acid-hydrolyzed corn stover and a wood acid hydrolysate. HMF was detected without interference from the other sugars in both samples.

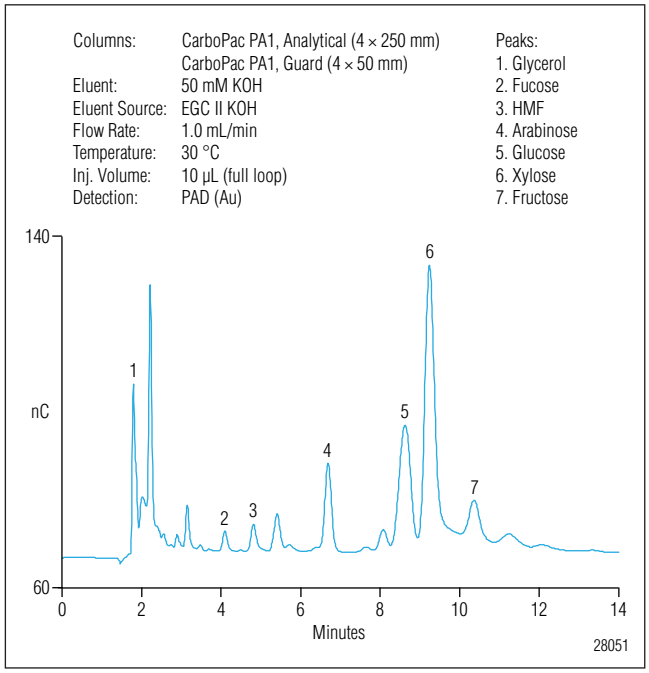

Figure 6. HMF in wood hydrolysate.

The total run time for these samples was 15 min, providing high sample throughput, suggesting that this method can be used for online monitoring of HMF during biomass processing.

Table 2. Linear Range and Precisions for HMF Standards							
Analyte	**Sample (Range µg/mL)**	**Corr. Coeff. (r^2)**	**RT (min)**	**Concentration Used for Precision Injections (µg/mL)**	**RT Precision (RSD)**	**Peak Area (nC*min)**	**Peak Area Precision (RSD)**
HMF	Honey (0.1–50)	0.9998	4.85	0.5	0.12	0.55	0.50
	Corn Stover (0.5–1000)	0.9965	4.85	5.0	0.10	4.91	0.14

Table 3. Recoveries of HMF in Spiked Honey and Corn Stover Samples				
Analyte	**Sample**	**Amount Found (µg/mL)**	**Amount Added (µg/mL)**	**Average Recovery ([%]n = 3 days)**
HMF	Thermally stressed honey	6.4	2.9	103
	Corn stover	10.0	5.3	113

Linear Range, Limit of Quantitation, Limit of Detection

To determine the linearity of the method, calibration standards were injected in triplicate covering the expected concentration range of HMF in food and biomass samples. Calibration plots produced correlation coefficient (r^2) values of 0.9998 in the range 0.1 to 50 µg/mL for food applications and 0.9965 in the range 0.5 to 1000 µg/mL for biomass applications (Table 2). A least squares regression fit with weighting was used to accurately represent the lower values of the calibration curve.

The limit of detection (LOD) was determined by measuring the peak-to-peak noise in a representative one-minute segment of baseline where no peaks elute, followed by analyzing a standard at a concentration expected to provide a chromatogram with a signal-to-noise (S/N) ratio of 3. Similarly, the lower limit of quantitation (LOQ) was determined by injecting a standard at a concentration that resulted in a S/N ratio of 10. Typical baseline noise for this method was 20 to 40 pC. The LOD and LOQ for this method were 0.04 µg/mL and 0.10 µg/mL, respectively.

Precision

The peak area and retention time (RT) precisions were determined for seven replicate injections of an HMF standard. The concentrations used for precision injections were 0.5 µg/mL for food applications and 5.0 µg/mL for biomass applications (Table 2).

The retention time precisions (RSD) for the two concentrations were 0.12 and 0.10, respectively. The corresponding peak area precisions were 0.5 and 0.14. The high retention time precisions were attributed to consistent generation of high-purity KOH using the eluent generator.

