Beverages Applications Notebook

Drinking Water

Thermo SCIENTIFIC

Table of Contents

Index of Analytes

Introduction to Beverages

The global beverage industry is growing each year with the introduction of new products, such as vitamin-fortified water, energy drinks, anti-aging water, and herbal nutritional supplements. With this growth, come many more analytical challenges. These challenges are compounded by the continuing and new needs to analyze classic favorites such as sodas, fruit juices, milk drinks, alcoholic beverages, and bottled water. One such example would be the melamine contamination in milk and infant milk formula.

For all beverages, the compositional quality and safety must be monitored to help track contamination, adulteration, product consistency, and to ensure regulatory compliance from raw ingredients (water, additives, and fruits) to the final product.

Thermo Fisher Scientific is a recognized leader in providing analytical solutions for sample preparation, liquid chromatography for compositional testing, and chromatography data management for compliance and quality testing of beverages. From inorganic ions, organic acids, biogenic amines, glycols and alcohols, carbohydrates and sugar alcohols, to vitamins, additives, and sugar substitutes, we are unique in our commitment to provide fast, accurate testing and labeling information for all applications in this industry.

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.

For more information on how the new lineup of Thermo Scientific products can expand your capabilities and provide the tools for new possibilities, choose one of our integrated solutions:

- Ion Chromatography and Mass Spectrometry
- 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.

Basic LC Systems: UltiMate 3000 Basic LC Systems are UHPLC compatible and provide reliable, high-performance solutions to fit your bench space and your budget.

IC and RFIC Systems

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.

Dionex ICS-1600: The Dionex ICS-1600 combines high sensitivity with convenience. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-1100: With dual-piston pumping and electrolytic suppression. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-900: Can routinely analyze multiple anions and cations in 10–15 min—fully automated with Displacement Chemical Regeneration (DCR).

MS Instruments

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

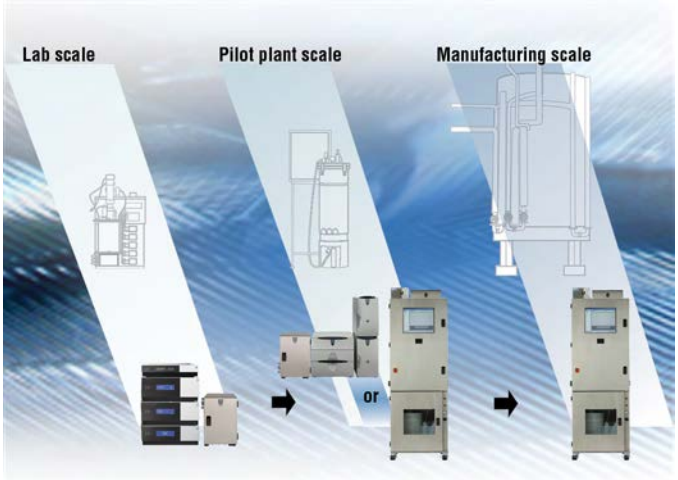
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, 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

Thermo
SCIENTIFIC

Analysis of Drinking Water

Ion Chromatographic Determination of Oxyhalides and Bromide at Trace Level Concentrations in Drinking Water Using Direct Injection

INTRODUCTION

The U.S. EPA Information Collection Rule (ICR) requires drinking water utilities serving more than 100,000 customers to report the concentration of target microorganisms present, the removal process used, and the concentration of disinfection byproducts (DBP) present in their waters.[1] The monitoring period began in July 1997 and will continue for 18 months. The ICR will affect approximately 300 drinking water utilities in the United States.

The U.S. EPA recommends Method 300.0 (B), which specifies ion chromatography with chemically suppressed conductivity detection, to monitor chlorite, bromate, and chlorate in drinking water.[2] Bromide, a precursor to bromate, must also be monitored down to levels as low as 10 ppb, because bromate has been classified as a potential carcinogen, even at low ppb levels, by the U.S. EPA and the World Health Organization. Under the ICR, utilities using hypochlorite solution as a disinfectant are required to monitor chlorate; those using chlorine dioxide are required to monitor chlorite, chlorate, and bromate; and those using ozone are required to monitor bromate. All utilities are required to monitor bromide.

This Application Note describes an isocratic separation using the IonPac® AS9-HC column to determine trace levels of oxyhalides and bromide in the presence of common anions in drinking water, as well as their detection limits and linear concentration ranges. The AS9-HC column method was developed to improve the separation between bromate and chloride, thus allowing lower detection limits of bromate in the presence of high chloride concentrations. Unlike previous IC methods for these ions, no pretreatment cartridges are required to remove high concentrations of common ions such as chloride, bicarbonate, and sulfate. The instrumentation, techniques, and representative applications of this method are discussed in this Note.

EQUIPMENT

Dionex DX-500 Ion Chromatography system
- consisting of:
- GP40 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC20 Chromatography Enclosure equipped with a rear-loading injection valve

4-L Plastic bottle assemblies (for external water mode)

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

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

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

Sodium and Potassium salts, ACS reagent grade, for preparing anion standards (see Table 1) (Fisher Scientific, EM Science)

CONDITIONS

Columns:	IonPac AS9-HC Analytical, 4 x 250 mm (P/N 51786) IonPac AG9-HC Guard, 4 x 50 mm (P/N 51791)
Eluent:	9 mM Sodium carbonate
Run Time:	25 min
Flow Rate:	1 mL/min
Sample Vol.:	200 µL
Detection:	Suppressed conductivity, ASRS™-II, 4 mm, AutoSuppression™ external water mode

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solutions

Stock Anion Standard Solution (1000 mg/L)

Dissolve the corresponding mass of the salt for each of the anions of interest (see Table 1) in 1000 mL of deionized water. Standards are stable for at least one month when stored at 4 °C.

Table 1 Masses of compounds used to prepare 1 liter of 1000 mg/L ion standards

Anion	Compound	Mass (g)
F^-	Sodium fluoride (NaF)	2.210
ClO_2^-	Sodium chlorite ($NaClO_2 \bullet 2H_2O$)	2.409
BrO_3^-	Sodium bromate ($NaBrO_3$)	1.180
Cl^-	Sodium chloride (NaCl)	1.648
NO_2^-	Sodium nitrite ($NaNO_2$)	1.500
Br^-	Sodium bromide (NaBr)	1.288
ClO_3^-	Sodium chlorate ($NaClO_3$)	1.276
NO_3^-	Sodium nitrate ($NaNO_3$)	1.371
$H_2PO_4^-$	Potassium phosphate, monobasic (KH_2PO_4)	1.433
SO_4^{2-}	Potassium sulfate (K_2SO_4)	1.814
HCO_3^-	Sodium bicarbonate ($NaHCO_3$)	1.377

Working Standard Solutions

Dilute each analyte to the required concentration with deionized water using the 1000-mg/L standards prepared above. Working standards containing less than 100-µg/L anions should be prepared daily.

Eluent Solution

9 mM Sodium carbonate

Dilute 18 mL of 0.5 M sodium carbonate concentrate to 1 L with deionized water. Transfer to an eluent container and blanket with helium at 0.055 MPa (8 psi).

Preservation Solution

45 g/L Ethylenediamine preservation solution

Dilute 10 mL of 99% ethylenediamine (Fluka) to 200 mL with water. Use 1 mL of this solution per liter of sample.

SAMPLE PREPARATION

Sparge samples for 5 minutes to remove any reactive gases (e.g., chlorine dioxide or ozone). To prevent oxidation of chlorite to chlorate or formation of bromate from hypobromite, preserve samples by adding 1 mL of ethylenediamine preservation solution per liter of sample.

DISCUSSION AND RESULTS

The IonPac AS9-HC provides baseline separation of chlorite, chlorate, bromate, and bromide from common anions and provides good resolution between bromate and chloride to allow a low detection level of 5 µg/L (5 ppb) for bromate in the presence of a high chloride (50 mg/L) concentration. Figure 1 shows a typical chromatogram of a separation of common anions and oxyhalides on the IonPac AS9-HC column.

For the best performance at low levels, it is critical that baseline noise be kept to a minimum. From start up, a system for trace analysis typically requires about 5 hours to

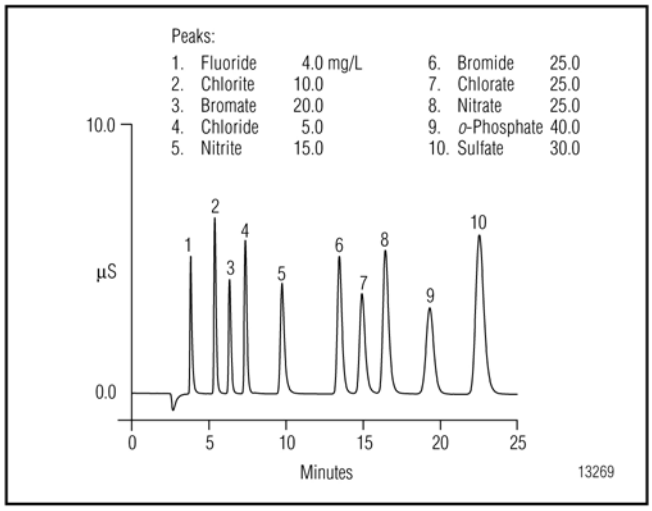


Figure 1 *Separation of common anions and oxyhalides on the IonPac AS9-HC column.*

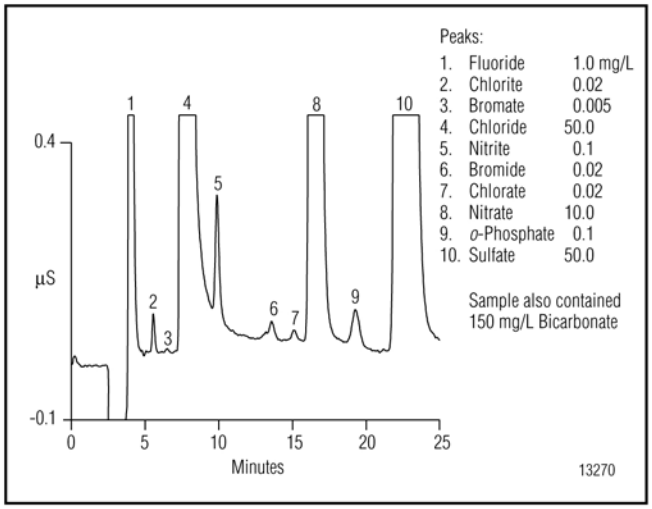


Figure 2 *Determination of µg/L levels of oxyhalides and bromide in simulated drinking water.*

establish a stable background conductivity. For this reason, it is best to keep the system running continuously. Ensure that there is an adequate supply of the eluents, external water, and waste containers to allow the system to run unattended. An equilibrated system will have a background conductivity between 20–24 µS. To keep a conductivity background at that low level throughout a whole day without significant drift, it is absolutely necessary to use external water mode rather than recycle mode. Peak-to-peak noise is typically 6–10 nS. Figure 2 shows the determination of low levels of oxyhalides and bromide in simulated drinking water. Seven injections of this same concentration standard were made to determine method detection limits of oxyhalides and bromide in simulated drinking water. Table 2 shows the results.

Table 2 Method detection limits for oxyhalides and bromide in simulated drinking water based on a 200-µL injection volume using the IonPac AS9-HC column

	Conc. (µg/L)	Std. Dev. (µg/L)	RSD (%)	MDL* (µg/L)
Chlorite	10	0.76	7.99	2.38
Bromate	5	0.55	12.55	1.73
Bromide	10	0.57	5.45	1.78
Chlorate	10	0.34	4.38	1.07

* MDL = SD x $t_{s,99}$ where $t_{s,99}$ = 3.14 for n = 7

Sample also contained: Chloride 50 mg/L
Bicarbonate 150 mg/L
Sulfate 50 mg/L

The data demonstrate that this method achieves the required method detection limits and exceeds the standard deviation requirements of the current ICR. It is important to establish a linear concentration range to accurately quantify oxyhalides and bromide in the presence of common anions in drinking water. Table 3 summarizes the results of the linearity analyses. In these analyses, seven different concentrations of each analyte were analyzed using a 200-µL sample injection. Table 3 shows that bromate is linear between 5–40 µg/L, and chlorite, chlorate, and bromide are linear between 20–500 µg/L.

Table 3 Linear concentration range study of oxyhalides and bromide in simulated drinking water using the IonPac AS9-HC

	Conc. Range (µg/L)	Std. Dev.* of Resp. Factor (µg/L)	RSD (%)	r^2
Bromate	5–40	2.09	1.17	0.9998
Chlorite	20–500	20.07	4.42	0.9999
Bromide	20–500	10.32	4.17	0.9991
Chlorate	20–500	4.05	1.46	0.9999

* Standard deviation was calculated as a function of the response factor. Response factor equals the ratio of area count to concentration.

The chosen ranges for oxyhalides and bromide are characteristic for the amounts of those analytes commonly found in drinking water. Concentrations outside these ranges were not tested. However, it is expected that the method is linear to higher concentrations. The linearity data also demonstrate method ruggedness and good column performance, which allow accurate and precise achievement of the ICR requirements. The concentration range experiment was repeated with the addition of ethylenediamine preservation solution to all of the samples to establish whether the presence of ethylenediamine would have any significant effect on chromatography and recovery of oxyhalides. No significant differences were observed.

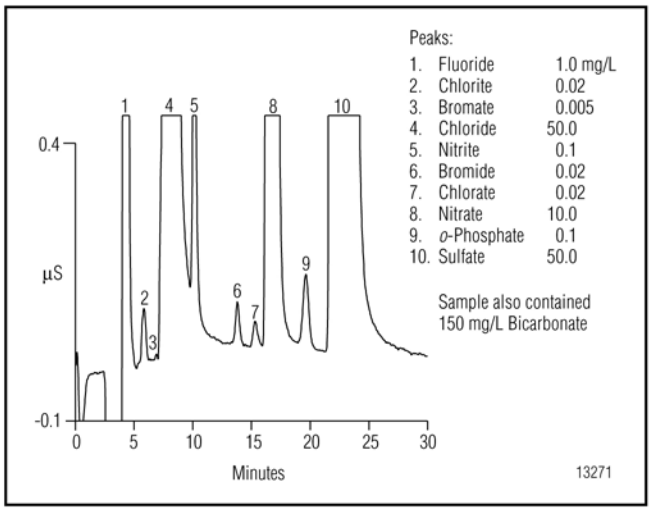


Figure 3 *Large loop injection for µg/L-level oxyhalide analysis.*

The effect of injection volume on simulated drinking water analysis was investigated. Figure 3 illustrates that a 500-µL injection is the largest amount of the simulated drinking water sample that can be injected onto the column without considerable loss of resolution between bromate and chloride. With a 750-µL injection, column ion-exchange sites are overloaded, which causes coelution of bromate and chloride. The IonPac AS9-HC column allows large sample loop injections (e.g., 200 µL) to achieve low detection limits even in the presence of high concentrations of chloride, sulfate, and bicarbonate.

SUMMARY

An improved column and method for the determination of oxyhalides and bromide in drinking water have been developed. Using this method, these anions can be detected in a concentration range that exceeds the ICR requirements. Linear concentration ranges have been established to accurately quantitate the oxyhalides and bromide in drinking water samples.

REFERENCES

1. Fed. Regist. **1996**, *61*(94), 24354.
2. U.S. Environmental Protection Agency. *U.S. EPA Method 300.0*. "The Determination of Inorganic Anions in Water by Ion Chromatography," August 1993.

LIST OF SUPPLIERS

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania, 15219-4785, USA. Tel: 1-800-766-7000.

EM Science, 480 Democrat Road, Gibbstown, New Jersey 08027, USA. Tel: 1-609-354-9200.

Fluka Chemika-BioChemika, Fluka Chemie AG, Industriestrasse 25, CH-9471 Buchs, Switzerland. Tel: 081 755 25 11.

Trace Level Determination of Bromate in Ozonated Drinking Water Using Ion Chromatography

INTRODUCTION

During the 1970s it was discovered that the chlorination of drinking water produced carcinogens, such as the trihalomethanes. Since then environmental regulatory agencies, as well as drinking water treatment technologists, have been aggressively researching alternative disinfection methods that minimize the production of byproducts with significant health risks. Ozonation has emerged as one of the most promising alternatives to chlorination. Ozonation, however, tends to oxidize bromide to bromate, which presents a potential problem since bromide is naturally present in source waters. Bromate has been judged by both the U.S. EPA and the World Health Organization as a potential carcinogen, even at the low µg/L level. Many regulatory agencies prefer to regulate potential carcinogens to the 10^{-5} health risk level or lower.* The U.S. EPA has recommended that bromate in ozonated water be controlled to < 10 µg/L while further health risk studies are underway. Accordingly, analytical methods must be found to quantify bromate at these levels, so as to aid in researching ozonation process design options to minimize this contaminant.

The following equations show the pathway by which bromide (Br^-) is oxidized by ozone to bromate (BrO_3^-) through the intermediate formation of hypobromite (OBr^-). These equations also show that ozone does not oxidize hypobromous acid (HOBr) to bromate. Since increased acid (H_3O^+) will favor the formation of hypobromous acid, this suggests that ozonation at a low pH will tend to minimize bromate formation (see Figure 1).

$$Br^- + O_3 + H_2O \longrightarrow HOBr + O_2 + OH^- \quad \{1\}$$
$$HOBr + H_2O \longrightarrow H_3O^+ + OBr^-$$
$$OBr^- + 2O_3 \longrightarrow BrO_3^- + 2O_2$$
$$HOBr + O_3 \longrightarrow \text{No Reaction}^1$$

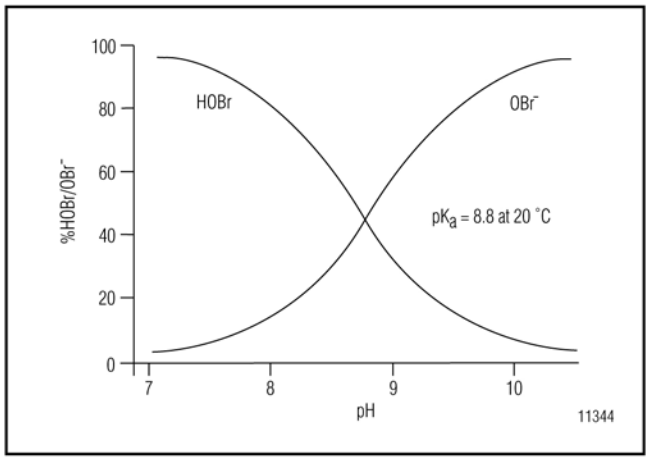


Figure 1 *A decrease in pH favors the formation of hypobromous acid.*

The final concentration of bromate is dependent on the concentration of bromide in the source water, ozone concentration, and duration of contact. Currently, the separation of bromate in a drinking water matrix is accomplished by using direct injection ion chromatography (IC) with suppressed conductivity detection.

Table 1 shows the method detection limits (MDLs) for bromate and other anions of interest that were achieved by U.S. EPA researchers with a 200-µL injected sample on a Dionex IonPac® AS9-SC column using a borate-based eluent. The detection limit for bromate using this methodology is 7.3 µg/L.[2] Injecting a larger sample impairs chromatographic efficiency and does not significantly improve MDLs. The disadvantage to this method is that the amount of bromate present in a typical ozonated water sample is near or below the current detection limit.

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

This application note reports the development of a modified IC method that significantly improves the method detection limits for bromate by sample preconcentration. This method is consistent with the proposed ASTM method for bromate.[3]

In this method, the sample is first preserved by sparging to remove reactive gases, such as chlorine dioxide or ozone. Ethylenediamine is then added to convert any hypobromite to the corresponding bromamines, thus preventing their ongoing conversion to bromate. The preserved sample is then spiked with a magnesium chloride and a sodium carbonate reagent. This sample is then passed through three treatment cartridges in the following sequence: OnGuard-Ba, OnGuard-Ag, and OnGuard-H. This treatment reduces the concentration of sulfate, chloride, carbonate, and metals. The magnesium and carbonate ions are added to ensure reliable sulfate reduction, while maintaining high recovery of bromate.

The treated sample is then loaded into a large sample loop (e.g., 2–5 mL) and the anions remaining in the sample, including bromate, are concentrated on a high capacity concentrator column. A weak borate eluent is then used to elute the concentrated anions through the analytical column set where they are separated and through the suppressed conductivity detector where they are quantified. After bromate is eluted, a strong borate eluent is used to purge the columns of remaining ions prior to analysis of the next sample.