Intraday and between-day precisions for HMF in honey and corn stover were evaluated over three consecutive days. The RT precisions were in the range of 0.06 to 0.67%, and peak area precisions were in the range of 0.33 to 3.13%. The high precisions suggest that this method can be used to measure HMF in complex matrices.

Accuracy

The accuracy of the method was verified by determining recoveries of HMF in spiked honey and acid-hydrolyzed corn stover samples over three consecutive days. The amount of HMF in a fresh honey sample was 0.17 µg/mL (Table 1: this equates to 17 mg of HMF per kg of honey). The thermally stressed honey had 3.4 µg/mL HMF (Figure 2), and was spiked with 2.9 µg/mL HMF. The treated corn stover sample (at 1000-fold dilution) had 4 µg/mL HMF and was spiked with 5.3 µg/mL HMF. Recoveries were calculated from the difference in response between the spiked and unspiked samples. Intraday concentration RSD was 1.7% for both honey and corn stover. The average recovery of HMF in honey was 103% and in corn stover was 112% (Table 3).

CONCLUSION

This study describes a HPAE-PAD method for the accurate determination of HMF in foods like honey and in biomass like acid-hydrolyzed corn stover. The method uses the CarboPac PA1 column with electrolytically generated hydroxide eluent. The method is shown to have a broad linear range, high precisions, and low detection limits. The disposable Au working electrode provides consistently high detector response, assuring greater instrument-to-instrument and lab-to-lab reproducibility. This configuration needs only addition of deionized water for continuous operation. In summary, the described HPAE-PAD-based HMF analysis method is accurate and reliable, and should be applicable to online monitoring of HMF levels in food and biomass applications.

SUPPLIERS

VWR, 1310 Goshen Parkway, West Chester, PA 19380, U.S.A., Tel: 800-932-5000.

Sigma-Aldrich Chemical Co., P.O. Box 2060, Milwaukee, WI 53201, U.S.A., Tel: 800-558-9160.

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Thermo
SCIENTIFIC

Column Selection Guide

Silica Columns

			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific					Example Applications
			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2	Acclaim Carbamate	
General Applications	*Neutral Molecules*	High hydrophobicity	√	√	√	√	√	√	√	√	√	√	√						*Fat-soluble vitamins, PAHs, glycerides*
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√	√	√							*Steroids, phthalates, phenolics*
		Low hydrophobicity	√			√	√					√	√						*Acetaminophen, urea, polyethylene glycols*
	Anionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							*NSAIDs, phospholipids*
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√		√							*Asprin, alkyl acids, aromatic acids*
		Low hydrophobicity				√			√	√		√	√						*Small organic acids, e.g. acetic acids*
	Cationic Molecules	High hydrophobicity	√	√	√	√	√	√		√	√	√							*Antidepressants*
		Intermediate hydrophobicity	√	√	√	√	√	√	√		√	√							*Beta blockers, benzidines, alkaloids*
		Low hydrophobicity	√			√			√		√	√	√						*Antacids, pseudoephedrine, amino sugars*
	Amphoteric/ Zwitterionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							*Phospholipids*
		Intermediate hydrophobicity	√	√	√	√	√	√			√								*Amphoteric surfactants, peptides*
		Low hydrophobicity				√	√		√	√	√	√	√						*Amino acids, aspartame, small peptides*
	Mixtures of Neutral, Anionic, Cationic Molecules	Neutrals and acids	√			√	√		√	√									*Artificial sweeteners*
		Neutrals and bases	√			√	√		√		√								*Cough syrup*
		Acids and bases				√			√										*Drug active ingredient with counterion*
		Neutrals, acids, and bases				√			√										*Combination pain relievers*
Specific Applications	*Surfactants*	Anionic	√	√	√	√	√								√				*SDS, LAS, laureth sulfates*
		Cationic													√				*Quats, benzylalkonium in medicines*
		Nonionic	√	√	√	√	√					√			√				*Triton X-100 in washing tank*
		Amphoteric	√	√	√	√	√								√				*Cocoamidopropyl betaine*
		Hydrotropes													√				*Xylenesulfonates in handsoap*
		Surfactant blends													√				*Noionic and anionic surfactants*
	Organic Acids	Hydrophobic							√	√				√					*Aromatic acids, fatty acids*
		Hydrophilic							√	√				√					*Organic acids in soft drinks, pharmaceuticals*
	Environmental Contaminants	Explosives														√	√		*U.S. EPA Method 8330, 8330B*
		Carbonyl compounds															√		*U.S. EPA 1667, 555, OT-11; CA CARB 1004*
		Phenols	√			√													*Compounds regulated by U.S. EPA 604*
		Chlorinated/Phenoxy acids				√													*U.S. EPA Method 555*
		Triazines	√			√													*Compounds regulated by U.S. EPA 619*
		Nitrosamines				√													*Compounds regulated by U.S. EPA 8270*
		Benzidines	√			√													*U.S. EPA Method 605*
		Perfluorinated acids				√													*Dionex TN73*
		Microcystins	√																*ISO 20179*
		Isocyanates					√					√							*U.S. OSHA Methods 42, 47*
		Carbamate insecticides																√	*U.S. EPA Method 531.2*
	Vitamins	Water-soluble vitamins				√	√		√										*Vitamins in dietary supplements*
		Fat-soluble vitamins	√	√	√	√	√	√		√									*Vitamin pills*
	Pharmaceutical Counterions	Anions							√	√									*Inorgaic anions and organic acids in drugs*
		Cations							√		√								*Inorgaic cations and organic bases in drugs*
		Mixture of Anions and Cations							√										*Screening of pharmaceutical counterions*
		API and counterions							√										*Naproxen Na^+ salt, metformin Cl^- salt, etc.*

Polymer Columns

		IonPac AS23	IonPac AS22	IonPac AS22-Fast	IonPac AS14/A	IonPac AS12A	IonPac AS9/HC/SC	IonPac AS4A/SC	IonSwift MAX-100	IonPac AS24	IonPac AS21	IonPac AS20	IonPac AS19	IonPac AS18	IonPac AS18-Fast	IonPac AS17-C	IonPac AS16	IonPac AS15	IonPac AS11(-HC)	IonPac AS10	IonPac AS7	IonPac AS5	IonPac Fast Anion IIIA	OmniPac PAX-100	OmniPac PAX-500
ANIONS	Inorganic Anions	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	√	√					
	Oxyhalides	√				√	√			√			√												
	Bromate	√					√			√			√												
	Perchlorate										√	√					√								
	Organic Acids								√							√		√	√	√					
	Phosphoric/Citric Acids																						√		
	Poly/High-Valence Anions								√			√					√		√		√	√			
	Hydrophobic Anions								√			√					√		√						
	Hydrophobic/Halogenated Anions								√			√							√					√	
	Anionic Neutral Molecules									√	√	√	√												√
CATIONS	Inorganic Cations																								
	Sodium/Ammonium																								
	Amines/Polyvalent Amines																								
	Aliphatic/Aromatic Amines																								
	Alkanol/Ethhanolamines																								
	Biogenic Amines																								
	Transition/Lanthanide Metals																								
	Hydrophobic Cations																								
	Cationic Neutral Molecules																								
BIO-MOLECULES	Amino Acids																								
	Phosphorylated Amino Acids																								
	Amino Sugars																								
	Oligoscharides																								
	Mono-/Di-Saccharides																								
	Glycoproteins																								
	Alditols/Aldoses mono/di Saccharides																								
	ds Nucleic Acids																								
	Single-Stranded Oligonucleotides																								
	Peptides																								
	Proteins																								
	Metal-binding Proteins																								
	Monoclonal antibodies																								
ORGANIC MOLECULES	Aliphatic Organic Acids																								
	Alcohols																								
	Borate																								
	Large Molecules, Anions																								
	Small Molecules																								
	Small Molecules/LC-MS																								
	Polar/Non-Polar Small Molecules																								
	Hydrophobic/Aliphatic Organic Acids																								
	Surfactant Formulations																								
	Explosives/EPA 8330																								
MODE	Anion Exchange / Carbonate	√	√	√	√	√	√	√																	
	Anion Exchange / Hydroxide								√	√	√	√	√	√	√	√	√	√	√	√	√	√			
	Cation Exchange																								
	Multi-Mode																							√	√
	Affinity																								
	Ion Exclusion																								
	Reversed Phase																								
	Anion Exchange/Other																								