Table 1 Method detection limits using the AS9-SC column

Anion	Spiking Conc. µg/L	Stats MDL[a] µg/L	Noise MDL[b] µg/L	Conservative MDL, µg/L
ClO_2^-	10.0	3.4	2.9	3.4
ClO_3^-	25.0	5.2	9.4	9.4
BrO_3^-	10.0	7.3	5.9	7.3
Br^-	10.0	3.9	8.3	8.3

[a] MDL = SD (t_s)
[b] MDL = 3 x noise
Conditions: 9 mM NaOH, 36 mM Boric acid, 1.0 mL/min, 200 µL injection

EQUIPMENT

Dionex DX 500 IC system consisting of:
- GP40 Gradient Pump
- LC20 Chromatography Enclosure with Second Channel Option
- CD20 Conductivity Detector with DS3 Detection Stabilizer
- EO1 Eluent Organizer
- DXP Sample Delivery Pump
- AS40 Automated Sampler
- AC2 Power Control Accessory

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm resistance or better (Type I reagent grade)

Boric acid, >99% pure (Aldrich, Milwaukee, Wisconsin, USA)

Sodium hydroxide, 50% (w/w) (Fisher Scientific, Pittsburgh, Pennsylvania, USA)

Potassium bromate (Fluka Chemie AG, Buchs, Switzerland)

Magnesium chloride (Aldrich, Milwaukee, Wisconsin, USA)

Sodium carbonate (Aldrich, Milwaukee, Wisconsin, USA)

Ethylenediamine, 99% (Aldrich, Milwaukee, Wisconsin, USA)

SAMPLE PRETREATMENT CARTRIDGES

OnGuard™-Ba Cartridges

OnGuard-Ag Cartridges

OnGuard-H Cartridges

CONDITIONS

Columns: IonPac AS9-SC Analytical, 4-mm i.d.
IonPac AG9-SC Guard, 4-mm i.d.
IonPac AG10 Guard, 4-mm i.d., or TAC-LP1 (Concentrator Column)

Metal Trap Column: MetPac™ CC-1

Eluent A: 40 mM Boric acid/20 mM Sodium hydroxide

Eluent B: 200 mM Boric acid/100 mM Sodium hydroxide

Gradient:

Time (min)	Eluent A (%)	Eluent B (%)	Valve A (setting)	Valve B (setting)
0.00	100	0	Load	Inject
1.50	100	0	Inject	Load
6.50	100	0	Load	Inject
12.49	100	0	Load	Inject
12.50	0	100	Load	Inject
17.49	0	100	Load	Inject
17.50	100	0	Load	Inject

Flow Rate: 2 mL/min

Injection Volume*: 5 mL (max.)

Concentrator Pump

Flow Rate: 2 mL/min

Detection: Suppressed conductivity

Suppressor: ASRS AutoSuppression, external water mode

Note: Loop volume should be checked by filling loop with water and determining actual volume by weight on an analytical balance.

*Use 0.037-inch i.d. tubing for sample loop (1 cm = 6.9 µL)

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solutions

Bromate (BrO_3^-) 1000 mg/L

Dissolve 1.31 g of potassium bromate ($KBrO_3$) in water and dilute to 1.00 L.

Preservation Solution

Ethylenediamine Preservation Solution (45 g/L)

Dilute 10 mL of ethylenediamine (99%) to 200 mL with water. Use 1 mL of this solution per liter of sample.

OnGuard-Ba Activating Reagents

0.50 M ($MgCl_2$)

Dissolve 48 g of magnesium chloride in water and dilute to 1 L.

0.17 M (Na_2CO_3)

Dissolve 18 g of sodium carbonate in water and dilute to 1 L.

Chromatography Eluent (A)

40 mM Boric Acid / 20 mM Sodium Hydroxide

Dissolve 2.47 g boric acid in 990 mL of water, add 1.6 g of 50% sodium hydroxide, and dilute to 1.00 L. Transfer this solution to an eluent container and vacuum degas for 10 minutes.

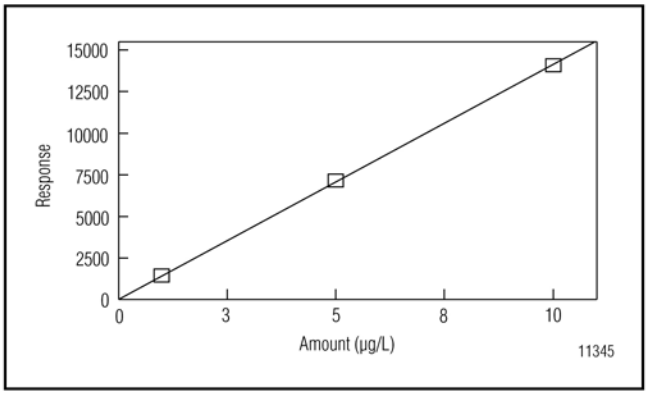


Figure 2 *Linearity plot for bromate at µg/L levels.*

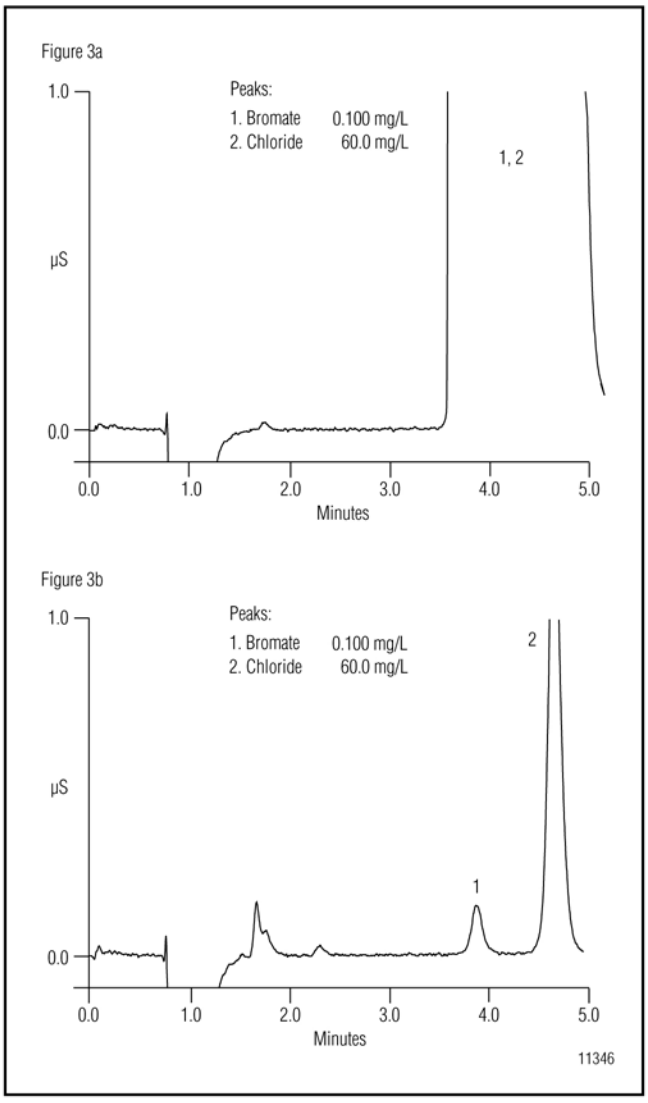


Figure 3a *An untreated sample in which chloride coelutes with bromate.*

Figure 3b *A sample pretreated with OnGuard-Ag in which bromate is resolved from chloride.*

Purge Eluent (B)

200 mM Boric Acid /100 mM Sodium Hydroxide

Dissolve 12.36 g of boric acid in 900 mL of water, add 8.0 g of 50% sodium hydroxide and dilute to 1.00 L. Transfer this solution to an eluent container and vacuum degas for 10 minutes.

Note: Care must be taken to minimize air contact with hydroxide reagent as absorbed carbon dioxide will change eluent characteristics. Keep eluent containers pressurized with an inert gas to prevent atmospheric carbon dioxide from entering.

Preparation of Calibration Standards

Calibration Standards

Prepare calibration standards at a minimum of three concentrations in deionized water from the stock standard solution. The lowest concentration of the bromate standard should slightly exceed 1 µg/L, the method detection limit (MDL). The other concentrations of the calibration curve should correspond to the expected range of concentrations found in the samples of interest. A typical calibration curve is shown in Figure 2.

SAMPLE PREPARATION

Samples should be sparged for 5 minutes to remove any reactive gases. Next, preserve samples with ethylenediamine to prevent oxidation of chlorite or formation of bromate from hypobromite by adding 1 mL of ethylenediamine preservation solution per liter of sample. Now add the OnGuard-Ba activating reagents: 1 mL of the 0.5 M magnesium chloride reagent and 1 mL of the sodium carbonate reagent to 100 mL of sample. All samples should be filtered through a 0.45-µm filter prior to injection. With high levels of sulfate, chloride, and carbonate in the sample matrix, the exchange sites on the AG10/AS9-SC columns are overloaded and bromate cannot be detected as a separate peak (Figure 3a). Sulfate is removed by passing the sample through the Dionex OnGuard-Ba cartridge. This cartridge removes sulfate by forming the precipitate barium sulfate.

Chloride is removed by passing the sample through the Dionex OnGuard-Ag cartridge. Chloride precipitates as silver chloride. Next, the sample is passed through the Dionex OnGuard-H cartridge. It minimizes the carbonate in the sample by converting it to carbonic acid, which is removed by sparging the sample with helium for 2–3 minutes.

Table 2 Determination of bromate in drinking water, 5 mL preconcentrated

Sample	Bromate Present (µg/L)	Bromate Added (µg/L)	Bromate Found[a] (µg/L)	n	SD (µg/L)	MDL[b] (µg/L)
Raw Water A	ND[c]	1.0	1.1	7	0.09	0.3
	ND	5.0	5.1	6	0.29	0.9
	ND	10.0	10.0	7	0.58	1.7
Raw Water B	1.1	0.0	1.1	7	0.04	0.1
Raw Water B	1.1	1.0	1.2	7	0.11	0.3
(Ozonated)	1.1	5.0	4.7	7	0.70	2.1
	1.1	10.0	10.0	5	1.52	5.1

[a] Reference to 10 µg/L fortification of matrix
[b] MDL = SD x (t_s) 99%
[c] ND = Not Detected (< 0.1 µg/L)

DISCUSSION AND RESULTS

Traditionally, a cation resin in the barium-form has been used to remove sulfate from the sample matrices by forming a barium sulfate precipitate ($K_{sp} = 1.1 \times 10^{-10}$). Using this method, however, the sulfate removal varies considerably. Our studies indicate that for consistent sulfate removal, a sample must have a sufficient amount of a divalent cation to displace the divalent barium from the resin so that it can react with sulfate.[4] Some samples have sufficient calcium and magnesium to initiate the barium displacement; whereas, others do not contain sufficient levels of calcium and magnesium. Therefore, to ensure consistent sulfate removal a divalent cation such as magnesium must be added. It has been determined that at least 120 mg/L in magnesium from magnesium chloride will provide sufficient barium displacement from a cation resin in the barium form (OnGuard-Ba) for removal of sulfate up to 500 mg/L. Furthermore, it has been found that a minimum level of carbonate is required to ensure high bromate recovery when sulfate is being removed by the OnGuard-Ba cartridge. The excess chloride can be removed with OnGuard-Ag treatment, which is also required for removing chloride that is normally present in the sample.

The OnGuard-Ag cartridge packing is a silver form, high capacity, strong acid, cation exchange resin that is designed to remove chloride from the sample matrices. The cartridge capacity is 1.5–1.8 meq per cartridge. By treating the sample with the OnGuard-Ag cartridge, the chloride level is reduced to approximately 0.4 mg/L; this level is sufficient to resolve bromate from chloride (see Figure 3b).

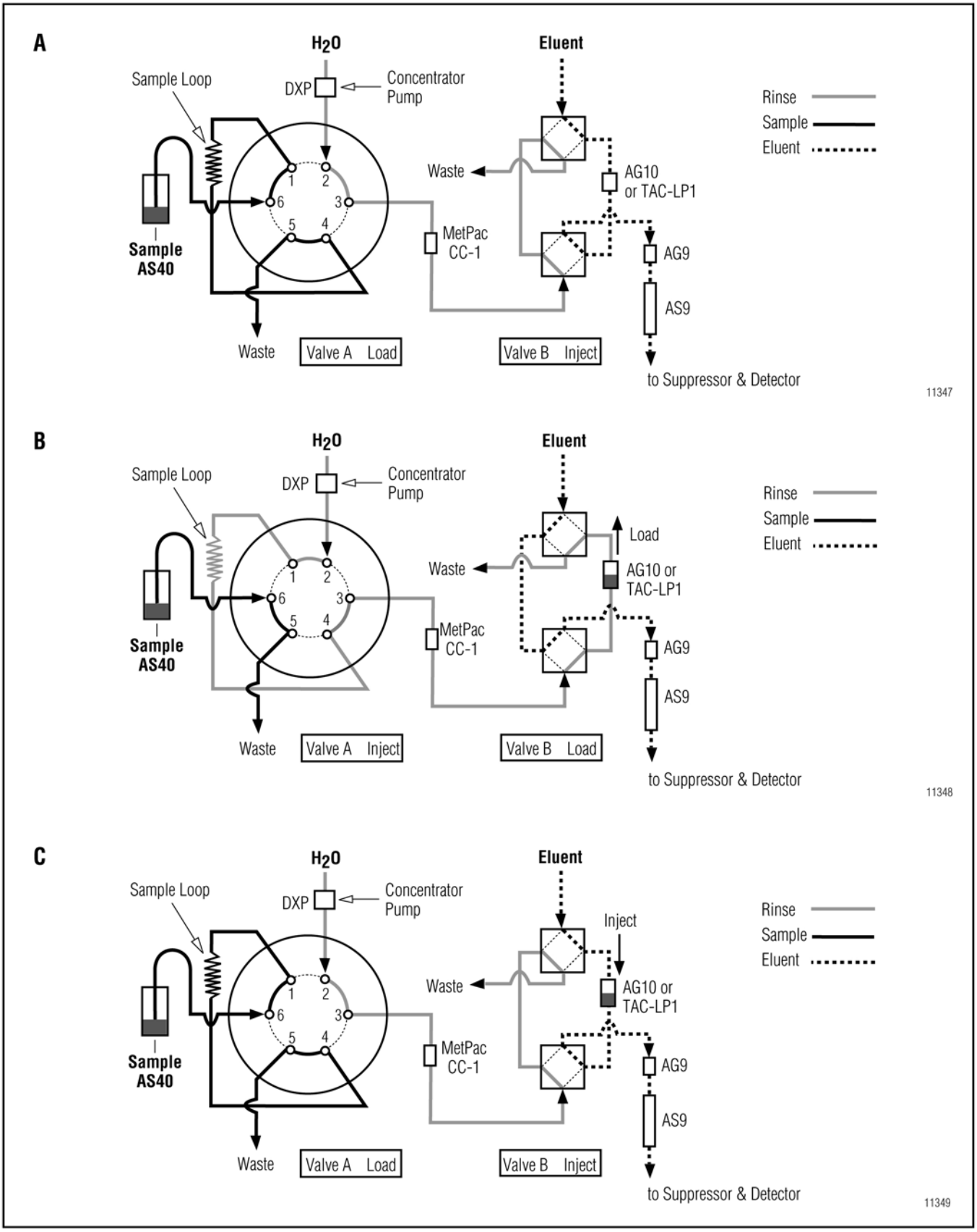


Figure 4 *Preconcentration suppressed IC system configuration. (A) sample loaded into loop; (B) sample washed onto concentrator column; (C) retained anions eluted to AG9/AS9-SC analytical column set.*

The lower sulfate and chloride levels allow for a larger sample volume to be concentrated, which improves the bromate response. The OnGuard-H sample pretreatment cartridge is a hydrogen-form cation resin. Its use followed by helium sparging minimizes the carbonate in the sample, which further improves preconcentration efficiency. The use of the OnGuard-Ag leaches a small amount of silver from the cartridge into the sample matrix. The accumulation of silver on the analytical column and concentrator column will affect column performance over time. The OnGuard-H cartridge also removes metal ions such as silver. To further avoid metal contamination, a Dionex MetPac CC-1 column is installed between the two injection valves (see Figure 4). The MetPac CC-1 metal chelating column not only removes the silver, but it also removes other metal cations that may foul the analytical column.

The determination of bromate utilizing this method is a three step process as illustrated in Figures 4a–4c: Step 1 loads the sample loop, Step 2 washes the sample onto the concentrator, Step 3 separates the anions of interest on the analytical column. Figure 4a illustrates the sample being loaded into the sample loop using an autosampler. During this first step, the GP40 pumps Eluent A to the AS9-SC column. After the loop is filled, the DXP Pump is turned "ON" and it washes the sample from the sample loop onto the concentrator column using deionized water (see Figure 4b). The sample loop is then rinsed 2.5 times its volume to ensure that all of the sample is transfered onto the concentrator. The concentrator column strongly retains anionic species such as bromate, chloride, and sulfate. Figure 4c shows the concentrator column being switched in-line with the IonPac AG9/AS9-SC columns. At this step, the retained anions are eluted to the analytical column. After the chloride elution, the remaining anions are purged off the analytical column using the purge eluent.

After purging for 5 minutes, the AG9/AS9-SC columns are equilibrated with the chromatography eluent for 7–10 minutes. The equilibration time is placed at the beginning of the analysis sequence, during which the sample loop is being filled and the sample is flushed onto the concentrator column. The total analysis time for this method is 25 minutes. Table 2 lists the bromate MDLs that have been achieved when preconcentrating raw water samples, obtained before and after ozonation.

Using this method, bromate (at the low µg/L level) can be measured in a matrix containing as much as 200 mg/L of chloride, carbonate, and sulfate as shown in Figure 5. Figure 6 shows the analysis of an ozonated drinking water sample spiked with 1 µg/L bromate.

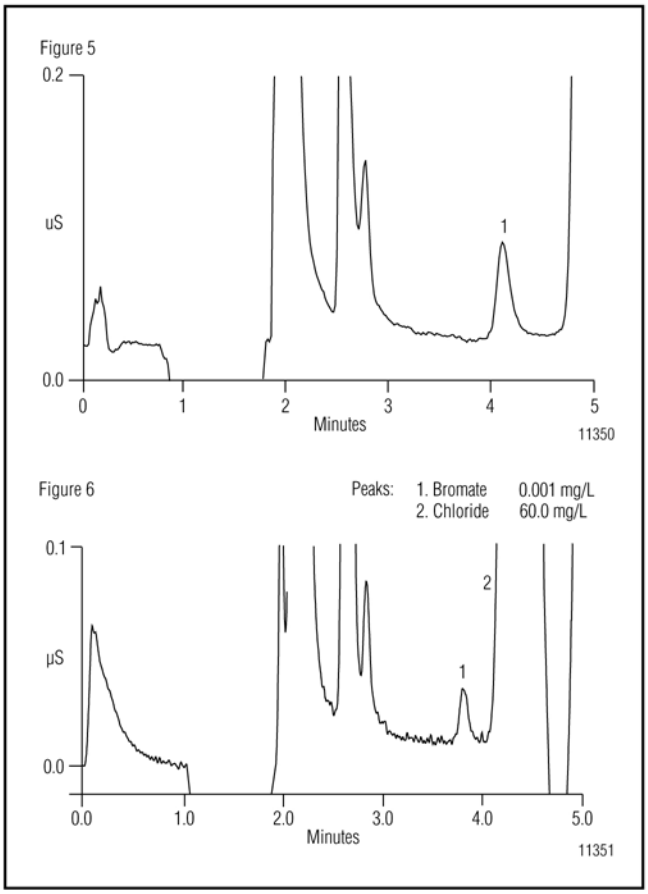


Figure 5 *Bromate in the presence of 200 mg/L of chloride, carbonate, and sulfate.*

Figure 6 *An ozonated drinking water sample is spiked with 1.0 µg/L of bromate.*

REFERENCES

1. Haag, W. R.; Hoigne, J. *Environmental Science and Technology,* **1983**, *17*, 261.
2. Hautman, D. P.; Bolyard, M. *J. Chromatogr,* **1991**, *602*, 7.
3. American Society for Testing and Materials. Proposed ASTM Method "Determination of Oxyhalides and Bromide in Water by Chemically Suppressed Ion Chromatography," under review by the ASTM D-19 Committee on Water.
4. Patent filed.

Analysis of Low Concentrations of Perchlorate in Drinking Water and Ground Water by Ion Chromatography

INTRODUCTION

Perchlorate (as ammonium perchlorate), which is widely used in solid rocket propellants, has recently been found in drinking water wells in areas where aerospace materials and munitions were manufactured and tested.[1] Perchlorate is a health concern because it interferes with the production of thyroid hormones. Current data suggest that an exposure level range of 4 to 18 µg/L (ppb) is acceptable.[2] Although perchlorate is not yet regulated in the U.S. under the Federal Safe Drinking Water Act, the State of California requires remedial action for drinking water sources containing greater than 18 µg/L of perchlorate.

This Application Note details a new method developed to quantify low levels of perchlorate. A large loop injection (1000 µL) is used with an IonPac® AS11 column and suppressed conductivity detection to quantify perchlorate in drinking water down to approximately 2.5 µg/L.

EQUIPMENT

Dionex DX-500 Ion Chromatography system consisting of:
- GP40 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC20 Chromatography Enclosure with a rear-loading valve

4-L Plastic bottle assemblies (two for external water mode)

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent grade, 18 MΩ-cm resistance or better

Sodium hydroxide, 50% (w/w) aqueous solution (Fisher Scientific or other)

Sodium perchlorate, 99% ACS reagent grade or better (Aldrich or other)

Potassium sulfate, 1000 mg/L aqueous solution (Ultra Scientific or other)

CONDITIONS

Columns:	IonPac AS11 Analytical, 4 × 250 mm (P/N 044076) IonPac AG11 Guard, 4 × 50 mm (P/N 044078)
Eluent:	100 mM Sodium hydroxide
Run Time:	12 min
Flow Rate:	1.0 mL/min
Sample Volume:	1000 µL
Detection:	Suppressed conductivity, ASRS® (4 mm), AutoSuppression® external water mode
System Backpressure:	600–900 psi (3.95–5.93 MPa)
Background Conductance:	2–5 µS

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solution

Stock Perchlorate Standard Solution (1000 mg/L)

Dissolve 1.231 g of sodium perchlorate in 1000 mL of deionized water to prepare a 1000 mg/L standard. Standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Dilute 1000 mg/L standard solution as required with deionized water to prepare the appropriate working standards.

Eluent Solution

100.0 mM Sodium Hydroxide

Weigh 992.0 g of deionized water into an eluent bottle. Degas water for approximately 5 minutes. Carefully add 8.0 g of 50% sodium hydroxide directly to the bottle. Mix then quickly transfer the eluent bottle to the instrument and pressurize the bottle with helium at 8 psi (0.055 MPa).

RESULTS AND DISCUSSION

For the best perfomance at low-ppb levels, it is critical that baseline noise be kept to a minimum. To minimize baseline noise, it is necessary to use the ASRS in external water mode rather than the recycle mode. An equilibrated system will produce a background conductance from 2–5 µS. Peak-to-peak noise is typically 10 nS and system backpressure is 600–900 psi (3.95–5.93 MPa). A system blank is determined by using deionized water as a sample. This blank establishes the baseline and confirms the lack of contamination in the system. The linear concentration range was determined to ensure accurate quantification of perchlorate in the 2.5–100 µg/L range. Figure 1 shows the results of a linearity study.

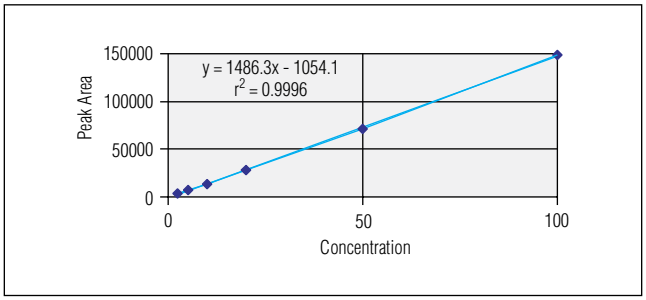


Figure 1. Perchlorate calibration.

This plot demonstrates that calibration of perchlorate is linear in the low-ppb range. Figure 2 shows a typical chromatogram of a 20 µg/L perchlorate standard. To determine the method detection limit (MDL), seven injections of the 2.5 µg/L perchlorate standard were made. Table 1 shows the results of a method detection limit study. The 1000-µL injection is large enough to achieve the desired detection limit without overloading the column. Note that this method is not intended for use with high (ppm) levels of perchlorate. The calculated MDL equals 880 ng/L (ppt).

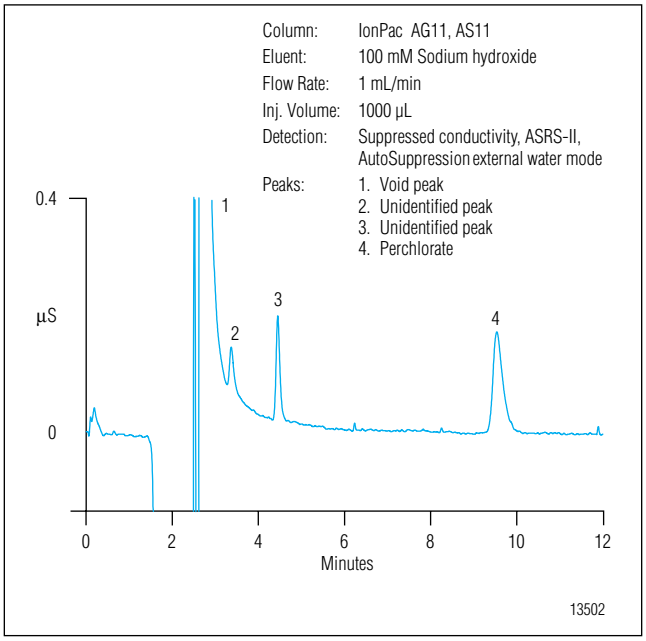


Figure 2. 20 µg/L perchlorate standard.

Table 1 MDL for Perchlorate Based on a 1000-µL Injection Volume

Injection #	Area counts	Retention time (min)
1	3391	9.48
2	3405	9.57
3	3504	9.50
4	3503	9.45
5	3435	9.47
6	3301	9.52
7	3315	9.43
Average	3408	9.49
SD	81	0.05
RSD	2.38	0.49

MDL=880 ng/L (ppt), MDL=SD*$t_{s,99}$ where $t_{s,99}$=3.14 for n=7

Figures 3 through 5 show chromatograms obtained for 2.5 µg/L perchlorate in three different matrices. Figure 3 shows the chromatogram of 2.5 µg/L perchlorate in deionized water. Figure 4 shows 2.5 µg/L perchlorate in tap water. Note that all other anions present in tap water elute in the void volume and do not interfere with perchlorate determination. Some environmental samples may contain low levels of perchlorate in the presence of a large amount of sulfate. Figure 5 shows the determination of 2.5 µg/L perchlorate in the presence of 700 mg/L sulfate. The high concentration of sulfate does not affect perchlorate recovery or the detection limit.

SUMMARY

The method outlined in this Application Note allows the determination of low-µg/L (ppb) levels of perchlorate. A linear concentration range has been established to accurately quantify perchlorate in drinking water and ground water samples.

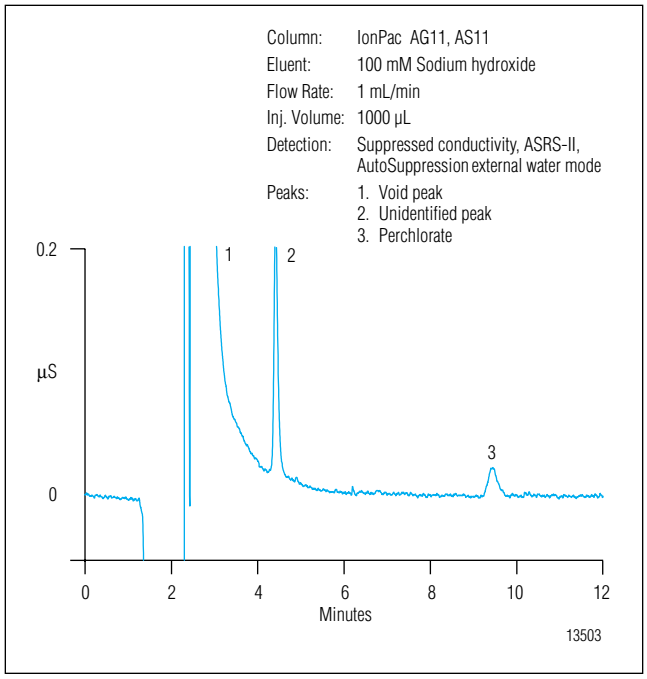


Figure 3. 2.5 µg/L perchlorate standard.

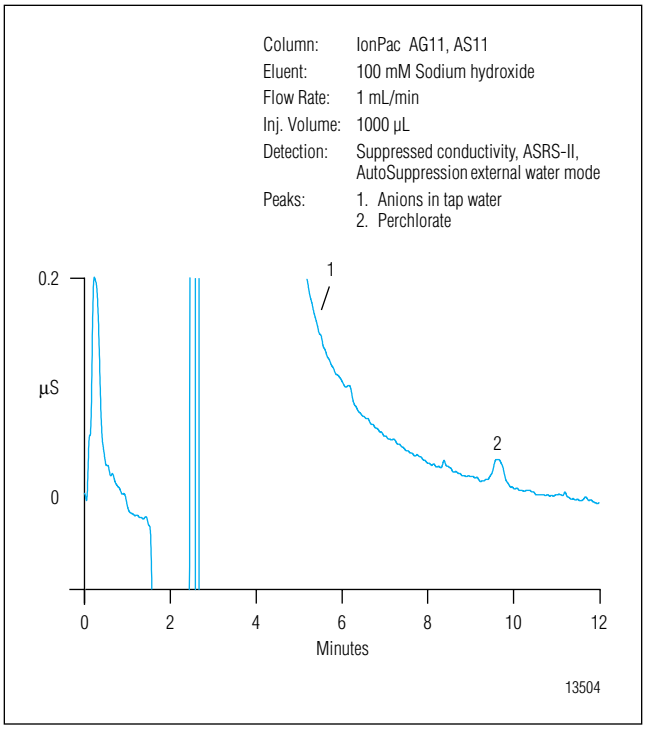


Figure 4. 2.5 µg/L perchlorate in tap water.

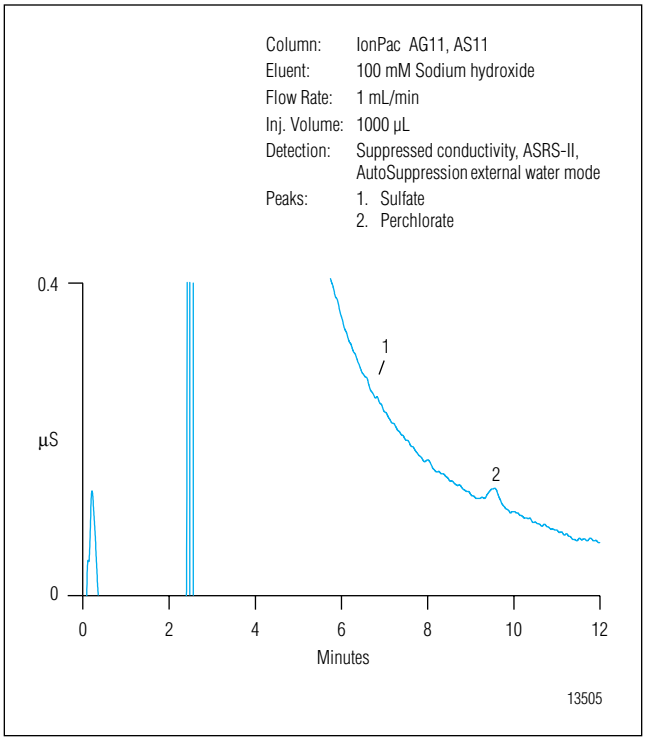


Figure 5. 2.5 µg/L perchlorate and 700 mg/L sulfate.

REFERENCES

1. "Perchlorate in California Drinking Water"; California Department of Health Services, September 1997.
2. Correspondence from Joan S. Dollarhide, National Center for Environmental Assessment, Office of Research and Development, to Mike Girrard, Chairman, Perchlorate Study Group, U.S. EPA, 1995.

SUPPLIERS

Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, Wisconsin, 53233, USA. Tel: 1-800-558-9160.

Fisher Scientific, 711 Forbes Ave., Pittsburgh, Pennsylvania, 15219-4785, USA. Tel: 1-800-766-7000.

Ultra Scientific, 250 Smith Street, North Kingstown, Rhode Island, 02852, USA. Tel: 401-294-9400.

DIONEX

Determination of Inorganic Anions in Drinking Water by Ion Chromatography

INTRODUCTION

The determination of common inorganic anions in drinking water is one of the most important applications of ion chromatography (IC) worldwide. The National Primary Drinking Water Standards in the United States specify a Maximum Contaminant Level (MCL) for a number of inorganic anions, including fluoride, nitrite, and nitrate. The MCLs are specified to minimize potential health effects arising from the ingestion of these anions in drinking water.[1] High levels of fluoride cause skeletal and dental fluorosis, and nitrite and nitrate can cause methemoglobulinemia, which can be fatal to infants. Other common anions, such as chloride and sulfate, are considered secondary contaminants. The National Secondary Drinking Water Standards in the U.S. are guidelines regarding taste, odor, color, and certain aesthetic characteristics. Although these guidelines are not federally enforced, they are recommended to all states as reasonable goals and many states adopt their own regulations governing these contaminants.[2]

Ion chromatography has been approved for compliance monitoring of these common inorganic anions in U.S. drinking water since the mid-1980s, as described in U.S. EPA Method 300.0.[3] Many other industrialized countries have similar health and environmental standards and a considerable number of regulatory IC methods have been published worldwide (e.g., in Germany, France, Italy, and Japan) for the analysis of anions in drinking water. In addition, many standards organizations (including ISO, ASTM, and AWWA) have validated IC methods for the analysis of inorganic anions in drinking water.[4,5]

This application note describes the determination of inorganic anions in drinking water and other environmental waters using conditions that are consistent with those in U.S. EPA Method 300.0.[3] The use of an optional column, the IonPac® AS14, is also discussed.

EQUIPMENT

Dionex DX-120 and DX-500 ion chromatography systems were used for this work. The DX-120 is a dedicated ion chromatograph; the DX-500 is a modular system, which in this case consisted of:

GP50 Gradient Pump

CD20 Conductivity Detector

LC20 Chromatography Enclosure with rear-loading injection valve

AS40 Automated Samplers (5-mL vials) and a PeakNet® Chromatography Workstation were used with both systems.

REAGENTS AND STANDARDS

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

0.18 M sodium carbonate/0.17 M sodium bicarbonate: IonPac AS4A Eluent Concentrate (P/N 39513)

0.35 M sodium carbonate/0.1 M sodium bicarbonate: IonPac AS14 Eluent Concentrate (P/N 53560)

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

Fluoride standard 1000 mg/L, 100 mL (P/N 37158)

Chloride standard 1000 mg/L, 100 mL (P/N 37159)

Sulfate standard 1000 mg/L, 100 mL (P/N 37160)

Nitrate standard 1000 mg/L, 100 mL (ULTRA Scientific, VWR P/N ULICC-004)

Phosphate standard 1000 mg/L, 100 mL (ULTRA Scientific, VWR P/N ULICC-005)

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

CONDITIONS

Part A

Columns: IonPac AG4A-SC, 4 × 50 mm (P/N 43175)
IonPac AS4A-SC, 4 × 250 mm (P/N 43174)

Eluent: 1.8 mM sodium carbonate/ 1.7 mM sodium bicarbonate

Run Time: <8 min

Flow Rate: 2.0 mL/min

Inj. Volume: 50 µL

Detection: Suppressed conductivity, ASRS® ULTRA, 4 mm, in recycle mode, 50 mA current

System Backpressure: ~1000 psi

Background Conductance: ~14 µS

Part B

Columns: IonPac AG14, 4 × 50 mm (P/N 46134)
IonPac AS14, 4 × 250 mm (P/N 46124)

Eluent: 3.5 mM sodium carbonate/1.0 mM sodium bicarbonate

Run Time: <14 min

Flow Rate: 1.2 mL/min

Inj. Volume: 50 µL

Detection: Suppressed conductivity, ASRS ULTRA, 4 mm, in recycle mode, 100 mA current

System Backpressure: ~1600 psi

Background Conductance: ~17 µS

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Standard Solutions

Stock Anion Standard Solutions (1000 mg/L)

For several of the analytes of interest, 1000-mg/L standard solutions are available from Dionex and other commercial sources. When commercial standards are not available, 1000-mg/L standards can be prepared by dissolving the appropriate amounts of the corresponding mass in 1000 mL of deionized water according to Table 1. Standards are stable for at least one month when stored at 4 °C.

Table 1. Masses of Compounds Used to Prepare 1 L of 1000-mg/L Anion Standards

Anion	Compound	Mass (g)
Fluoride	Sodium fluoride (NaF)	2.210
Chloride	Sodium chloride (NaCl)	1.648
Nitrite	Sodium nitrite ($NaNO_2$)	1.499
Bromide	Sodium bromide (NaBr)	1.288
Nitrate	Sodium nitrate ($NaNO_3$)	1.371
o-Phosphate	Potassium phosphate, monobasic (KH_2PO_4)	1.433
Sulfate	Sodium sulfate (Na_2SO_4)	1.522

Working Standard Solutions

Composite working standards at lower analyte concentrations are prepared from the 1000-mg/L standards described above. Working standards containing less than 100 mg/L anions should be prepared daily. Table 2 shows the linear concentration range investigated for each anion, as well as the concentration of the standard used to calculate the method detection limits (MDLs) and the concentration of the quality control sample (QCS) used to determine the retention time and peak area precision.

Table 2. Concentration of Linearity, MDL, and Reproducibility Standards

Anion	Seven-Point Calibration Range (mg/L)	MDL Calculation Standard (mg/L)	QCS Standard for RSD Calculation (mg/L)
Fluoride	0.1–100	0.025	2
Chloride	0.2–200	0.010	20
Nitrite	0.1–100	0.025	2
Bromide	0.1–100	0.050	2
Nitrate	0.1–100	0.045	10
o-Phosphate	0.1–100	0.045	2
Sulfate	0.2–200	0.050	60

Eluent Solutions

Dilute 20 mL of the appropriate eluent concentrate to 2.0 L with deionized water. Transfer to a 2-L eluent container and pressurize the container with helium at 8 psi.

SAMPLE PREPARATION

The vial caps in the AS40 Automated Sampler contain a 20-µm filter, so no additional filtration was used in conjunction with this mode of sample introduction. If an AS40 is not used, filter all samples through appropriate 0.45-µm syringe filters, discarding the first 300 µL of the effluent. The domestic wastewater sample was treated with a C_{18} Sep-Pak cartridge (Waters Corporation) to remove hydrophobic organic material in order to prolong column lifetimes.[6] The C_{18} cartridge was preconditioned with 5 mL of methanol, followed by 5 mL of deionized water. The sample (5 mL) was then passed through the cartridge, with the first 1 mL of the effluent being discarded. Aqueous soil extracts were prepared by the extraction of 3.0 g of soil in 30 mL of deionized water in an ultrasonic bath for 30 min, followed by filtration with a 0.45-µm filter.

RESULTS AND DISCUSSION

A variety of methods have been used for the analysis of inorganic anions, including traditional spectroscopic techniques such as colorimetry; wet chemical methods such as gravimetric analysis, turbidimetry, and titrimetry; and electrochemical techniques such as ion selective electrodes (ISE) and amperometric titrations.[4] However, many of these methods are not specific and suffer from interferences or limited sensitivity, can be labor-intensive, and are often difficult to automate. Because many of the individual test procedures described above can be replaced by one chromatographic separation, IC was quickly accepted by regulatory agencies worldwide for the determination of anions in drinking water and other environmental waters.

IonPac AS4A-SC Column

U.S. EPA Method 300.0(A) specifies the use of an IonPac AS4A anion-exchange column with an eluent of 1.8 mM sodium carbonate/1.7 mM sodium bicarbonate for the separation of common anions.[3]The method specifies the use of an AMMS® (Anion MicroMembrane™ Suppressor) operated in the chemical regeneration mode; however, an ASRS (Anion Self-Regenerating Suppressor) provides equivalent method performance with added convenience. Conductivity is used as a bulk property detector for the measurement of inorganic anions.

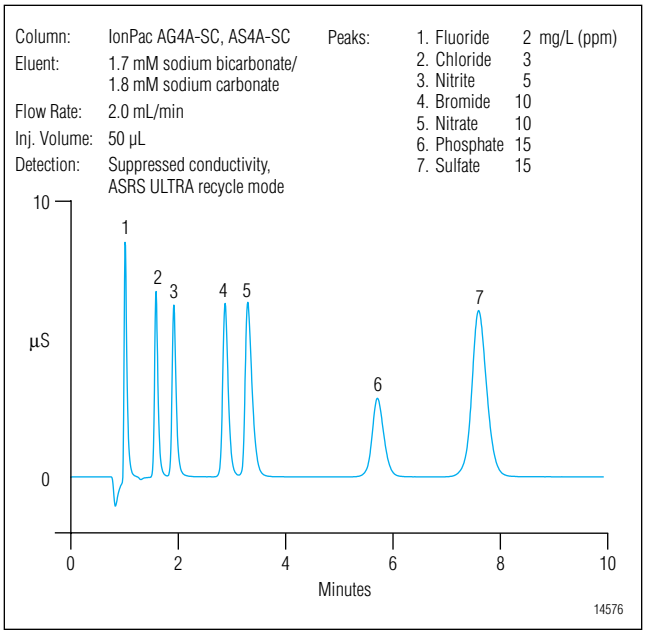


Figure 1. Separation of a low-ppm inorganic anion standard using an IonPac AS4A-SC column.

Figure 1 shows a typical chromatogram of a standard containing low-ppm levels of common inorganic anions separated using an IonPac AS4A-SC column as described in Part A of the "Conditions" section. The AS4A-SC has selectivity similar to that of the AS4A column, although the substrate of the AS4A-SC is ethylvinylbenzene (EVB) crosslinked with 55% divinylbenzene (DVB), which makes the column 100% solvent-compatible. All the anions are well-resolved within a total run time of less than 8 min. The method linearity was determined for the inorganic anions over a seven-point calibration range. MDLs were calculated for each of the anions according to the procedure described in U.S. EPA Method 300.0.[3] The MDLs are estimated by injecting seven replicates of reagent water fortified at a concentration of 3 to 5 times the estimated instrument detection limit. The MDL is then calculated as *(t)* x (SD) 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) and SD = standard deviation of the replicate analysis.

Table 3. Linearity, MDL, Retention Time, and Peak Area Precision Performance Obtained Using the IonPac AS4A-SC Column[a]

Anion	Range (mg/L)	Linearity (r^2)	Calculated MDL[b] (µg/L)	Retention Time Precision (% RSD[c])	Area Precision (% RSD)
Fluoride	0.1–100	0.9971	5.9	0.48%	0.67%
Chloride	0.2–200	0.9996	2.3	0.30%	0.47%
Nitrite	0.1–100	0.9997	5.7 (1.8 as NO_2-N)	<0.05%	0.53%
Bromide	0.1–100	0.9967	9.7	<0.05%	0.13%
Nitrate	0.1–100	0.9969	6.2 (1.4 as NO_3-N)	0.40%	0.17%
o-Phosphate	0.1–100	0.9967	17.8 (5.8 as PO_4-P)	0.30%	0.35%
Sulfate	0.2–200	0.9975	6.7	<0.05%	0.14%

[a] Dionex DX-120 system.