		IonPac CS18	IonPac CS17	IonPac CS16	IonPac CS15	IonPac CS14	IonPac CS12A	IonPac CS11	IonPac CS10	IonPac CS5A	OmniPac PCX-100	OmniPac PCX-500	AminoPac PA10	AminoPac PA1	CarboPac PA200	CarboPac PA100	CarboPac PA20	CarboPac PA10	CarboPac PA1	CarboPac MA1	DNAPac PA200	DNAPac PA100	ProPac WAX/SAX	ProPac WCX/SCX	ProPac IMAC	ProPac HIC	ProPac PA1	ProSwift	IonPac ICE-AS6	IonPac ICE-AS1	IonPac ICE-Borate	IonPac NS1
ANIONS	Inorganic Anions																														
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	Poly/High-Valence Anions																														
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CATIONS	Inorganic Cations	√	√	√	√	√	√	√	√																						
	Sodium/Ammonium			√	√		√																								
	Amines/Polyvalent Amines	√	√																												
	Aliphatic/Aromatic Amines		√			√	√																								
	Alkanol/Ethhanolamines	√	√		√																										
	Biogenic Amines	√	√																												
	Transition/Lanthanide Metals									√																					
	Hydrophobic Cations	√	√			√					√																				
	Cationic Neutral Molecules											√																			
BIO-MOLECULES	Amino Acids												√	√																	
	Phosphorylated Amino Acids												√																		
	Amino Sugars												√		√	√	√	√													
	Oligoscharides														√	√			√	√											
	Mono-/Di-Saccharides															√	√	√	√												
	Glycoproteins																						√	√	√			√			
	Alditols/Aldoses mono/di Saccharides															√	√	√	√												
	ds Nucleic Acids																			√	√										
	Single-Stranded Oligonucleotides																			√	√							√			
	Peptides																								√	√	√	√			
	Proteins																						√	√	√	√	√	√			
	Metal-binding Proteins																								√						
	Monoclonal antibodies														√	√			√				√	√	√	√	√	√			
ORGANIC MOLECULES	Aliphatic Organic Acids																												√	√	√
	Alcohols																												√	√	
	Borate																														√
	Large Molecules, Anions																														√
	Small Molecules																														
	Small Molecules/LC-MS																														
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	Hydrophobic/Aliphatic Organic Acids																														
	Surfactant Formulations																														√
	Explosives/EPA 8330																														
MODE	Anion Exchange / Carbonate																														
	Anion Exchange / Hydroxide														√	√	√	√	√	√											
	Cation Exchange	√	√	√	√	√	√	√	√	√														√			√	√			
	Multi-Mode										√	√																			
	Affinity																								√						
	Ion Exclusion																												√	√	√
	Reversed Phase																										√	√			√
	Anion Exchange/Other												√	√						√	√		√					√			