[b] MDL = $\sigma^* t_{s,99}$ where $t_{s,99}$ = 3.14 for n = 7.

[c] RSD = Relative Standard Deviation, n = 7.

Table 3 shows the concentration ranges investigated, the resulting linear coefficients of determination (r^2), and the calculated MDL for each anion. The retention time and peak area precision (expressed as % RSD) were determined from seven replicate injections of a quality control standard, as described in Table 2. Table 3 also shows typical retention time and peak area precision data that can be obtained for inorganic anions using the IonPac AS4A-SC column with a DX-120 system.

The performance of methods used for environmental analysis are typically validated through single- and multi-operator precision and bias studies on spiked samples. Table 4 shows typical recovery results for single-operator data obtained using the IonPac AS4A-SC column for common anions spiked into drinking water, raw (unprocessed) drinking water, and other environmental water matrices. In this instance, the samples were spiked with the analytes at approximately the same levels as specified in U.S. EPA Method 300.0.[3]

Table 4. Anion Recoveries for Spiked Water Samples Obtained Using the IonPac AS4A-SC Column

	Drinking Water		Raw Water		Surface Water	
Anion	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)
Fluoride	1	93.9	1	96.5	1	109.0
Chloride	10	97.4	20	83.2	40	81.4
Nitrite	2	91.6	2	102.1	4	105.0
Bromide	2	98.7	2	96.7	2	101.0
Nitrate	5	92.4	5	94.4	10	96.7
o-Phosphate	10	95.0	10	95.4	10	107.9
Sulfate	20	97.5	40	106.8	40	106.4
	Domestic Wastewater		**Industrial Wastewater**		**Soil Extract**	
Anion	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)
Fluoride	1	57.0	1	88.0	2	99.0
Chloride	20	82.7	20	100.8	5	100.2
Nitrite	2	217.0*	2	98.0	2	102.5
Bromide	2	86.5	2	92.0	2	91.0
Nitrate	5	6.8*	5	96.2	5	90.2
o-Phosphate	20	101.6	20	98.8	20	111.7
Sulfate	40	90.6	40	105.9	20	96.6

*Sample stored for longer than recommended holding time; inappropriate recovery due to microbial action.

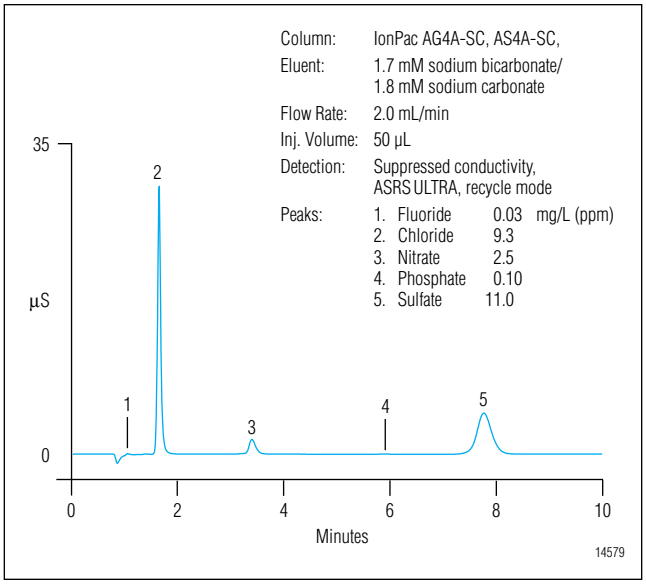


Figure 2. Determination of inorganic anions in drinking water using an IonPac AS4A-SC column.

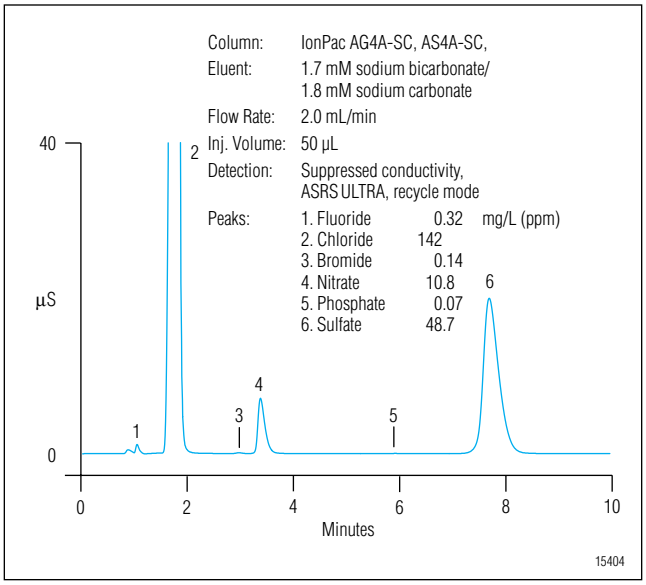


Figure 3. Determination of anions in surface water using an IonPac AS4A-SC column

Figure 2 shows a typical chromatogram of inorganic anions in Sunnyvale, California drinking water obtained using the IonPac AS4A-SC column. Figure 3 shows a chromatogram of inorganic anions in surface water obtained from a lake in Utah. In general, Table 4 shows that acceptable recovery data (i.e., 80–120%) was obtained for the inorganic anions in most matrices. The one exception was the domestic wastewater sample, where fluoride gave a recovery of < 60% under these conditions. Fluoride concentrations of < 1.5 mg/L are subject to interference from mg/L levels of small organic acids, such as formate and acetate, when using the AS4A column.[3] In this sample, the recoveries for nitrite and nitrate were also not as expected. At the time of these analyses, the sample had been stored (at 4 °C) for longer that the recommended holding time for nitrite/nitrate of 2 days.[3] In this case, the unexpected recoveries were due to the presence of nitrifying/denitrifying microbes in the sample rather than any chromatographic resolution problems.

IonPac AS14 Column

Although U.S. EPA Method 300.0 specifies the use of an IonPac AS4A column, Section 6.2.2.1 states that "an optional column may be used if comparable resolution of peaks is obtained and the quality control requirements of Section 9.2 can be met." The IonPac AS14 column is packed with a grafted, methacrylate-based anion-exchange resin. In contrast, the AS4A-SC column is packed with a functionalized latex on the surface of an EVB/DVB-based material. The AS14 column provides complete resolution of fluoride from formate and/or acetate, in addition to improved resolution of fluoride from the void peak.

The improved selectivity and higher capacity of the AS14 column (65 µeq/column compared to 20 µeq/column for the AS4A) also allows improved resolution of chloride and nitrite, which is important in environmental water analysis. One drawback of using the grafted, higher capacity AS14 column is lower peak efficiencies than those obtained using the latex-agglomerated AS4A-SC column. Figure 4 shows a typical chromatogram of a standard containing low-ppm levels of common anions separated using the IonPac AS14 column as described in Part B of the "Conditions" section. Fluoride is clearly resolved from the void volume and the overall selectivity is improved compared to the chromatogram shown in Figure 1, although the total run time is increased to 14 min.

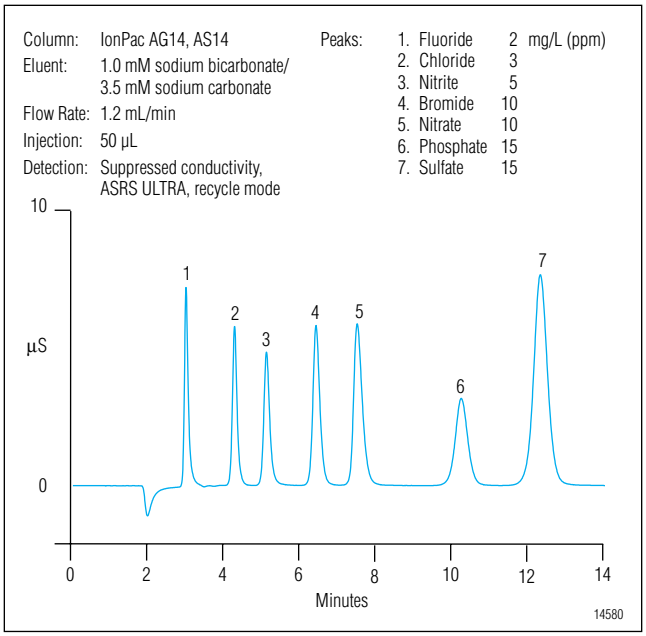


Figure 4. Separation of a low-ppm inorganic anion standard using an IonPac AS14 column.

Table 5. Linearity, MDL, Retention Time, and Peak Area Precision Performance Obtained Using the IonPac AS14 Column[a]

Anion	Range (mg/L)	Linearity (r^2)	Calculated MDL[b] (µg/L)	Retention Time Precision (% RSD)	Area Precision (% RSD[c])
Fluoride	0.1–100	0.9980	3.5	0.23%	0.17%
Chloride	0.2–200	0.9995	2.9	0.41%	0.51%
Nitrite	0.1–100	0.9997	6.5 (2.0 as NO_2-N)	0.40%	0.37%
Bromide	0.1–100	0.9976	7.8	0.56%	0.51%
Nitrate	0.1–100	0.9970	7.7 (1.7 as NO_3-N)	0.66%	0.54%
o-Phosphate	0.1–100	0.9963	20.2 (6.6 as PO_4-N)	0.15 %	0.57%
Sulfate	0.2–200	0.9973	8.2	0.15%	0.59%

[a] Dionex DX-500 system.
[b] MDL = $\sigma^* t_{s,99}$ where $t_{s,99}$ = 3.14 for n = 7.
[c] RSD = Relative Standard Deviation, n = 7.

Table 6. Anion Recoveries for Spiked Water Samples Obtained Using the IonPac AS14 Column

Anion	Drinking Water		Raw Water		Surface Water	
	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)
Fluoride	1	91.5	1	85.1	1	101.0
Chloride	10	94.6	20	84.0	40	83.6
Nitrite	2	103.1	2	92.0	4	100.2
Bromide	2	96.1	2	95.6	2	93.3
Nitrate	5	87.2	5	89.4	10	93.2
o-Phosphate	10	93.8	10	94.2	10	106.4
Sulfate	20	96.1	40	106.6	40	106.1
Anion	**Domestic Wastewater**		**Industrial Wastewater**		**Soil Extract**	
	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)	Amount Added (mg/L)	Recovery (%)
Fluoride	1	90.8	1	90.1	2	101.1
Chloride	20	87.3	20	96.7	5	96.7
Nitrite	2	0.0*	2	98.2	2	89.3
Bromide	2	96.8	2	96.2	2	89.9
Nitrate	5	15.3*	5	95.1	5	92.8
o-Phosphate	20	94.3	20	95.9	20	111.0
Sulfate	40	91.5	40	102.0	20	94.7

*Sample stored for longer than recommended holding time; inappropriate recovery due to microbial action.

The method linearity using the AS14 column was again determined over a seven-point calibration range and the MDLs were calculated according to U.S. EPA Method 300.0.[3] Table 5 shows the concentration ranges investigated, the resulting linear coefficients of determination (r^2), and typical calculated MDLs for each anion. The retention time and peak area precision (expressed as % RSD) were determined from seven replicate injections of the quality control sample. Table 5 also shows typical retention time and peak area precision data that can be obtained for inorganic anions using the IonPac AS14 column with a DX-500 system.

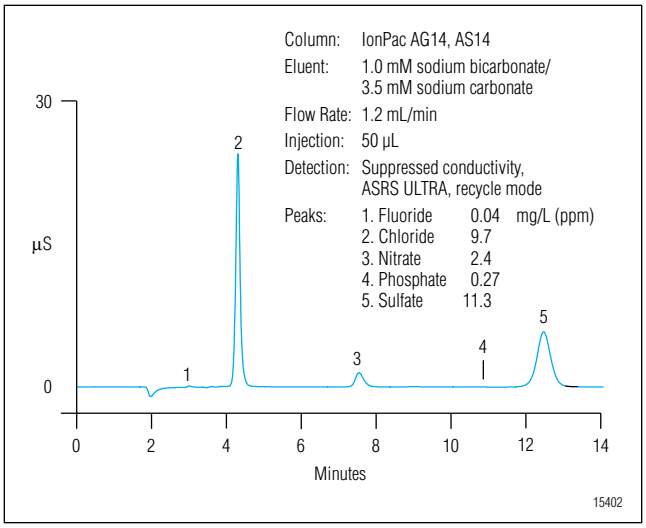


Figure 5. Determination of inorganic anions in drinking water using an IonPac AS14 column.

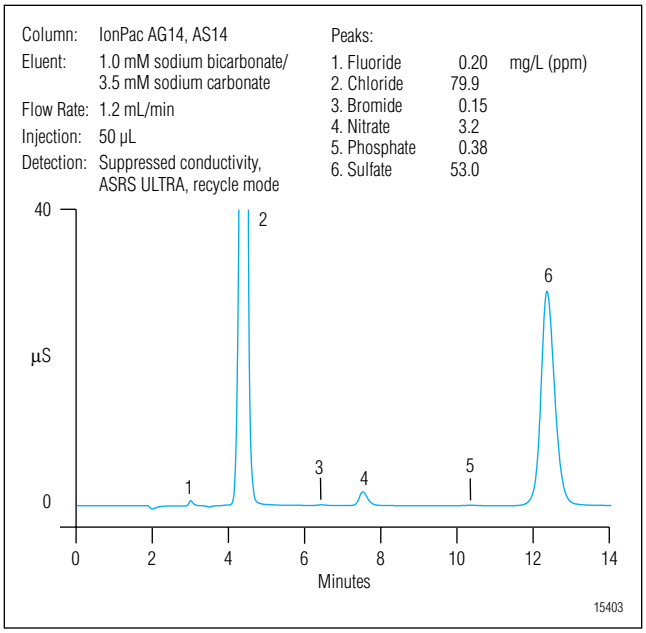


Figure 6. Determination of anions in raw water using an IonPac AS14 column.

The performance of the AS14 method was also validated through single-operator precision and bias studies on spiked samples. Table 6 shows typical recovery results for single-operator data obtained using the IonPac AS14 column for common anions spiked into drinking water, raw drinking water, and other environmental water matrices.

Figure 5 shows a chromatogram of inorganic anions in the same Sunnyvale, California drinking water obtained using the IonPac AS14 column, and Figure 6 shows a chromatogram of inorganic anions in raw water. Table 6 shows that acceptable recovery data (i.e., 80–120%) was obtained for the inorganic anions in all matrices when using the AS14 column, with the exception of nitrite and nitrate in the domestic wastewater sample. However, as discussed previously, this was due to the presence of nitrifying/denitrifying microbes rather than any chromatographic resolution problems.

CONCLUSION

Both the IonPac AS4A-SC and AS14 columns provide suitable performance for the determination of inorganic anions in drinking waters as outlined in U.S. EPA Method 300.0. The AS4A-SC column is recommended for the rapid analysis of anions in low-ionic-strength, well-characterized samples such as drinking, raw, and surface water. The AS14 column provides improved fluoride resolution from the system void peak and complete resolution of fluoride from formate and/or acetate. The improved selectivity, along with higher capacity, makes the AS14 column a better choice for higher-ionic-strength water samples and more complex matrices, such as domestic and industrial wastewaters.

REFERENCES

1. *Fed. Regist.* **1998;** *63* 171.
2. *Fed. Regist.* **1994;** *59* 145.
3. "The Determination of Inorganic Anions in Water by Ion Chromatography"; U.S. Environmental Protection Agency Method 300.0; Cincinnati, Ohio, 1993.
4. Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D., Eds.; Standard Methods for the Examination of Water and Wastewater, 18th ed.; Am. Public Health Assoc.: Washington, DC, 1992.
5. "Standard Test Method for Anions in Water by Chemically Suppressed Ion Chromatography"; Standard D4327-97; American Society for Testing and Materials: West Conshohocken, Pennsylvania, 1999; Vol. 11.01, p 420–427.
6. Jackson, P. E.; Haddad, P. R.; Dilli, S. "The Determination of Nitrate and Nitrite in Cured Meats using High Performance Liquid Chromatography." *J. Chromatogr.,* **1984**, *295*, 471–478.

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 1-800-766-7000, www.fishersci.com.

VWR International, 1310 Goshen park, West Chester, PA 19380 USA, Tel: 800-932-5000, www.vwr.com.

Waters Corporation, 34 Maple Street, Milford, MA 01757 USA, Tel: 1-800-252-4752, www.waters.info.

Determination of Low Concentrations of Perchlorate in Drinking and Groundwaters Using Ion Chromatography

INTRODUCTION

Ammonium perchlorate is a key ingredient in solid rocket propellants. Perchlorate has recently been found in drinking water wells in regions of the U.S. where aerospace material, munitions, or fireworks were developed, tested, or manufactured.[1] Perchlorate poses a human health concern because it can interfere with the thyroid gland's ability to utilize iodine to produce thyroid hormones. Current data suggest that 4 to 18 µg/L (ppb) is an acceptable exposure level.[1] The State of California requires remedial action for drinking water sources containing more than 18 µg/L perchlorate.

The determination of perchlorate at trace levels is a difficult analytical task and ion chromatography represents the only viable means for the quantification of such low concentrations of perchlorate. In 1997, the California Department of Health Services (CDHS) developed an IC method to support the California action level of 18 µg/L.[2] The CDHS method uses an IonPac® AS5 column and an eluent of 120 mM sodium hydroxide containing 2 mM p-cyanophenol, which is added to minimize hydrophobic interactions with the resin.[3] In 1998, Dionex developed an updated method for determining low perchlorate concentrations using an IonPac AS11 column with an eluent of 100 mM sodium hydroxide and suppressed conductivity detection.[4,5]

This application note describes an improved method to quantify low levels of perchlorate. This method uses an IonPac AS16 column to separate perchlorate from the other anions typically found in drinking and groundwaters. The IonPac AS16 is a high-capacity, very hydrophilic, hydroxide-selective column designed for the fast separation of polarizable anions (e.g., thiosulfate, iodide, and perchlorate). Compareö to other anion-exchange columns, the polarizable anions are eluted with higher efficiency and improved peak shape, without the addition of organic solvents. The IonPac AS16 column is the column specified in U.S. EPA Method 314.0, which is the analytical method to be prescribed for the analysis of perchlorate in the assessment phase of the Unregulated Contaminant Monitoring Rule.[6]

Because perchlorate is well separated from other inorganic anions using the IonPac AS16 column at a lower hydroxide eluent concentration than needed for the IonPac AS11, the EG40 Eluent Generator can be used. This application note shows that perchlorate can be quantified at the 2-µg/L level using an IonPac AS16 column, EG40-generated hydroxide eluents, a large-loop injection, and suppressed conductivity detection.

EQUIPMENT

Dionex DX-500 IC system consisting of:
- GP50 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC30 Chromatography Oven
- EG40 Eluent Generator with an EluGen® Hydroxide Cartridge

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

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent-grade, 18 MΩ-cm resistance or better

Sodium perchlorate, 99% ACS reagent-grade or better (Sigma-Aldrich)

ACS reagent-grade sodium salts (Fisher, Sigma-Aldrich, Fluka, EM Science) were used to make standards of other anions for interference studies.

CONDITIONS

Columns:	IonPac AS16 Analytical 4 × 250 mm (P/N 055376) IonPac AG16 Guard 4 × 250 mm (P/N 055377)
Eluent:	65 mM potassium hydroxide
Eluent Source:	EG40
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Sample Volume:	1000 µL
Detection:	Suppressed conductivity, ASRS® ULTRA, 4 mm, AutoSuppression® external water mode; Power setting—300 mA
System Backpressure:	2600 psi
Background Conductance:	1–4 µS
Run Time:	12 min

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Perchlorate Standard Solution

Dissolve 1.4120 g of sodium perchlorate monohydrate in 1000 mL of deionized water to prepare a 1000-mg/L standard solution. This standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Appropriate dilutions of the 1000-mg/L perchlorate standard solution were made for studies of method linearity and the method detection limit (MDL). Method linearity was determined by diluting 2, 10, 20, 50, and 100 µL of the 1000-mg/L perchlorate standard to 1 L to prepare working standard solutions at 2, 10, 20, 50, and 100 µg/L and making two injections of each working standard. Seven injections of the 2-µg/L standard were made for the MDL study.

INTERFERENCE STUDIES

To determine if other anions interfere with perchlorate determinations, 1-mL samples containing 100 ppb of the chosen anion and 20 ppb of perchlorate were injected. Arsenate, arsenite, bromate, bromide, carbonate, chlorate, chloride, chromate, cyanide, humic acid, iodate, iodide, molybdate, nitrate, nitrite, phosphate, phthalate, selenate, sulfate, sulfite, thiocyanate, and thiosulfate were tested as possible interferences.

To ascertain the effect of high levels of common anions on perchlorate recovery, solutions containing 50, 200, 600, and 1000 mg/L carbonate, chloride, or sulfate and 20-µg/L perchlorate were prepared. The effect of sulfate on perchlorate recovery was further investigated by preparing solutions containing 50, 200, 600, and 1000 mg/L sulfate and either 2- or 200-µg/L perchlorate. One-milliliter aliquots of each of these samples were analyzed. To determine the effect of very high chloride concentrations, a sample was prepared that contained 10,000 mg/L chloride and 100 µg/L perchlorate. An aliquot of this sample was treated with an OnGuard® Ag cartridge (P/N 39637) followed by an OnGuard H cartridge (P/N 39596). Prepare the OnGuard cartridges by passing 10 mL of deionized water though them at 2 mL/min. (For details on cartridge preparation, refer to the OnGuard cartridge manual, P/N 032943.) After the cartridges have been prepared, pass 5 mL of the undiluted sample through the cartridge. Discard the first 3 mL and collect the remainder for injection.

SYSTEM PREPARATION AND SETUP

For determinations of target anions at trace concentrations, it is essential to have low baseline noise. To ensure a quiet baseline, the following steps must be taken during the system setup. The ASRS ULTRA is operated in the external water mode rather than the recycle mode. A 1000 psi backpressure coil must be added to the degas module on the eluent generator. Refer to the EG40 manual (P/N 031373) for details on adding backpressure to the degas module. The final system backpressure should be in the range of 2400– 2600 psi when using the EG40 Eluent Generator. Prior to sample analysis, determine a system blank by analyzing 1 mL of deionized water using the method described above. An equilibrated system has a background conductance between 1 and 4 µS with the peak-to-peak noise should not exceed ions 5–10 nS, and no peaks eluting with the same retention time as perchlorate (9.6 ± 0.2 min).

RESULTS AND DISCUSSIONS

Figure 1 shows a chromatogram of a 20-µg/L perchlorate standard. Perchlorate elutes at 9.6 min. The method linearity range was determined to ensure accurate quantification of perchlorate. Figure 2 shows that the method is linear from 2 to 100 µg/L, a concentration range appropriate for this application. This method is also linear in a larger concentration range (2–100 ppm, $r^2 = 0.9999$). The excellent linearity over a wide concentration range is a result of the high capacity for perchlorate and its symmetrical peak shape using the IonPac AS16 column. The method detection limit was established by making seven replicate injections of a 2-µg/L perchlorate standard. Table 1 shows the results of this study. The MDL calculated using the method described in U.S. EPA Method 300.0 is 151.4 ng/L.[7] Figure 3 shows a chromatogram of a 2-µg/L perchlorate standard.

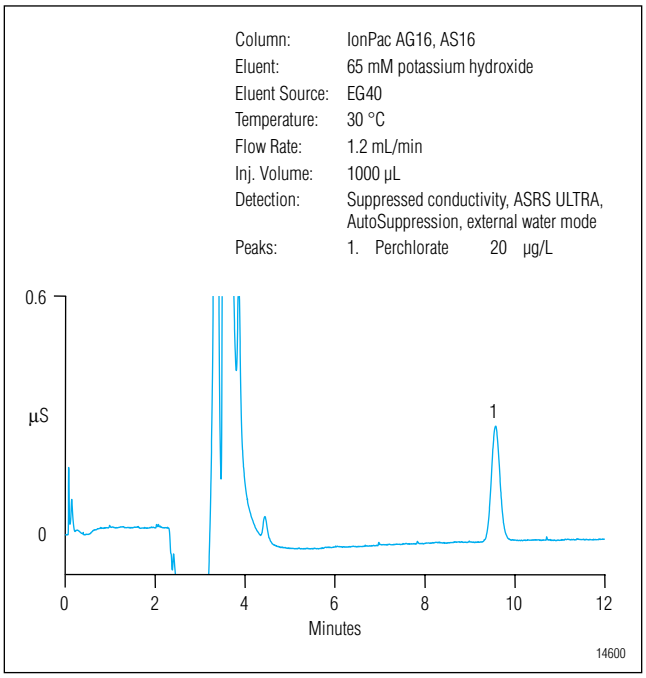


Figure 1. Perchlorate standard at 20 µg/L.

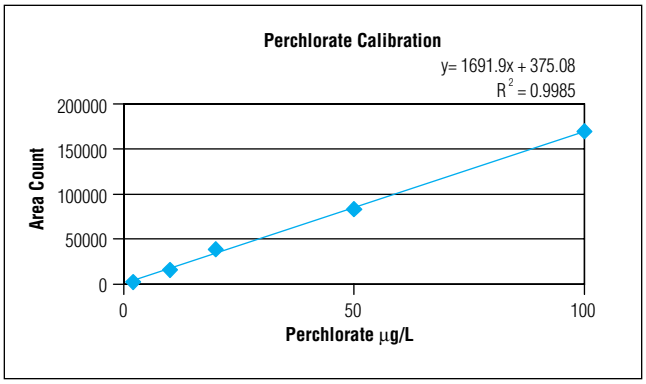


Figure 2. Perchlorate calibration.

Table 1. Determination of the Method Detection Limit for Perchlorate

Injection #	Peak Area	Retention Time (min)
1	2416	9.82
2	2314	9.82
3	2313	9.83
4	2323	9.85
5	2414	9.73
6	2317	9.82
7	2384	9.72
Average	2354	9.80
SD	48.22	0.05
RSD	2.05	0.53
MDL*	151.4 ng/L	

MDL = SD$t_{s,99}$ where $t_{s,99}$ = 3.14 for n = 7

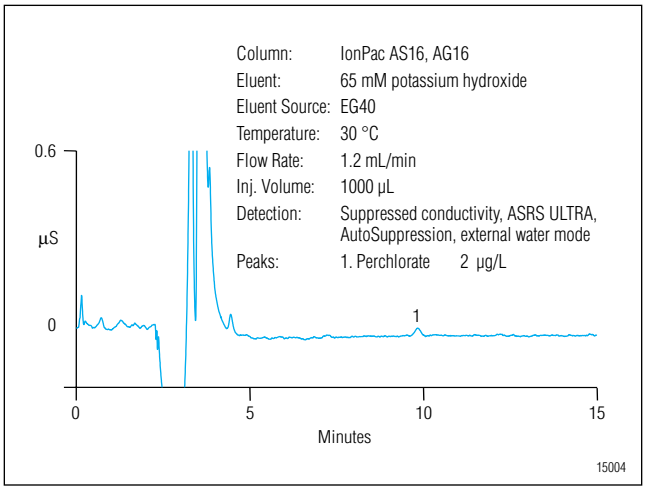


Figure 3. Perchlorate standard at 2 µg/L.

INTERFERENCE STUDIES

Common Anions

Twenty-two anions were injected using the conditions described in this Application Note to study whether they interfere with the determination of perchlorate. Included in these 22 anions were polarizable anions that are typically well retained on anion-exchange columns. The results of this study are shown in Table 2. All 22 anions elute well before perchlorate, most in less than 4 minutes, and therefore do not interfere with the determination of perchlorate.

Groundwater samples may contain high concentrations of common anions, particularly carbonate, chloride, or sulfate. The method outlined in this Application Note can be used to determine low concentrations of perchlorate in the presence of high concentrations of these common anions. The effect of mg/L levels of these anions on perchlorate recovery was investigated by injecting solutions of 20µg/L perchlorate in the presence of 50, 200, 600, and 1000 mg/L carbonate, chloride, or sulfate. Quantitative recoveries were obtained for perchlorate at the 20-µg/L level in all cases, as shown in Table 3.

Because sulfate is the most likely interference in groundwaters, the effect of sulfate on perchlorate recovery was further investigated. Perchlorate (200 µg/L) was determined in the presence of 50-, 200, 600, and 1000 mg/L sulfate. The recovery of perchlorate from these samples was 78, 89, 77, and 90%, respectively. The same study was also done with 2 µg/L perchlorate. For that experiment the recoveries were 115, 107, 109, and 110%, respectively. Figure 4 shows an overlay of chromatograms of 200 µg/L perchlorate in the presence of 50 to 1000 mg/L sulfate, demonstrating that high concentrations of sulfate do not significantly affect the retention time or peak shape for perchlorate.

Table 2. Comparison of the Retention Times of 22 Anions and Perchlorate on the IonPac AS16 Column (1000 µL injected)*

Anion	Anion Retention Time (min)	Perchlorate Retention Time (min)
Arsenate	<4	9.78
Arsenite	<4	9.75
Bromate	<4	9.72
Bromide	<4	9.73
Carbonate	<4	9.72
Chlorate	<4	9.72
Chloride	<4	9.68
Chromate	<4	9.68
Cyanide	<4	9.65
Humic acid	<4	9.67
Iodate	<4	9.65
Iodide	5.28	9.65
Molybdate	<4	9.63
Nitrate	<4	9.65
Nitrite	<4	9.63
Phosphate	<4	9.63
Phthalate	<4	9.62
Selenate	<4	9.60
Sulfate	<4	9.60
Sulfite	<4	9.60
Thiocyanate	7.72	9.60
Thiosulfate	<4	9.58

* An eluent of 50 mM hydroxide at 1.5 mL/min was used for this study.

Table 3. Effect of mg/L Levels of Common Anions on Perchlorate Recovery (20 µg/L) on the IonPac AS16 Column (1000 µL injected)*

Anion	Anion Concentration (mg/L)	Perchlorate Recovery
Carbonate	50	97.6%
Carbonate	200	94.4%
Carbonate	600	95.4%
Carbonate	1000	93.5%
Chloride	50	96.1%
Chloride	200	96.7%
Chloride	600	109.6%
Chloride	1000	97.4%
Sulfate	50	94.4%
Sulfate	200	96.3%
Sulfate	600	94.7%
Sulfate	1000	95.5%

* An eluent of 50 mM hydroxide at 1.5 mL/min was used for this study.

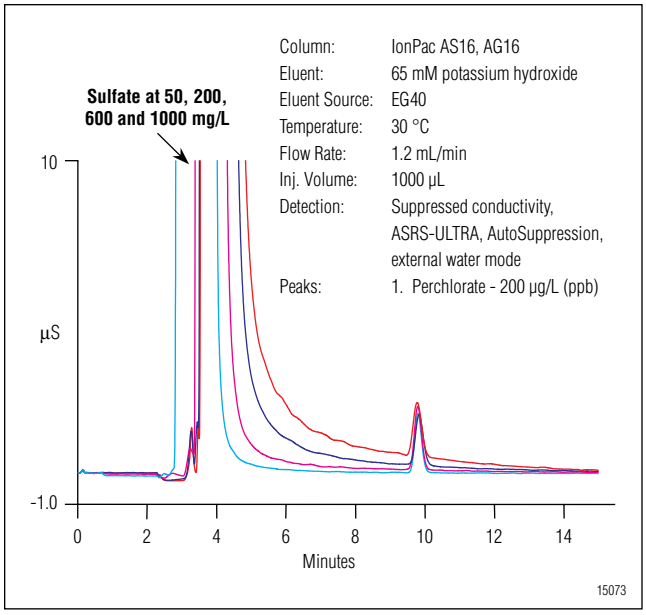


Figure 4. Effect of sulfate on perchlorate recovery on the IonPac AS16 column.

Extreme Chloride Matrices

Low concentrations of perchlorate are sometimes found in matrices containing a very high chloride concentration (e.g., brines). The sample used for this study had a chloride concentration of 10,000 mg/L and a perchlorate concentration of 100 µg/L. One approach for determining perchlorate in an extreme chloride matrix is to reduce the sample's chloride concentration. This can be achieved by pretreating the sample with an OnGuard Ag cartridge. The cartridge packing is a high- capacity, strong acid, cation-exchange resin in the Ag^+ form that is designed to remove chloride from the sample by precipitating it as silver chloride. Figure 5B shows an analysis of the sample (10,000 mg/L chloride and 100 µg/L perchlorate) after treatment with an OnGuard Ag cartridge. This treatment allows perchlorate to be quantified with good recovery (92.6%). Analysis of the untreated sample is shown in Figure 5A.

Another approach for determining perchlorate in an extreme chloride matrix is to dilute the sample and/or reduce the eluent concentration. The same sample containing chloride at 10,000 mg/L and perchlorate at 100 µg/L was diluted 10-fold and the diluted solution was

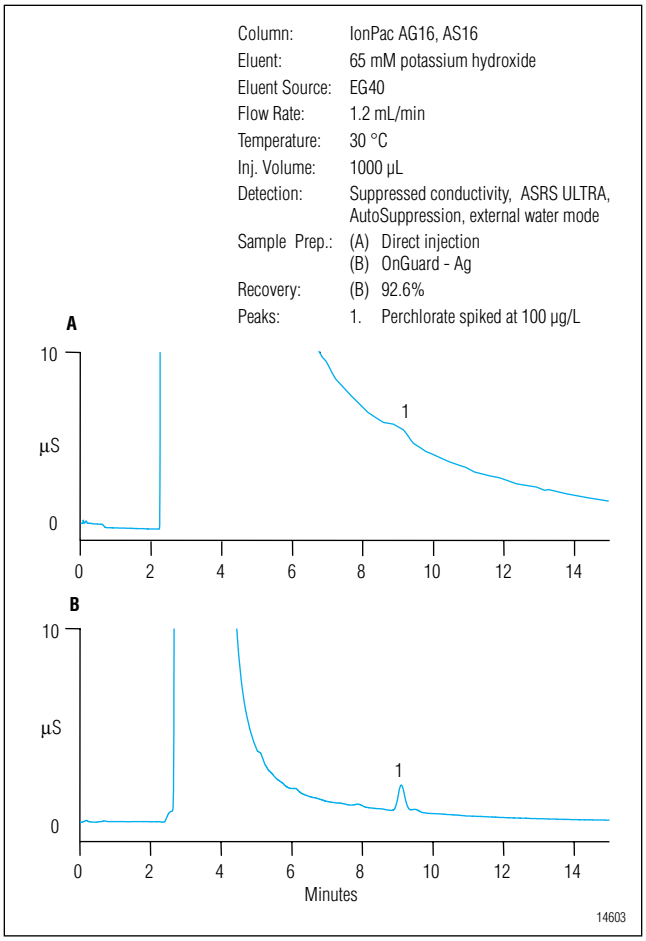


Figure 5. Determination of perchlorate in high chloride (10,000 mg/L) matrices.

analyzed using two different eluent strengths, 35 mM and 65 mM KOH. Figure 6A shows that when using 65 mM KOH, perchlorate is difficult to quantify because it elutes on the tail of the large chloride peak. When the weaker eluent (35 mM KOH) is used, perchlorate elutes at 14 min and is easier to quantify (Figure 6B).

When choosing an approach for analyzing perchlorate in samples containing high concentrations of chloride, the perchlorate concentration must be considered. For a sample containing low levels of perchlorate (<40 µg/L), use the OnGuard Ag cartridge. In samples where the concentration of perchlorate is higher, sample dilution and a 35 mM KOH eluent is recommended.

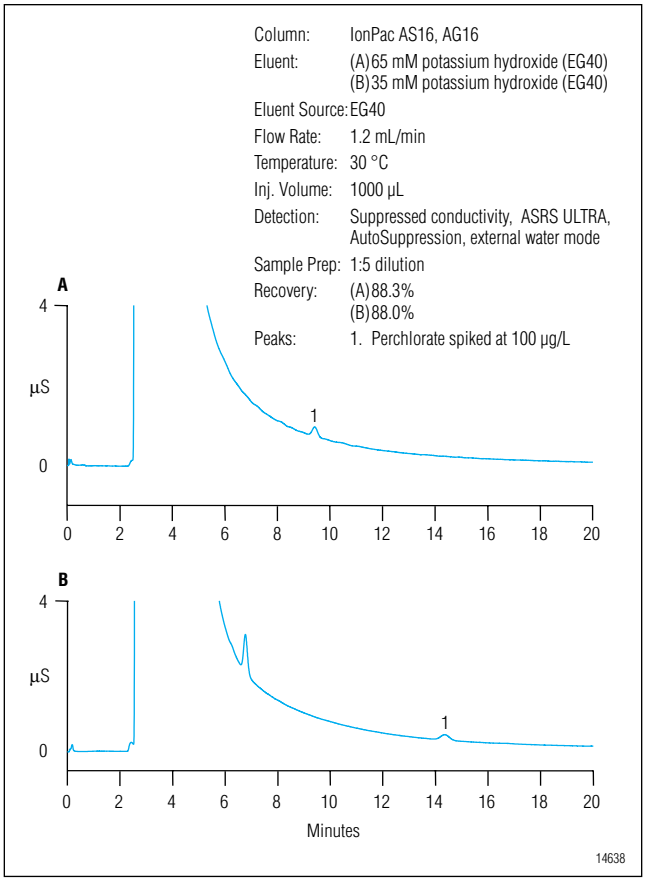


Figure 6. Determination of perchlorate in high chloride (10,000 mg/L) matrices.

CONCLUSION

The method described in this application note can be used to determine low-µg/L concentrations of perchlorate in drinking and ground waters. The use of IC with the AS5 or AS11 columns has previously been shown to provide an interference-free method for the analysis of perchlorate in modest-ionic-strength drinking water and groundwater samples[8]; the AS16 column provides similar results. The AS16 also is compatible with the EG40 and its higher capacity makes it most appropriate for the analysis of perchlorate in higher- ionic-strength samples.

SUPPLIERS

EM Science, P.O. Box 70, 480 South Democrat Road, Gibbstown, NJ 08027 USA, Tel: 800-222-0342, www.emscience.com.

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

Fluka Chemika-BioChemika, Fluka Chemie AG, Industriestrasse 25, CH-9471, Buchs, Switzerland, Tel: +81 755 25 11, www.sigma-aldrich.com.

Sigma-Aldrich Chemical Company, P.O. Box 14508, St. Louis, MO 63178 USA, Tel: 1-800-325-3010, www.sigmaaldrich.com.

REFERENCES

1. "Perchlorate in California Drinking Water" Update; California Department of Health Services, September 1999.
2. California Department of Health Services, Determination of Perchlorate by Ion Chromatography, June, 1997.
3. Okamoto, H. S., Rishi, D. K., Steeber, W. R., Baumann, F. J., and Perera, S. K. *J. of American Water Works Assoc*. **1999** *91(10),* 73–84.
4. Dionex Corporation. "Analysis of Low Concentrations of Perchlorate in Drinking Water and Ground Water by Ion Chromatography", Application Note 121; Sunnyvale CA.
5. Jackson, P. E., Laikhtman, M., and Rohrer, J. S. *J. of Chromatography* A **1999** *850,* 131–135.
6. Federal Register, September 17, 1999, Vol. 64, No. 180, 50555–50620.
7. U.S. EPA Method 300.0. "The Determination of Inorganic Anions in Water by Ion Chromatography"; August 1993; U. S. Environmental Protection Agency.
8. Chaudhuri, S., Okamoto, H. S., Pia, S. and Tsui, D. Inter-Agency Perchlorate Steering Committee Analytical Subcommittee Report 1999.

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.

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.

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]

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.

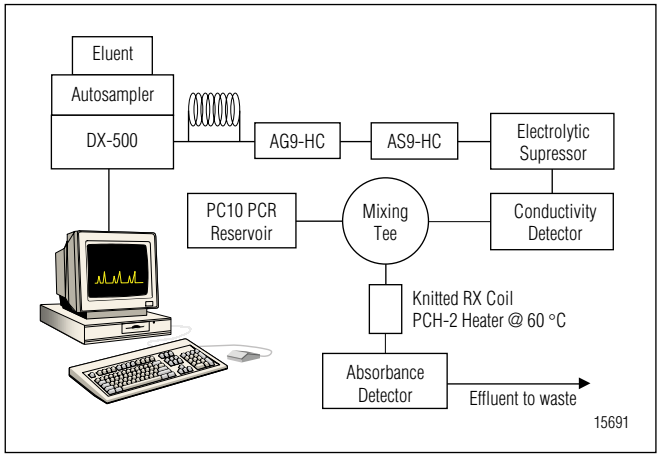


Figure 1. IC system configuration for EPA Method 317.0.

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.

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.

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).

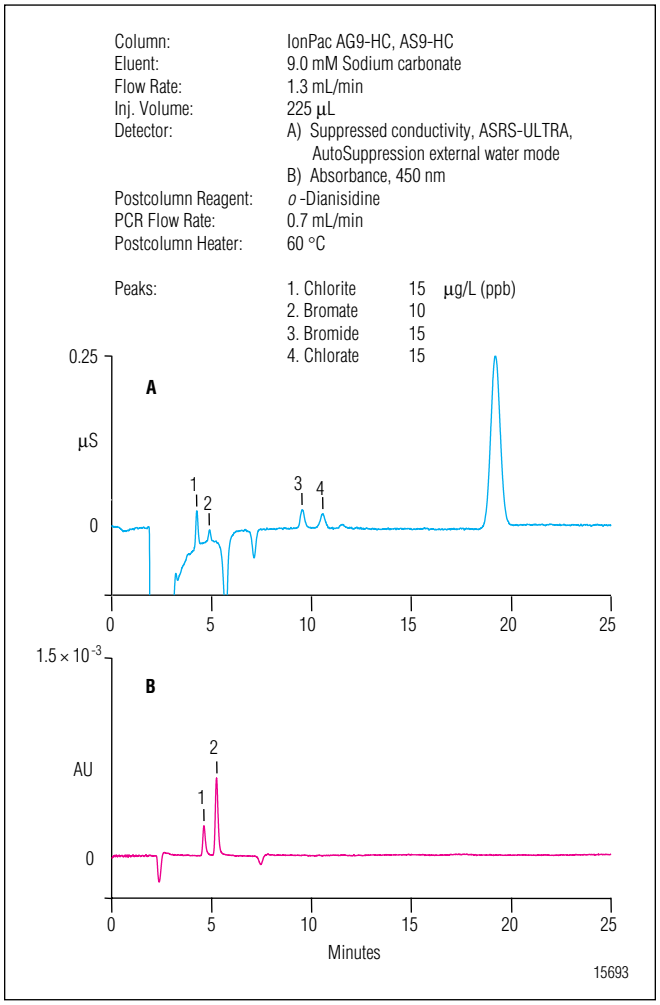


Figure 2. Separation of a low-ppb DBP anion standard using an IonPac AS9-HC column: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o-dianisidine.