Column Specifications

IC Anion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS24	2 × 250 mm	Hydroxide	Recommended column for haloacetic acids prior to MS or MS/MS detection	7 µm	55%	-	-	140 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS23	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	6 µm	55%	-	-	80 µeq 320 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS22	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	6.5 µm	55%	-	-	52.5 µeq 210 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS21	2 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to MS or MS/MS detection	7.0 µm	55%	-	-	45 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS20	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to suppressed conductivity detection.	7.5 µm	55%	-	-	77.5 µeq 310 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS19	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	7.5 µm	55%	-	-	60 µeq 350 µeq	Alkanol quaternary ammonium	Low
IonPac AS18	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for the analysis of common inorganic anions.	7.5 µm	55%	65 nm	8%	75 µeq 285 µeq	Alkanol quaternary ammonium	Low
IonPac AS17-C	2 × 250 mm 4 × 250 mm	Hydroxide	Trace anions in HPW matrices. Carboxylated resin, no sulfate blank. Low capacity for fast analysis of common inorganic anions using gradient elution with the Eluent Generator.	10.5 µm	55%	75 nm	6%	7.5 µeq 30 µeq	Alkanol quaternary ammonium	Low
IonPac AS16	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. Polyvalent anions including: polyphosphates and polycarboxylates	9 µm	55%	80 nm	1%	42.5 µeq 170 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS15	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	9 µm	55%	-	-	56.25 µeq 225 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS15-5mm	3 × 150 mm	Hydroxide	Fast run, high capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	5 µm	55%	-	-	70 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS14A- 5 µm	3 × 150 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	5 µm	55%	-	-	40 ueq	Alkyl quaternary ammonium	Medium
IonPac AS14A	4 × 250 mm	Carbonate	For analysis of common inorganic anions.	7 µm	55%	-	-	120 µeq	Alkyl quaternary ammonium	Medium
IonPac AS14	2 × 250 mm 4 × 250 mm	Carbonate	Moderate capacity for fast analysis of common inorganic anions.	9 µm	55%	-	-	16 µeq 65 µeq	Alkyl quaternary ammonium	Medium-High

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS12A	2 × 200 mm 4 × 200 mm	Carbonate	Moderate capacity for analysis of inorganic anions and oxyhalides. Trace chloride and sulfate in high carbonate matrices.	9 µm	55%	140 nm	0.20%	13 µeq 52 µeq	Alkyl quaternary ammonium	Medium
IonPac AS11-HC	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the determination of organic acids and inorganic anions in uncharacterized samples.	9 µm	55%	70 nm	6%	72.5 µeq 290 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac AS11	2 × 250 mm 4 × 250 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	13 µm	55%	85 nm	6%	11 µeq 45 µeq	Alkanol quaternary ammonium	Very Low
IonPac AS10	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the analysis of inorganic anions and organic acids in high nitrate samples.	8.5 µm	55%	65 nm	5%	42.5 µeq 170 µeq	Alkyl quaternary ammonium	Low
IonPac AS9-HC	2 × 250 mm 4 × 250 mm	Carbonate	High-capacity column for inorganic anions and oxyhalides. Trace bromate in drinking water.	9 µm	55%	90 nm	18%	48 µeq 190 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS9-SC	4 × 250 mm	Carbonate	Low capacity for fast analysis of inorganic anions and oxyhalides. Specified column in US EPA Method 300.0 (B).	13 µm	55%	110 nm	20%	30-35 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS4A-SC	2 × 250 mm 4 × 250 mm	Carbonate	Low capacity for fast analysis of common inorganic anions. Specified column in U.S. EPA Method 300.0 (A).	13 µm	55%	160 nm	0.50%	5 µeq 20 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac Fast Anion IIIA	3 × 250 mm	Hydroxide	Recommended column for phosphoric and citric acids in cola soft drinks.	7.5 µm	55%	-	-	55 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS7	4 × 250 mm	Specialty Eluents	Polyvalent anions including chelating agents, polyphosphates and polyphosphonates. Cyanide, sulfide, hexavalent chromium, and arsenic speciation.	10 µm	2%	530 nm	5%	100 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS5A	4 × 150 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	5 µm	2%	60 nm	4%	35 µeq	Alkanol quaternary ammonium	Low
IonPac AS5	4 × 250 mm	Hydroxide	Metal-EDTA complexes, metal-cyanide complexes, and oxyanions.	15 µm	2%	120 nm	1%	20 µeq	Alkanol quaternary ammonium	Low