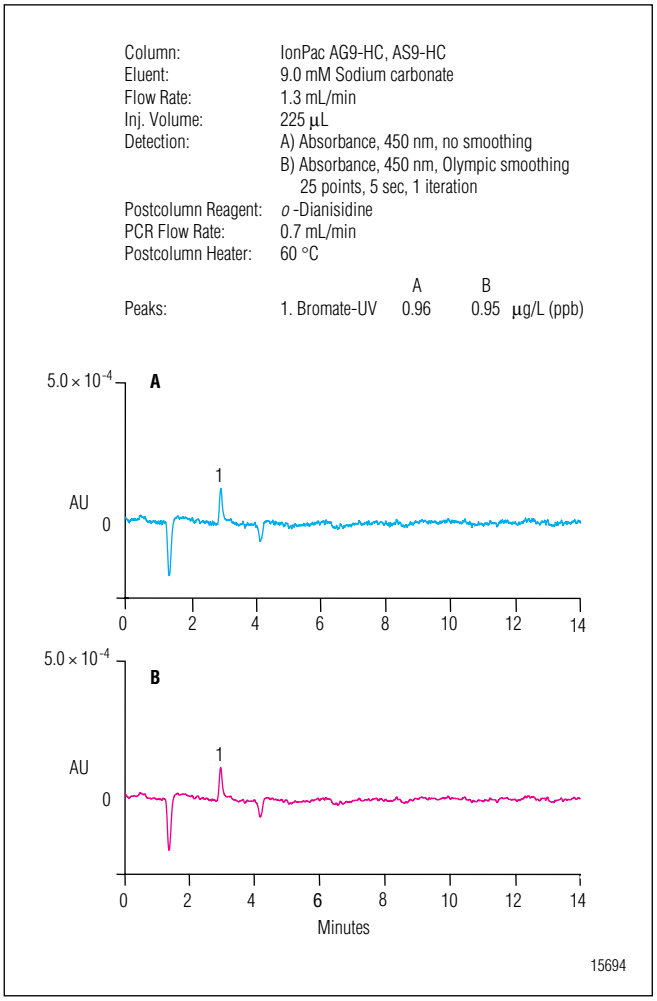


Figure 3. Effect of smoothing on bromate determination: A) unsmoothed data and B) smoothed data (Olympic, 25 points, 5 sec, 1 iteration).

Table 2 Linear Ranges and MDLs for Oxyhalides and Bromide

Solute	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.

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 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

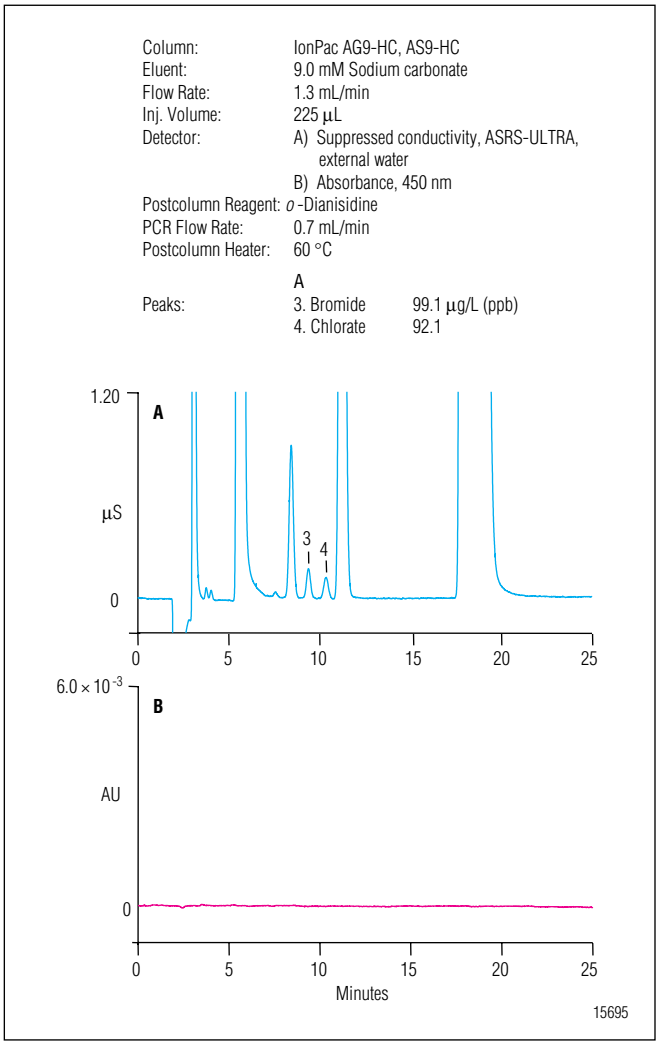


Figure 4. Determination of DBP anions in tap water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

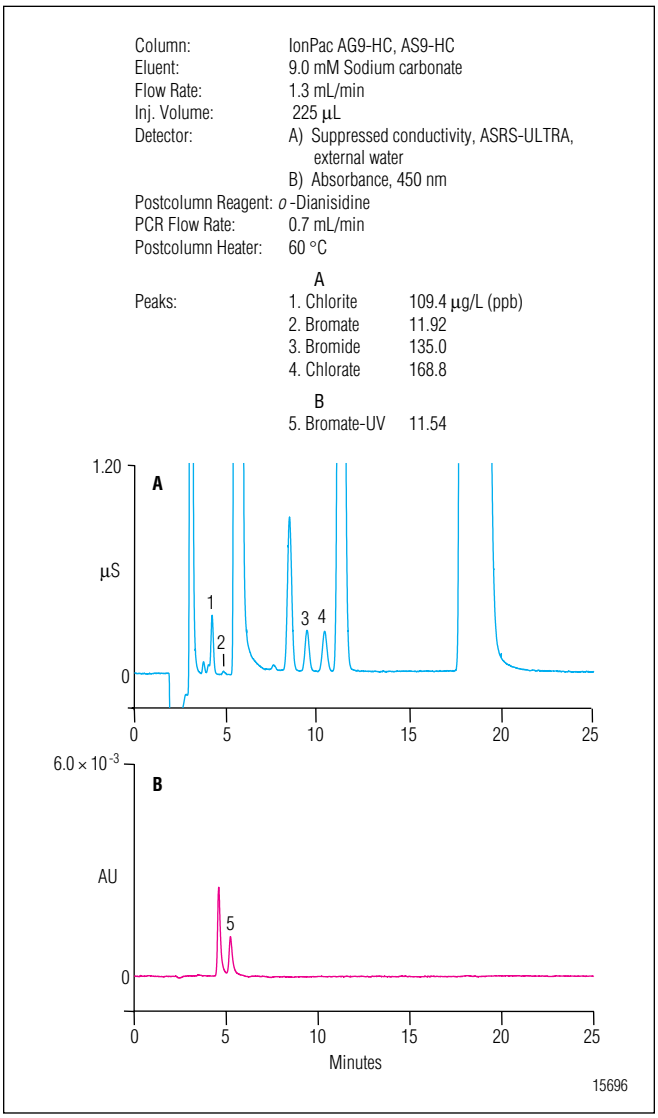


Figure 5. Determination of DBP anions in spiked tap water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

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.

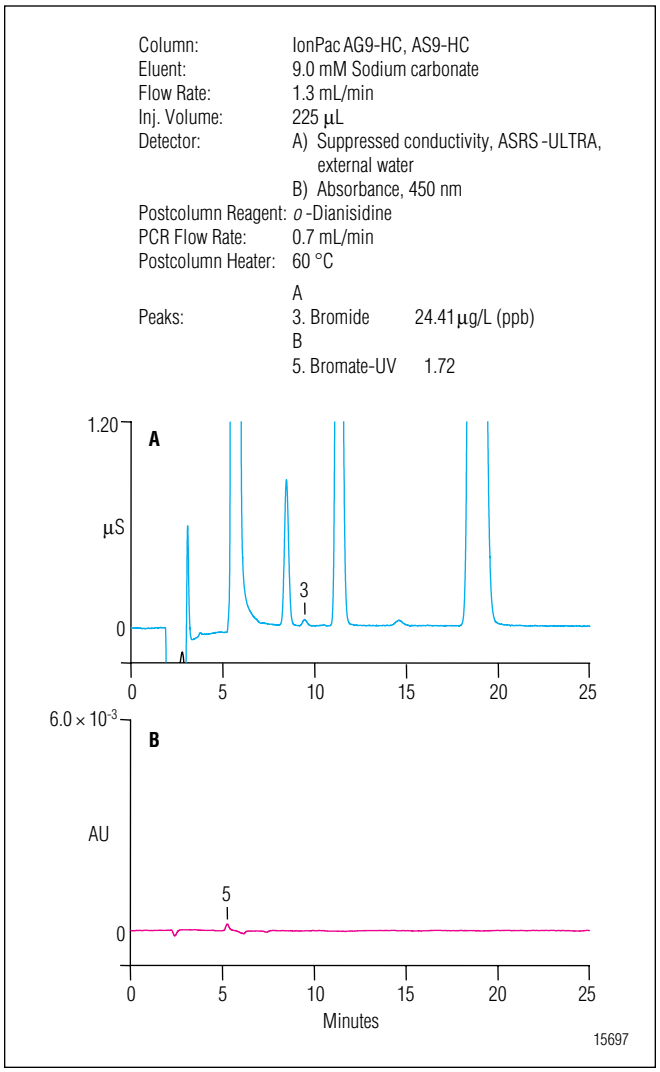


Figure 6. Determination of DBP anions in bottled water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

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

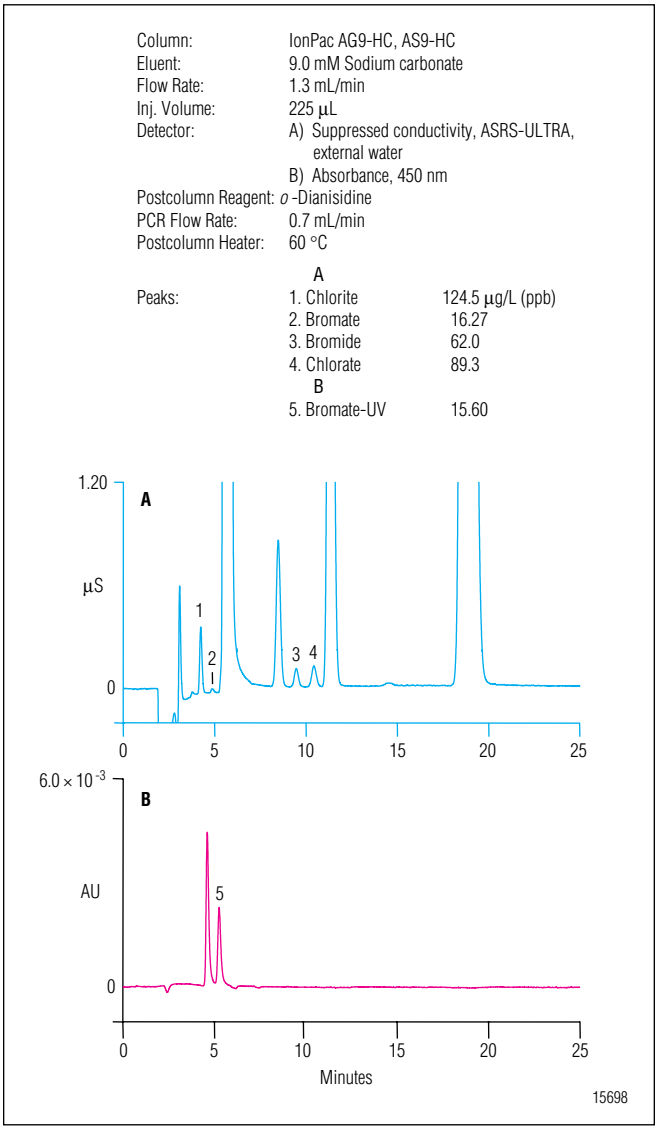


Figure 7. Determination of DBP anions in spiked bottled water: A) suppressed conductivity detection and B) visible absorbance detection after PCR with o*-dianisidine.*

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

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.

REFERENCES

1. Joyce, R.J.; Dhillon, H.S. *J. Chromatogr. A* **1994,** *671,* 165.
2. Wagner, H.P.; Pepich, B.V.; Hautman, D.P.; Munch, D.J. *J. Chromatogr. A* **1999,** *850,* 119.
3. Kruithof, J.C.; Meijers, R.T. *Water Supply* **1995,** *13,* 117.
4. *Fed. Regist.,* **1994**, 59 (145), 38709.
5. *Fed. Regist.,* **1998,** 63 (241), 69389.
6. Jackson, L.K.; Joyce, R.J.; Laikhtman, M.; Jackson, P.E. *J. Chromatogr. A* **1998,** *829,* 187.
7. *U.S. EPA Method 300.0,* U.S. Environmental Protection Agency, Cincinnati, OH, 1993.
8. *U.S. EPA Method 300.1,* U.S. Environmental Protection Agency, Cincinnati, OH, 1997.
9. Walters, B.D.; Gordon, G.; Bubnis, *B. Anal. Chem.* **1997,** *69,* 4275.
10. Weinberg, H.S.; Yamada, H. *Anal. Chem.* **1998,** *70,* 1.
11. Nowack, B.; Von Gunten, U. *J. Chromatogr. A* **1999,** *849,* 209.
12. Achilli, M.; Romele, L. *J. Chromatogr. A* **1999**, *847,* 271.
13. Charles, L.; Pepin, D. *Anal. Chem.* **1998,** *70,* 353.
14. Creed, J.T.; Magnuson, M.L.; Brockhoff, C.A. *Environ. Sci. Technol.* **1997,** *31,* 2059.
15. *U.S. EPA Method 317.0,* U.S. Environmental Protection Agency, Cincinnati, OH, 2000.
16. *Method 4500-ClO_2.C;* Greenberg, A.E.; Clesceri, L.S.; Eaton, A.D. (Eds.); Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA: Washington, DC, 1992.
17. Hautman, D.P.; Bolyard, M. *J. Chromatogr.* **1992,** *602,* 65.

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.

Fast Analysis of Anions in Drinking Water by Ion Chromatography

INTRODUCTION

The U.S. National Primary Drinking Water Standards specify a Maximum Contaminant Level (MCL) for a number of inorganic anions, including fluoride, nitrite, and nitrate. The MCLs are specified to minimize potential health effects arising from ingestion of these anions in drinking water. Other common anions, such as chloride and sulfate, are considered secondary contaminants and guidelines exist regarding taste, odor, color, and certain aesthetic effects. U.S. EPA Method 300.0[1] describes the use of ion chromatography (IC) with a Dionex IonPac® AS4A anion exchange column, a carbonate/bicarbonate eluent, and suppressed conductivity detection for the determination of these inorganic anions in environmental waters, such as drinking water, wastewater, and aqueous soil extracts. The scope of the method allows optional columns and suppression devices to be used provided that comparable resolution of peaks is obtained and the method quality control requirements can be met.

In this paper, we describe the use of the IonPac AS14A anion exchange column[2] with a new Atlas™ Electrolytic Suppressor (AES™)[3] for the routine high-throughput determination of common inorganic anions in drinking water matrices. The IonPac AS14A provides greater speed and efficiency, ruggedness equivalent to the AS4A-SC column, improved separation of fluoride from the void volume (water dip), and better overall separation selectivity. The AES is a continuously electrolytically regenerated suppressor based on the MonoDisc™ suppression technology. The Atlas electrolytic suppressor offers lower baseline noise and improved ruggedness and reliability. The analytical throughput, potential interferences, linear range, method detection limits, system stability, and analyte recoveries obtained using the AS14A column with the Atlas suppressor for drinking water are described in this Application Note.

EQUIPMENT

Dionex DX-600 ion chromatography system configured for Atlas anion suppression consisting of:

- GS50 or GP50 Gradient Pump
- ED50A Electrochemical Detector or CD25A Conductivity Detector
- AS40 Automated Sampler with 0.5-mL sample vials
- LC30 Chromatography Oven with a rear-loading valve

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent grade, 18 MΩ-cm resistance or better

AS14A Eluent Concentrate (100X), P/N 056937

All anion standards were 99% ACS reagent grade or better:

- Sodium fluoride, CAS 7681-49-4 (Fisher Scientific or other)
- Sodium chloride, CAS 7647-14-5 (J.T. Baker or other)
- Sodium nitrite, CAS 5347-50-0 (Fisher Scientific or other)
- Sodium bromide, CAS 7647-15-6 (EM Sciences or other)
- Sodium nitrate, CAS 7631-99-4 (Aldrich Chemical Company or other)
- Potassium phosphate, monobasic, anhydrous, CAS 7778-77-0 (Sigma Chemical Company or other)
- Sodium sulfate, anhydrous, CAS 7757-82-6 (EM Sciences or other)

CONDITIONS

Columns:	IonPac AS14A Analytical, 3 x 150 mm, 5-µm particle diameter (P/N 056901)
	IonPac AG14A Guard, 3 x 30 mm, 5-µm particle diameter (P/N 056899)
Eluent:	8.0 mM Sodium carbonate and 1.0 mM sodium bicarbonate
Temperature:	30 ˚C
Run Time:	6 min
Flow Rate:	0.8 mL/min
Sample Volume:	25 µL
Detection:	Suppressed conductivity, Atlas AAES™ (P/N 056116), recycle mode, 45 mA
System Backpressure:	2610–2890 psi (18–20 MPa)
Background Conductance:	23–25 µS

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solution

Starting Anion Standard Solution (10,000 mg/L)

Sodium fluoride—Formula weight = 42.00, anionic mass percent = 45.26%. Dissolve 2.209 g of sodium fluoride solid in 100 mL deionized water.

Sodium chloride—Formula weight = 58.45, anionic mass percent = 60.67%. Dissolve 1.648 g of sodium chloride solid in 100 mL deionized water.

Sodium nitrite—Formula weight = 69.00, anionic mass percent = 66.68%. Dissolve 1.500 g of sodium nitrite solid in 100 mL deionized water.

Sodium bromide—Formula weight = 102.91, anionic mass percent = 77.66%. Dissolve 1.288 g of sodium bromide solid in 100 mL deionized water.

Sodium nitrate—Formula weight = 85.01, anionic mass percent = 72.96%. Dissolve 1.371 g of sodium nitrate solid in 100 mL deionized water.

Potassium phosphate (monobasic)—Formula weight = 136.09, anionic mass percent = 71.27%. Dissolve 1.403 g of potassium phosphate solid in 100 mL deionized water.

Sodium sulfate—Formula weight = 142.06, anionic mass percent = 83.82%. Dissolve 1.193 g of sodium sulfate solid in 100 mL deionized water.

Stock Anion Standard Solution (1000 mg/L)

Dilute each 10,000 mg/L starting anion solution 10-fold in deionized water.

Working Standard Solutions

Dilute 1000 mg/L stock anion standard solutions together as required with deionized water to prepare the appropriate working standard mixtures. The five levels of working standards used in this study for calibration and quality checks are presented in Table 1. These concentration ranges were chosen to bracket the concentrations typical for drinking water samples. The intermediate standard (level 3) was used as a quality check and to evaluate long-term response stability.

LABORATORY FORTIFIED BLANK (LFB) AND MATRIX (LFM)

Dilute 1000 mg/L standard solutions together as required with deionized water to prepare 20X fortification concentrate (see Table 1). The 20X fortification concentrate (5.0 mL) was diluted in deionized labora-

tory water (95.0 mL) to produce the LFB, and 5.0 mL was added to 95.0 mL of Sunnyvale, California drinking water to make the LFM. The concentrations of LFB and LFM are given in Table 1. The LFB and LFM are used to calculate the spike recovery of anions from deionized water and drinking water.

Table 1 Anion Standards and Controls

	Anion Standards,* mg/L (ppm) Levels					LFB*	LFM**
	1	2	3	4	5	DI Water Blank Fortification (ppm)	Matrix Fortification (ppm)
Fluoride	0.1	0.5	2.5	5	10	1	1
Chloride	0.5	5	25	50	100	10	10
Nitrite	0.1	1	5	10	20	2	2
Bromide	0.1	1	5	10	20	2	2
Nitrate	0.1	1	5	10	20	5	5
Phosphate	0.2	1.5	7.5	15	30	10	10
Sulfate	0.5	5	25	50	100	20	20

25-µL Injections
LFB = Laboratory fortified blank
LFM = Laboratory fortified matrix
* Anion standards and LFBs were prepared in laboratory water.
** LFMs were prepared in tap water collected from Sunnyvale, California.

Eluent Solution

8.0 mM Sodium Carbonate/1.0 mM Sodium Bicarbonate

Weigh 1980 g deionized water into an eluent bottle. Degas water for approximately 20 min. Carefully add 20.0 mL of the AS14A Eluent Concentrate (100X) to the degassed water. Mix and then quickly transfer the eluent bottle to the instrument and pressurize the bottle with helium at 8 psi (0.055 MPa). For experiments involving stability studies longer than 1 day, the eluent was prepared as described above but added to the previous eluent.

RESULTS AND DISCUSSION

The use of the IonPac AS14A (3 mm) column at 0.8 mL/min can reduce the run time to 6 minutes (Figure 1) from the usual 10 min for the 0.5 mL/min flow rates. The run times for other IonPac columns commonly used for drinking water analysis are presented in Table 2. Actual run times are typically longer because additional time is needed to fill the injection loop of the autosampler prior to injection and to download data. This additional time varies with the autosampler used. The run times can be reduced by several means: increased eluent strength, increased temperature, and increased flow rate. The AS4A operated at 2 mL/min can reduce the run times to 9 min, but cannot completely resolve the fluoride peak from the water dip. The AS14 column was designed to elute fluoride away from the water dip, but the run times are longer. The AS14A (3 mm) column was designed to elute fluoride from the water dip and also reduce the run times. The performance of this column is maintained

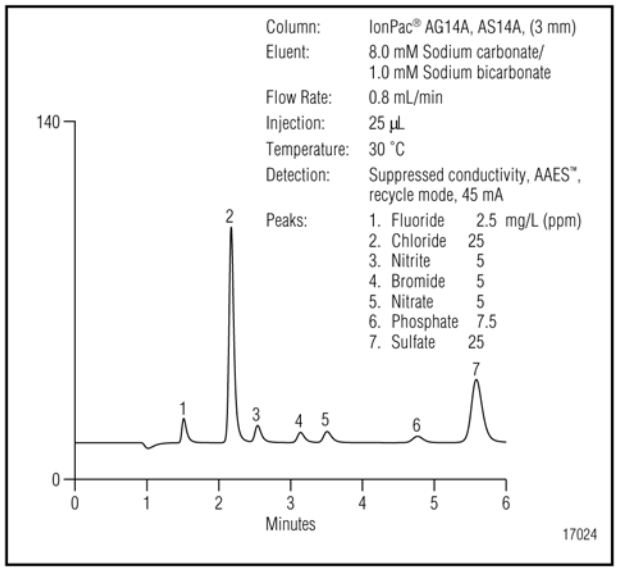


Figure 1. Seven common anion standards using the AS14A (3 mm) at 0.8 mL/min with the AAES suppressor.

Table 2 Comparison of Run Times and Throughput for Dionex IonPac Columns Recommended for Drinking Water Analysis

Column Set	Flow Rate (mL/min)	Sodium Carbonate/ Sodium Bicarbonate Eluent Concentration (mM)	Run Time (min)*	Actual Run Time (min)**	No. of Inject. per 24 h
AS4A/ AG4A (4 mm)	2.0	1.8 mM/1.7 mM	9	10.1	143
AS14/ AG14 (4 mm)	1.2	3.5 mM/1.0 mM	14	15.1	95
	1.2	4.8 mM/0.6 mM	11	12.1	119
	1.5	4.8 mM/0.6 mM	9	10.1	143
	2.0	4.8 mM/0.6 mM	7	8.1	178
AS14A/ AG14A (3mm)	0.5	8.0 mM/1.0 mM	10	11.1	130
	0.8	8.0 mM/1.0 mM	6	7.1	203

* Time from injection to end of shoulder of last peak (sulfate) plus ~1 min
** Actual time per injection, which includes AS40 autosampler loading, injection, and data transfer.

even at higher flow rates, making it well suited for high-throughput water analysis. Using the AS40 autosampler, each injection was calculated to take 7.1 minutes of actual time, and therefore 203 injections were possible over 24 h, surpassing the throughput of any other IonPac column (see Table 2).

The Atlas suppressor is appropriate for this application. Although the Atlas has lower capacity than the ASRS® and AMMS® membrane-based suppressors, the relatively low ionic strength of the carbonate eluent used in this application is well within its operating range. The advantage of Atlas suppression is that baseline noise can be minimized relative to the membrane suppression. In the Recycle mode, the Atlas suppressor provides both low noise and the convenience of long-term maintenance-free operation. An equilibrated system will produce peak-to-peak noise between 0.50–3.5 nS for this application. Figure 2 compares baseline noise between ASRS-ULTRA and Atlas suppressors. The magnitude of the baseline noise can be further reduced by using the External Water mode of suppression as in membrane-based suppressors. All results presented in this Note used the Recycle mode. Both the ASRS-ULTRA and the Atlas suppressor can be used for this high throughput application using the AS14A at higher flow rates, but the Atlas generally produces lower noise and thus lower detection limits.

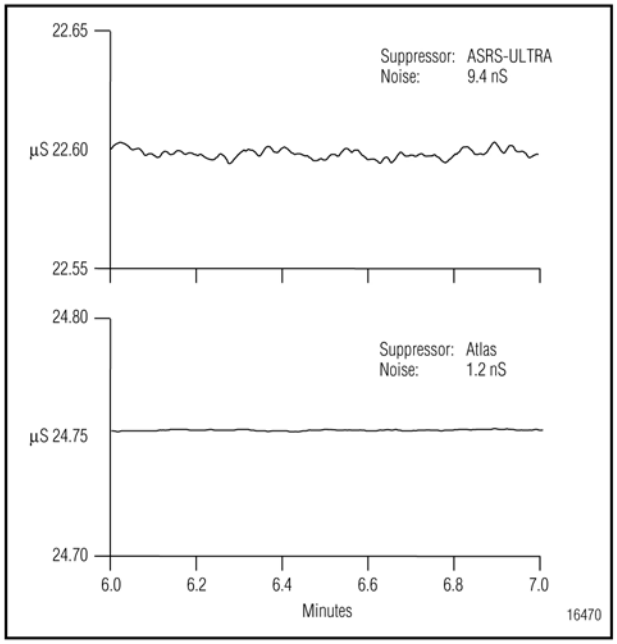


Figure 2. Baseline noise for ASRS and Atlas suppressors using the IonPac AS14A (3 mm) at 0.8 mL/min (8 mM Sodium carbonate and 1 mM sodium bicarbonate eluent).

Because the Atlas suppressor produces lower noise, the limits of detection (LOD) and quantitation (LOQ) are reduced. Table 3 compares the estimated LODs and LOQs of seven anions for both ASRS-ULTRA and Atlas suppressors. The LOD is defined in this Note as the corresponding concentration of each respective standard anion peak height that is equivalent to three times the average of 40 one-minute interval peak-to-peak noise measure. The LOQ is ten times this average. The LOD for the Atlas ranged from 0.8–10 ppb, and from 6–74 ppb for the ASRS-ULTRA. In this study, the Atlas had about a seven times lower detection limit for each anion. Noise values will vary with each suppressor and will affect this comparison. In general, Atlas suppressors should yield lower detection limits than ASRS suppressors.

Table 3 Lower Limits of Detection and Quantification Using the AS14A (3 mm) at 0.8 mL/min with ASRS and Atlas Suppression

	Lower Limits of Detection (LOD)* (ppb)		Lower Limits of Quantification (LOQ)** (ppb)	
	ASRS	Atlas	ASRS	Atlas
Fluoride	6	0.8	20	3
Chloride	7	1	24	3
Nitrite	20	3	68	9
Bromide	29	4	95	13
Nitrate	24	4	82	12
Phosphate	74	10	245	32
Sulfate	25	3	83	10

* LOD based on 3 times the peak-to-peak noise.
** LOQ based on 10 times the peak-to-peak noise.

The lower detection limits using Atlas suppression permit the detection of trace anions in drinking water. Figure 3 shows the separation of anions in Sunnyvale, CA drinking water using the AS14A (3 mm) column at 0.8 mL/min with Atlas suppression. Bromide was measured at a concentration of 20 ppb in this drinking water and the LOD for this ion was 4 ppb, which is above the measured concentration of this drinking water sample based on the Atlas results. The measure of bromide at this concentration is often difficult to achieve by other methods.

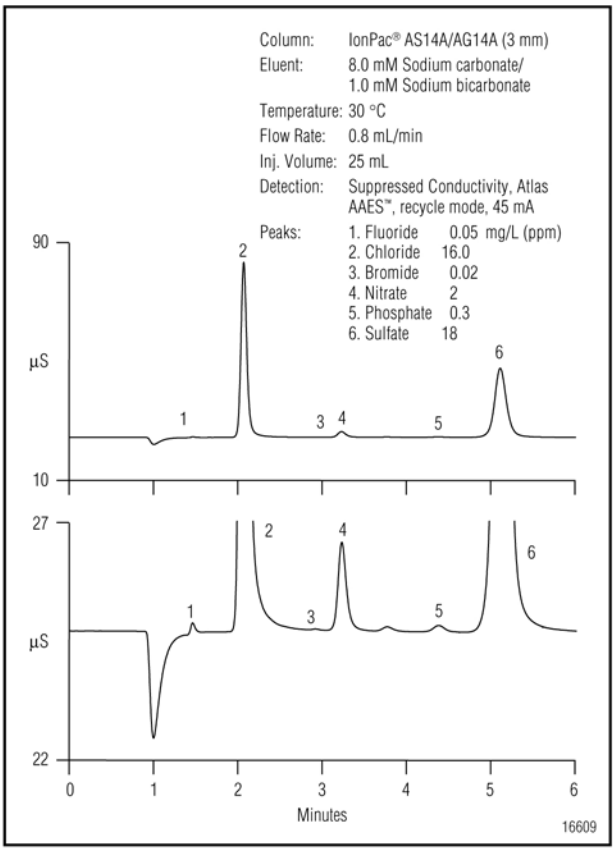


Figure 3. Anions in Sunnyvale, CA drinking water in August separated using the IonPac® AS14A (3 mm) at 0.8 mL/min.

Peak area precision generally improves at higher concentrations. Sunnyvale drinking water was analyzed sequentially for over 6 days in groups of 20 injections interspersed with duplicate quality control samples (blank, level 3 standard, LFB, LFM). The measured anion concentrations for the 940 injections of drinking water sample were plotted with the calculated RSDs (Figures 4 and 5). Chloride (17 ppm) and sulfate (19 ppm) concentrations were measured with high precision (1.2 and 1.1% RSD, respectively). At low to trace level concentrations, high stability was also observed. Nitrate (1.9 ppm) was 1.2% RSD, phosphate (0.33 ppm) was 4.9% RSD, fluoride (0.047 ppm) was 5.5%, and bromide (0.021 ppm) was 14% RSD. This measured concentration of bromide (21 ppb) was only slightly higher than the lower limit of quantification (13 ppb) for this anion using the Atlas (Table 3), thus the precision was poor. When ASRS-ULTRA suppression is used under these conditions, the precision decreases for anions close in concentration to the LOD (Table 4).

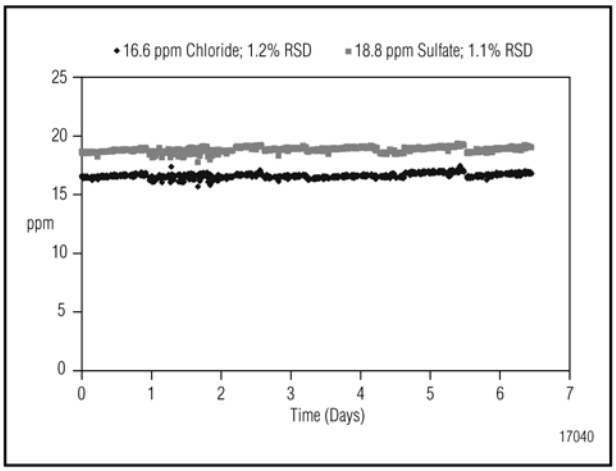


Figure 4. Stability of high level anions in Sunnyvale, CA drinking water using the AS14A (3 mm) at 0.8 mL/min with the Atlas suppressor.

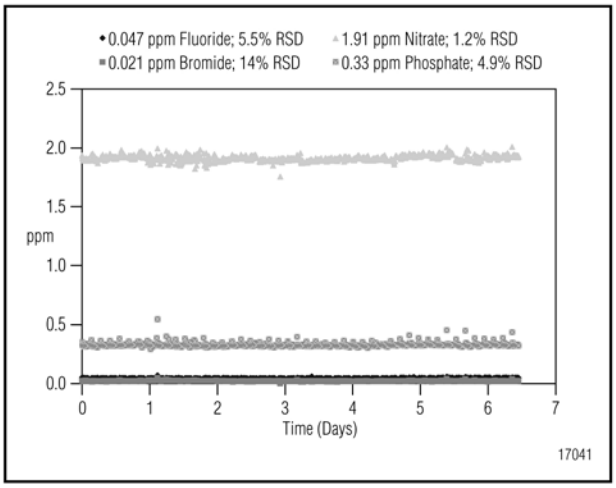


Figure 5. Stability of low level anions in Sunnyvale, CA drinking water using the AS14A (3 mm) at 0.8 mL/min with the Atlas suppressor.

Table 4 Peak Area Precision for the Same Sunnyvale Water Sample Over 3 Days Using ASRS and Atlas Suppressors

	Measured Concentration (ppm)	RSD	
		ASRS %	Atlas %
Fluoride	0.05	21.0	5.1
Chloride	17	1.0	1.1
Nitrite	< 0.003	ND	ND
Bromide	0.02	ND	14.0
Nitrate	2	1.2	1.2
Phosphate	0.3	7.3	5.0
Sulfate	19	0.9	1.1

ND = Not Detected (< 50% of the injections below the detection limit)
IonPac® AS14A and AG14A (3 mm) at 0.8 mL/min, 25 µL injections

The r^2 values ranged from 0.997 to 1.000 for the seven anions (Figure 6). Linearity extends above the concentrations selected for this study and presented in this Application Note. These concentrations used for calibration were designed to appropriately encompass the concentrations typically observed in drinking water samples. The response factors for each anion were calculated from the ratio of peak area to ppm concentration for a 25-µL injection. The response for each anion remained stable over 6 days of continuous operation (Figure 7), with RSDs ranging from 1.0 to 2.3%.

The recovery of anions from either deionized water (LFB) or drinking water (LFM) was calculated using the calibration curve generated at the beginning of the study and remained high over 6 days of continuous operation. The mean % recovery ranged from 96–103% for the LFB, and 92–102% for the LFM (Table 5). At no time did the percent recovery for the LFB drop below the 90% threshold for U.S. EPA Method 300.0, nor did the recovery from LFM drop below the 80% threshold. The retention times remained stable for all anions over 6 days of continuous operation, with RSDs ranging from 0.4–0.9%. At 4.5 days, a slight shift in retention time was observed corresponding in time with the replenishment of an eluent bottle, indicating some interday variance in retention times might occur from minor variations in eluent preparation. The optimized settings for the retention time acceptance window in the PeakNet software and the high level of separation of anions prevented misidentification of peaks.

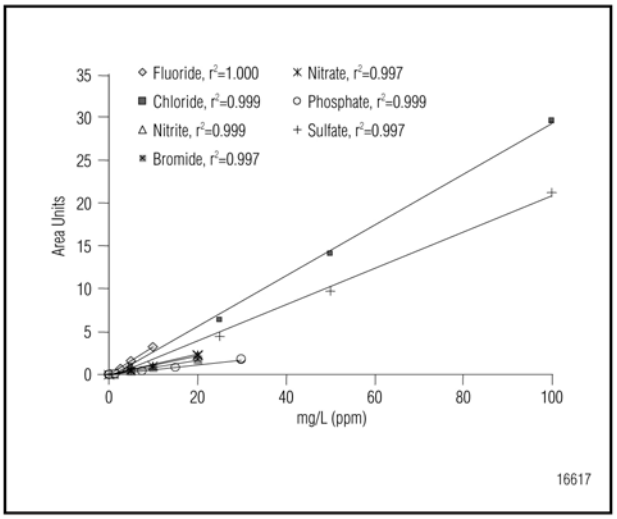


Figure 6. Calibration curves for common anions using the AS14A (3 mm) at 0.8 mL/min with the Atlas suppressor.

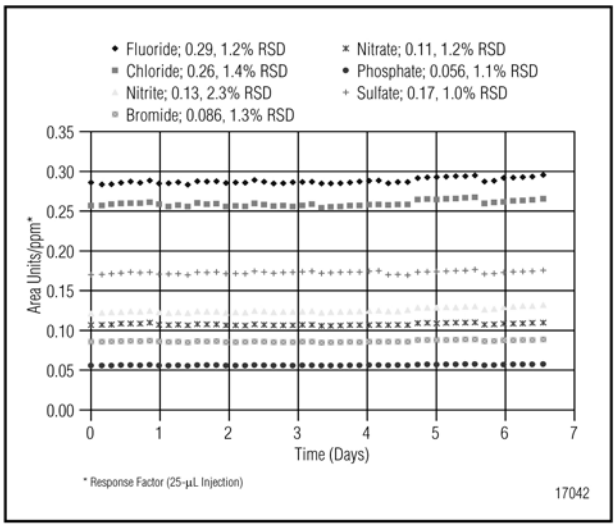


Figure 7. Stability of peak area response factors over 6 days using the AS14A (3 mm) at 0.8 mL/min with the Atlas suppressor.

Table 5 Recoveries of Common Anions From Deionized Lab Water (LFB) and Drinking Water Sample Matrix (LFM) Over 6 Days Using the AS14A (3 mm) at 0.8 mL/min with the Atlas Suppressor

	Recovery			
	LFB		LFM	
Anion	Mean%	RSD%	Mean%	RSD%
Fluoride	96	1.1	97	1.2
Chloride	99	1.4	98	2.8
Nitrite	98	2.5	92	3.2
Bromide	98	1.3	98	1.4
Nitrate	102	1.0	97	1.2
Phosphate	98	1.0	99	1.0
Sulfate	103	0.9	102	1.7

This method is useful for a variety of drinking water samples. Figures 8 and 9 show the separation of anions from fluorinated drinking water (Palo Alto, CA) and from Sierra Nevada mountain water low in ions (Twain Harte, CA), respectively. Even at high fluoride levels, this peak continues to elute out of the water dip using the AS14A (3 mm) column at 0.8 mL/min. When concentrations of ions become low, the use of Atlas suppression makes the detection of trace ions possible and with greater precision and accuracy. Figures 3 and 10 show water collected from the same Sunnyvale source during different seasons (August and February, respectively). Table 6 summarizes the measured results for the water samples tested and shows that this method is capable of monitoring seasonal changes in drinking water anions.

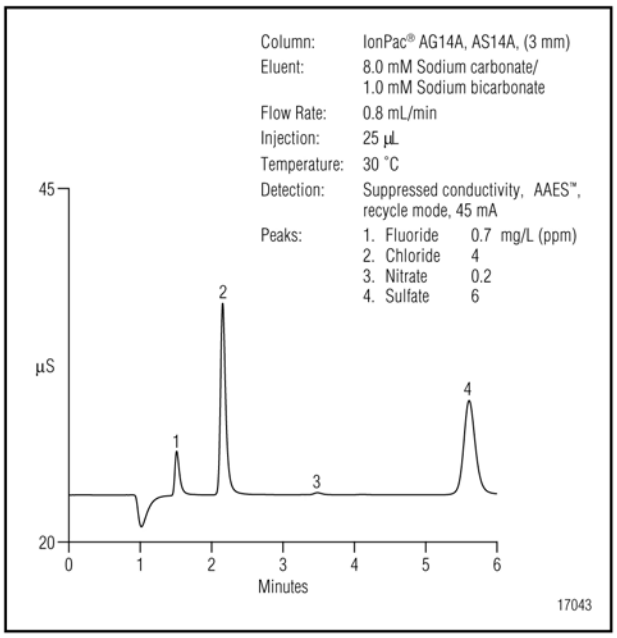


Figure 8. Anions in Palo Alto, CA drinking water separated using the IonPac AS14A (3 mm) at 0.8 mL/min.

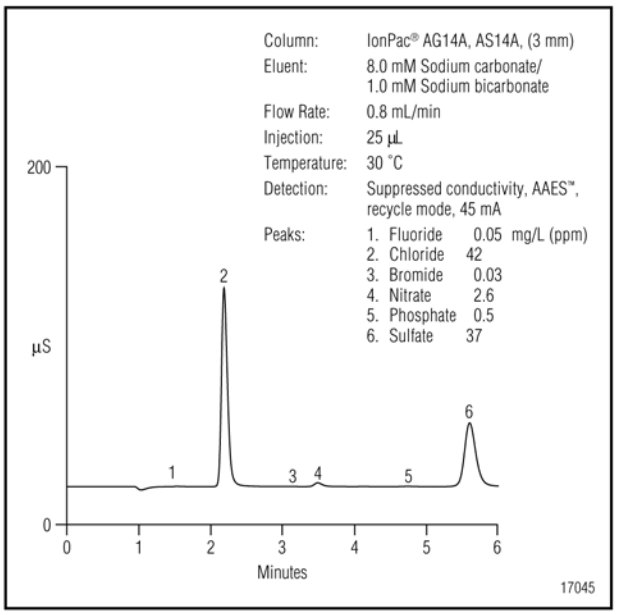


Figure 10. Anions in Sunnyvale, CA drinking water in February separated using the IonPac AS14A (3 mm) at 0.8 mL/min.

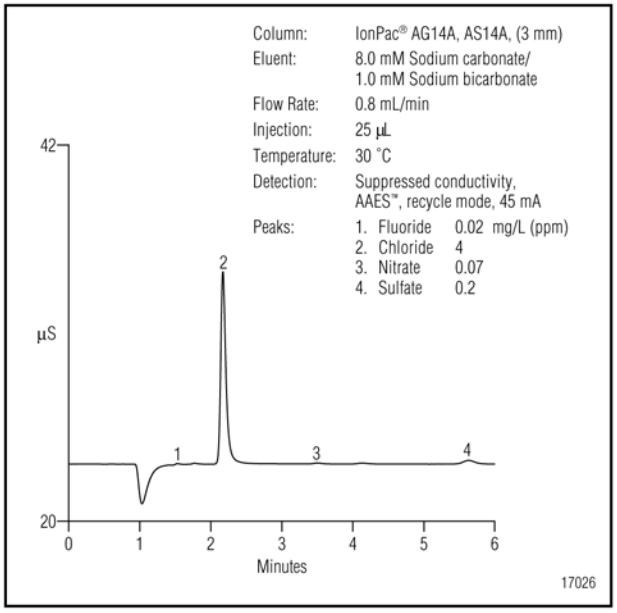


Figure 9. Anions in Twain Harte, CA drinking water separated using the IonPac AS14A (3 mm) at 0.8 mL/min.