IC Cation Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac CS18	2 × 250 mm	MSA	Recommended column for polar amines (alkanolamines and methylamines) and moderately hydrophobic and polyvalent amines (biogenic and diamines). Nonsuppressed mode when extended calibration linearity for ammonium and weak bases is required	6 µm	55%	-	-	0.29 µeq	Carboxylic acid	Medium
IonPac CS17	2 × 250 mm 4 × 250 mm	MSA	Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines)	7 µm	55%	-	-	0.363 µeq 1.45 µeq	Carboxylic acid	Very Low
IonPac CS16	3 × 250 mm 5 × 250 mm	MSA	Recommended column for disparate concentration ratios of adjacent-eluting cations such as sodium and ammonium. Can be used for alkylamines and alkanolamines.	5 µm	55%	-	-	3.0 µeq 8.4 µeq	Carboxylic acid	Medium
IonPac CS15	2 × 250 mm 4 × 250 mm	MSA	Disparate concentration ratios of ammonium and sodium. Trace ethanolamine in high-ammonium or high- potassium concentrations. Alkanolamines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid/ crown ether	Medium
IonPac CS14	2 × 250 mm 4 × 250 mm	MSA	Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations.	8.5 µm	55%	-	-	0.325 µeq 1.3 µeq	Carboxylic acid	Low
IonPac CS12A-MS	2 × 100 mm	MSA	IC-MS screening column for fast elution and low flow rates required for interfacing with IC-MS	8.5 µm	55%	-	-	0.28 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A-5 µm	3 × 150 mm	MSA	Recommended column for high efficiency and fast analysis (3 min) of mono- and divalent cations.	5 µm	55%	-	-	0.94 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A	2 × 250 mm 4 × 250 mm	MSA	Recommended column for the separation of mono- and divalent cations. Manganese morpholine, alkylamines, and aromatic amines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS11	2 × 250 mm	HCl + DAP	Separation of mono- and divalent cations. Ethanolamines if divalent cations are not present.	8 µm	55%	200 nm	5%	0.035 µeq	Sulfonic acid	Medium
IonPac CS10	4 × 250 mm	HCl + DAP	Separation of mono- and divalent cations.	8.5 µm	55%	200 nm	5%	0.08 µeq	Sulfonic acid	Medium
IonPac CS5A	2 × 250 mm 4 × 250 mm	Pyridine dicarboxylic acid	Recommended column for transition and lanthanide metals analysis. Aluminum analysis.	9 µm	55%	140 nm 75 nm	10% 20%	0.02 µeq/ 0.005 µeq 0.04 µeq/ 0.01 µeq	Sulfonic acid/ alkanol quaternary ammonium	-

Ion-Exclusion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydro-phobicity
IonPac ICE-AS1	4 × 250 mm 9 × 250 mm	Heptafluorobutyric acid	Organic acids in high ionic strength matrices. Fast separation of organic acids.	7.5 µm	8%	-	-	5.3 µeq 27 µeq	Sulfonic acid	Ultra Low
IonPac ICE-AS6	9 × 250 mm	Heptafluorobutyric acid	Organic acids in complex or high ionic strength matrices.	8 µm	8%	-	-	27 µeq	Sulfonic and carboxylic acid	Moderate
IonPac ICE-Borate	9 × 250 mm	MSA/ Mannitol	Trace concentrations of borate	7.5 µm	8%	-	-	27 µeq	Sulfonic acid	Ultra Low

Acclaim General and Specialty Columns

Column	Bonded Phase	USP Type	Endcapped	Substrate	Particle Shape	Particle Size	Metal Impurity (ppm) Na, Fe, AL	Average Pore Diameter	Surface Area (m^2/g)	Total Carbon Content
Mixed-Mode WAX	Proprietary alkyl amine	na	Proprietary	Ultrapure silica	Spherical	5 µm	<10 ppm	120 Å	300	na
Mixed-Mode HILIC	Proprietary alkyl diol	na	Proprietary			5 µm		120 Å	300	na
Mixed-Mode WCX	Proprietary alkyl carboxyl	na	Proprietary			5 µm		120 Å	300	na
Organic Acid (OA)	Proprietary	na	Yes			5 µm		120 Å	300	17%
Surfactant and Explosives E1/2	Proprietary	na	Yes			5 µm		120 Å	300	na
120 C18	C18	L1	Yes			2, 3 and 5 µm		120 Å	300	18%
120 C8	C8	L7	Yes			3 and 5 µm		120 Å	300	11%
300 C18	C18	L1	Yes			3 µm		300 Å	100	7%
Polar Advantage	Sulfamido C16	na	Yes			3 and 5 µm		120 Å	300	17%
Polar Advantage II	Amide C18	na	Yes			2, 3 and 5 µm		120 Å	300	17%
HILIC	Proprietary hydrophilic		Yes			3 µm		120 Å	300	
Phenyl-1	Proprietary alkyl phenyl		Yes			3 µm		120 Å	300	
Carbamate	Proprietary alkyl group		Yes			3 and 5 µm		120 Å	300	
Trinity			Yes					120 Å	300	

Bio Columns

Protein

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
MAbPac SEC-1									
MAbPac SCX-10									
ProPac WCX-10	Weak Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10-µm diameter nonporous substrate to which is grafted a polymer chain bearing carboxylate groups.	55%	6 mg/ mL lysozyme	0.2–2 mL/min	80% ACN, acetone. Incompatable with alcohols and MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-10	Strong Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups.	55%	3 mg/ mL lysozyme	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-20									
ProPac WAX-10	Weak Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups.	55%	5 mg/ mL BSA/ mL	0.2–2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (21 MPa)	2–12.0
ProPac SAX-10	Strong Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups.	55%	15 mg/ mL BSA	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProSwift RP-1S	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Standard permeability	5.5 mg/mL Insulin	2–4 mL/min	Most common organic solvents	2800 psi (19.2 Mpa)	1–14
ProSwift RP-2H	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith High permeability	1.0 mg/mL Lysozyme	1–10 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift RP-4H									
ProSwift RP-3U	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Ultrahigh permeability	0.5 mg/mL Lysozyme	1– 16 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethac-rylate with quaternary amine functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift SCX-1S	Strong Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethac-rylate with sulfonic acid fuctional group	Monolith Standard permeability	30 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2–12.0
ProSwift WAX-1S	Weak Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with tertiary amine (DEAE) functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift WCX-1S	Weak Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with carboxylic acid (CM) functional group	Monolith Standard permeability	23 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts.	55%	>60 mg lysozyme/ mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21MPa)	2–12
ProSwift ConA-1S									
ProPac HIC-10	Reversed-Phase	Protein separation using hydrophobic interaction with salt gradient elution	Spherical 5 µm, ultrapure silica, 300 A, surface area 100 m^2/ g,	n/a	340 mg lysozyme per 7.8 x 75 mm column	1.0 mL/ min	2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup	4,000 psi	2.5–7.5

Carbohydrate

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
CarboPac MA1	Reduced mono- and disaccharide analysis.	7.5 µm diameter macroporous substrate fully functionalized with an alkyl quaternary ammonium group	15%	No latex	1450 µeq (4 × 250 mm)	Hydroxide	0.4 mL/min	0%	2000 psi (14 MPa)	0–14
CarboPac PA1	General purpose mono-, di-, and oligosaccharide analysis	10 µm diameter nonporous substrate agglomerted with a 500 nm MicroBead quaternary ammonium functionalized latex	2%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–5%	4000 psi (28 MPa)	0–14
CarboPac PA10	Monosaccharide compositonal anaylysis	10 µm diameter nonporous substrate agglomerated with a 460 nm MicroBead di-functionalized latex	55%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	3500 psi (24.5 MPa)	0–14
CarboPac PA20	Fast mono-, and disaccharide analysis	6.5 µm diameter nonporous substrate agglomerated with a 130 nm MicroBead quaternary ammonium functionalized latex	55%	5%	65 µeq (3 × 150 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	3000 psi (21 MPa)	0–14
CarboPac PA100	Oligosaccharide mapping and analysis	8.5 µm diameter nonporous substrate agglomerated with a 275 nm MicroBead di-functionalized latex	55%	6%	90 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	4000 psi (28 MPa)	0–14
CarboPac PA200	High resolution oligosaccharide mapping and analysis	5.5 µm diameter nonporous substrate agglomerated with a 43 nm MicroBead quaternary ammonium functionalized latex	55%	6%	35 µeq (3 × 250 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	4000 psi (28 MPa)	0–14

DNA

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Max. Backpressure	pH Range
DNAPac PA100	Single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	13-µm diameter nonporous substrate agglomerated with a 100-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.5 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNAPac PA200	High resolution single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	8-µm diameter nonporous substrate agglomerated with a 130-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.2 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNASwift										

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