Table 6 Summary of Drinking Water Samples Using AS14A (3 mm) at 0.8 mL/min with the Atlas Suppressor

	Sunnyvale 8/8/00 (ppm)	Sunnyvale 2/22/01 (ppm)	Palo Alto 2/21/01 (ppm)	Twain Harte 2/20/01 (ppm)
Fluoride	0.047	0.052	0.728	0.016
Chloride	16.6	42.1	4.1	3.5
Nitrite	< 0.003	< 0.003	< 0.003	< 0.003
Bromide	0.021	0.033	<0.004	<0.004
Nitrate	1.91	2.60	0.17	0.066
Phosphate	0.328	0.483	<0.010	<0.010
Sulfate	18.8	36.8	6.45	0.228

SUMMARY

The IonPac AS14A (3 mm) used at a faster flow rate reduces run times and increases sample throughput. The increase in flow rate from 0.5 to 0.8 mL/min increased the number of injections per day by 56%. The Atlas suppressor lowers baseline noise, which lowers detection limits and therefore improves the detection of trace-level ions. Good long-term performance was realized using the AS14A with Atlas suppression at 0.8 mL/min.

REFERENCES

1. U.S. EPA Method 300.0, "The Determination of Inorganic Anions in Water by Ion Chromatography", United States Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268.
2. Jackson, P.E.; Donovan, B; Pohl, C.A.; Kiser, R.E. "A New Block-Grafted Anion Exchange Phase for Environmental Water Analysis Using Ion Chromatography." *J. Chromatog.*, **2001**, *920*, 51–60.
3. Small, H.; Riviello, J. "Electrically Polarized Ion-Exchange Beds in Ion Chromatography: Ion Reflux." *Anal. Chem.* **1998**, *70 (11)*, 2205–2212.

SUPPLIERS

Aldrich Chemical Co., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, Wisconsin 53233, USA. Tel: 1-800-558-9160. www.sigma-aldrich.com

EM Science, 480 South Democrat Road, Gibbstown, NJ 08027, USA. Tel: 1-800-222-0342. www.emscience.com

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA, 15275-1126, USA. Tel: 1-800-766-7000. www.fishersci.com

J.T. Baker Inc., 222 Red School Lane, Phillipsburg, NJ 08865, USA. Tel: 1-800-582-2537. www.jtbaker.com

Sigma Chemical Co., P.O. Box 952968, St. Louis, MO 63195-2968, USA. Tel: 1-800-521-8956. www.sigma-aldrich.com

Determination of Chlorite, Bromate, Bromide, and Chlorate in Drinking Water by Ion Chromatography with an On-Line-Generated Postcolumn Reagent for Sub-µg/L Bromate Analysis

INTRODUCTION

Public water suppliers treat drinking water with disinfectants to protect public health and give drinking water a pleasant taste and odor. Unfortunately, some of the chemical disinfectants or by-products of the disinfection process are themselves harmful. For example, chlorine dioxide generates the inorganic oxyhalide disinfection by-products (DBPs) chlorite and chlorate; hypochlorite treatment may also generate the DBP chlorate;[1] and ozonating source water that contains elevated levels of natural bromide can produce the DBP bromate.[2] Both the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) have listed bromate as a potential carcinogen at the low-µg/L level.[3]

EPA's Stage 1 Disinfectants/Disinfection By-Products rule (D/DBP) specifies a maximum contaminant level (MCL) of 10 µg/L for bromate, an MCL of 1000 µg/L[4] for chlorite, and prescribes EPA Method 300.1[5] for compliance monitoring of bromate and chlorite in drinking water. It is expected that when the EPA promulgates Stage 2 of the D/DBP rule, the MCL for bromate will remain at 10 g/L and the EPA will propose additional methods for compliance monitoring to add flexibility and improved performance. Until then, the EPA is evaluating new methods with improved performance for D/DBP monitoring, including EPA Method 317.0 (IC-PCR, Dionex Application Note 136), EPA Method 321.8 (IC/ICP-MS), and EPA Method 326.0 (IC-PCR).[6–8]

This application note describes an improved ion chromatography (IC) method to quantify oxyhalide DBP anions and bromide at low concentration levels in reagent water, bottled water, and finished drinking water using an approach that is technically equivalent to U.S. EPA Method 326.0. The oxyhalide anions chlorite, chlorate, bromide, and bromate are separated on an IonPac® AS9-HC column and measured by using suppressed conductivity detection (as in EPA Method 300.1), followed by postcolumn reaction (PCR) to enhance detection of bromate. Sensitivity for bromate is improved by more than a factor of 10 through the use of a postcolumn reaction in which hydroiodic acid (HI) generated *in situ* from potassium iodide (KI) reacts with bromate in the column effluent to form the triiodide anion (I_3^-) as shown in the following set of reactions:[9]

$$BrO_3^- + 3I^- + 3H^+ \rightleftharpoons 3HOI + Br^-$$
$$3HOI + 3I^- + 3H^+ \rightleftharpoons 3I_2 + 3H_2O$$
$$3I_2 + 3I^- \rightleftharpoons 3I_3^-$$

Triiodide is then detected by its strong absorbance at 352 nm.

Because the HI PCR reagent is generated on-line and used immediately, reagent purity and stability should be more easily ensured than in EPA Method 317.0. It is also advantageous from a safety and exposures standpoint to use the *in situ* generated HI versus the toxic o-dianisidine (ODA) PCR reagent employed in Method 317.0.

Method 326.0 allows for the determination of all three key oxyhalide anions and bromide at low- µg/L levels using conductivity detection. Bromate can be quantified down to 0.5 µg/L using PCR with UV absorbance detection. Although Method 326.0 is not yet promulgated by the U.S. EPA Office of Ground Water and Drinking Water, the conductivity portion of the method has been determined acceptable for compliance monitoring for the oxyhalide DBPs and bromide.

EQUIPMENT

A Dionex DX-600 ion chromatographic system consisting of:

- GP50 Gradient Pump with Vacuum Degas Option
- ED50A Conductivity Detector with AS50 Conductivity Cell (P/N 55400)
- AD25 UV/Vis Absorbance Detector with 10-mm Cell
- AS50 Automated Sampler with Thermal Compartment
- PC10 Pneumatic Postcolumn Delivery Module (P/N 50601)
- Anion MicroMembrane™ (AMMS®) III Suppressor

PCH-2 Reaction Heater (P/N 39348)

Knitted Reaction Coil, 500 L, Potted (for PCH-2) (P/N 39349)

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

Chromeleon® Chromatography Workstation

REAGENTS AND STANDARDS

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

0.5 M sodium carbonate (Na_2CO_3) Anion Eluent Concentrate (Dionex P/N 37162)

Potassium iodide (KI) (Sigma P-8256) or (Fisher P-410)

Ammonium molybdate tetrahydrate $[(NH_4)_6 Mo_7O_{24}•4H_2O]$ (Aldrich 22,136-6)

Iron (II) sulfate heptahydrate ($FeSO_4•7H_2O$) (Aldrich 21,542-2)

Ethylenediamine (EDA) (Alfa Products 11932)

Dichloroacetic acid (DCAA) (Fluka 35810)

Sulfuric acid, (18M) (J.T. Baker INSTRA-ANALYZED 9673-33)

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

Bromate standard, 1000 mg/L, $NaBrO_3$ in H_2O (SPEX CertiPrep AS-$BRO_3$9-2Y)

Bromide standard, 1000 mg/L, NaBr in H_2O (ULTRA Scientific ICC-001)

Chlorate standard, 1000 mg/L, $NaClO_3$ in H_2O (SPEX CertiPrep AS-$CLO_3$9-2Y)

Chlorite standard, 1000 mg/L, $NaClO_2$ in H_2O (SPEX CertiPrep AS-$CLO_2$9-2Y)

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)

CONDITIONS

Columns:	Dionex IonPac AG9-HC, 50 × 4 mm i.d. Guard Column (Dionex P/N 51791) Dionex IonPac AS9-HC, 250 × 4 mm i.d. Analytical Column (Dionex P/N 51786)
Eluent:	9.0 mM sodium carbonate (Na_2CO_3)
Flow Rate:	1.3 mL/min
Temperature:	30 C
Sample Volume:	225 µL
Detection:	Suppressed Conductivity, Anion Atlas® Electrolytic Suppressor (AAES™) (P/N 056116) AutoSuppression® external water mode, 78 mA Temperature compensation, 1.7%/°C
Expected Background:	~23–26 µS
Expected Backpressure:	~2400 psi
Run Time:	20 min

PCR

Detection:	Absorbance at 352 nm
PCR Reagent Flow:	0.26 M potassium iodide at 0.4 mL/min
AMMS III:	0.3 N sulfuric acid at 2.5 mL/min
Postcolumn Heater Temp:	80 °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-micron filter.

Eluent Solution

9 mM sodium carbonate

Dilute 36 mL of 0.5 M sodium carbonate concentrate to 2 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.

Ethylenediamine (EDA) Preservative Solution

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

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

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

Sulfuric Acid Solution (0.5 N)

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

Ammonium Molybdate Solution (2.0 mM)

Add 0.247 g of ammonium molybdate tetrahydrate [$(NH_4)_6\ Mo_7O_{24} \cdot 4H_2O$)] to about 50 mL of reagent water in a 100-mL volumetric flask. Dissolve and bring to volume with reagent water. Store in an opaque plastic bottle and prepare fresh monthly.

Postcolumn Reagent

Add 43.1 g of potassium iodide to about 500 mL of reagent water in a 1-L volumetric flask and mix to dissolve. Add 215 µL of the ammonium molybdate solution. Bring to volume with reagent water and mix. Remove dissolved gasses by sparging with helium or by sonicating under vacuum for 20 min. Immediately place it in the PC-10 reagent delivery vessel and blanket with helium. Protect from light by covering the PC-10 module with aluminum foil. The reagent is stable for 24 h under these conditions.

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 deionized water and dilute to 100 mL.

Prepare a mixed anion calibration stock standard at 20 mg/L by combining 2 mL 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 one month when stored at less than 6 °C.

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 two weeks when stored at less than 6 °C.

Working Standard Solutions

Use deionized water to prepare appropriate dilutions of the calibration stock standards as needed. Prepare mixed calibration standards containing all four anions fresh each day as needed.

SAMPLE PREPARATION

Sparge the water samples taken from a treatment plant employing chlorine dioxide or ozone with an inert gas (e.g., nitrogen, argon, or helium) for 5 min. Add 1.00 mL of EDA preservation solution per 1 L of sample to prevent conversion of residual hypochlorite or hypobromite to chlorate or bromate. This solution also prevents metal-catalyzed conversion of chlorite to chlorate. Samples preserved in this manner are stable for at least 14 days when stored in amber bottles at 4 °C.[10]

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 ($NaClO_2$)	0.1344*

* Mass of pure (>99%) sodium chlorite. For accurate results, determine the exact purity of $NaClO_2$ by using the iodometric titration procedure[14] and adjust the mass of the compound used accordingly. For example, for technical-grade sodium chlorite (80% pure) use (0.1344 g)(100%/80%) = 0.1680 g.

Most samples preserved as above can be filtered through a 0.45-micron filter (Gelman IC Acrodisk P/N 4485 or equivalent) 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 bromate quantification by PCR.

The treatment procedure to remove chlorite requires two portions of the water sample. Place one 10-mL aliquot of the sample into a 20-mL microbeaker. Place a second 10-mL aliquot into a second 20-mL beaker. Fortify one aliquot of the sample 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 0.5 N sulfuric acid solution 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-micron nylon filter to remove precipitated ferric hydroxide. 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.[11]

SYSTEM PREPARATION AND SETUP

Configure the IC with the AG9/AS9-HC columns and PCR system as depicted in Figure 1 and as described in the PC10 postcolumn delivery system installation instructions. Verify that the pump flow rate is within specifications and recalibrate if necessary. A GP50 should deliver water at 1.0 –0.005 mL/min against a constant backpressure of 2000 psi. Verify that the UV absorbance detector wavelength accuracy is within specifications. Recalibrate if necessary. It is good practice to periodically record the visible lamp output (i.e., the reference cell current in nA) and elapsed time to assist in potential troubleshooting. Consult the pump and detector manuals for procedural details.

Install a 1-mL sample syringe and set the AS50 syringe speed to 4 or 5 to make fast large-loop injections. Install a calibrated 225- µL sample loop made from 111 cm of 0.02-in. i.d. PEEK tubing. Enter the correct sample "Loop Size" and "Sample Syringe Volume" in the AS50 Plumbing Configuration screen.

Prepare the AAES for use by hydrating the eluent chamber. Use a disposable plastic syringe to slowly push approximately 3 mL of DI water through both the "Eluent In" port and "Regen In" port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor monodisks and membranes. Because the effluent from the conductivity detector cell will undergo a postcolumn reaction, install the AAES in the external water mode by following the *Installation Instructions and Troubleshooting Guide for the Anion Atlas Electrolytic Suppressor* (Document No. 031770). Make sure that the pressure downstream from the Atlas suppressor does not exceed the recommended operating pressure of 20–100 psi. Use 0.02-in. i.d. PEEK tubing from the Atlas suppressor to the mixing tee, to the PCR coil, to the absorbance detector, and to waste, and keep it as short as is practical to minimize backpressure. Adjust the head pressure on the external water reservoir to deliver a flow rate of 5–10 mL/min (~10–15 psi). Use an AAES current of 78 mA.

Prepare the AMMS III (P/N 56750) for use by hydrating the eluent chamber. Use a disposable plastic syringe to slowly push approximately 3 mL of 0.2 N sulfuric acid through the "Eluent Out" port and 5 mL of 0.2 N sulfuric acid through the "Regen In" port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the AMMS III in the chemical regeneration mode by following the *Installation Instructions and Troubleshooting Guide for the Anion Micromembrane Suppressor* (Document No. 031727). Adjust the head pressure on the 0.3 N sulfuric acid reservoir to deliver a flow rate of 2–3 mL/min (~10–15 psi if a short piece of 0.01-in. i.d. PEEK tubing is connected to the AMMS III "Regen Out" port and trimmed accordingly).

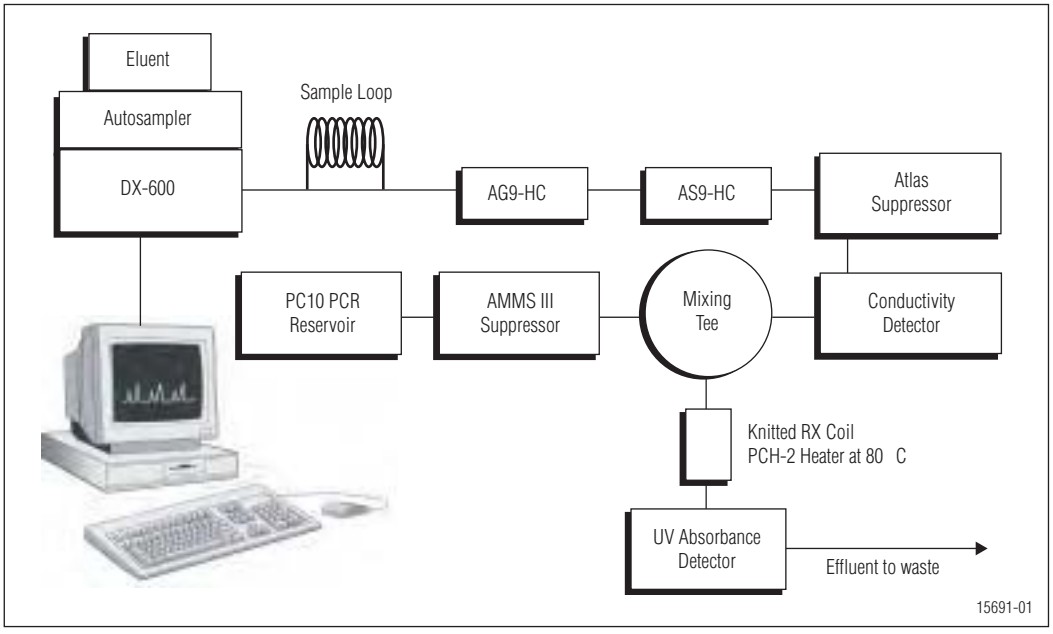


Figure 1. IC system configuration for EPA Method 326.0.

Pump the eluent at 1.3 mL/min and set the PC10 pneumatic pressure to 70 psi. To measure the PCR flow rate, collect the effluent from the detector (i.e., the total flow from the IC pump and PCR module) in a 10-mL graduated cylinder for 5 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 PC10 postcolumn delivery module and remeasure the flow rate until the correct PCR flow rate of 0.4 mL/min is established. Variations in the PCR flow rate affect the postcolumn reaction time, pH, dilution, mixing rate, and ratio of the reactants. Stable day-to-day results depend on a well-controlled PCR flow rate. Confirm this flow rate on a daily basis and whenever detector response for a calibration check standard deviates beyond quality control acceptance criteria.

The storage solution 10 mM $NaHCO_3$ is shipped with the AS9-HC. After equilibrating the column with 9.0 mM carbonate eluent for 20 min, analyze a system blank of reagent water. An equilibrated system has a background conductance ~26 µS, with the peak-to-peak noise typically 1–2 nS per min. The background absorbance at 352 nm should be less than 200 mAU with peak-to-peak noise of less than 50 µAU per min. There should be no peaks eluting within the retention time window of the bromate anion. The column is equilibrated when two consecutive injections of a standard produce the same retention time for bromate.

RESULTS AND DISCUSSION

Figure 2 shows the chromatograms of a mixed anion standard containing 5 µg/L each of chlorite, bromate, bromide, and chlorate. The top trace (A) was obtained with the conductivity detector and the bottom trace (B) was obtained with the UV/Vis absorbance detector after postcolumn reaction with acidified KI. The bromate peak is baseline resolved from chlorite on both detector channels. However, the response on the absorbance detector after PCR with acidified KI is significantly enhanced compared to the response obtained on the conductivity detector.

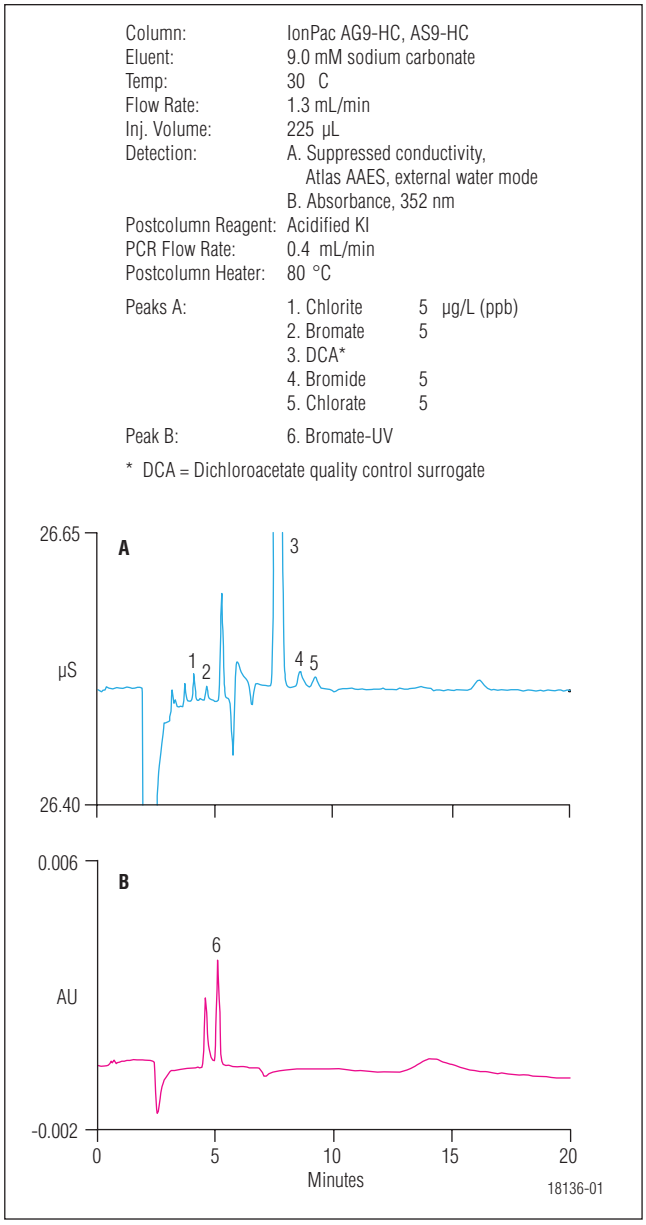


Figure 2. Separation of a low-ppb inorganic anion standard using an IonPac AS9-HC column; (A) suppressed conductivity detection and (B) UV absorbance detection after PCR with acidified KI.

Table 2 summarizes the calibration data and method detection limits (MDLs) obtained for the oxyhalide DBP anions and bromide using dual conductivity and UV detection. The MDL for each analyte was established by making eight replicate injections of a reagent

Table 2. Linear Ranges and MDLs for Oxyhalides and Bromide

Solute	Range (µg/L)	r^2	MDL Standard (µg/L)	Calculated MDL*(µg/L)
Chlorite	5.0–1000	0.9999	5.0	1.10
Bromate-conductivity	5.0–1000	0.9994	5.0	0.82
Bromide	5.0–1000	1.0000	5.0	1.10
Chlorate	5.0–1000	0.9999	5.0	0.85
Bromate-UV	0.5–15	0.9999	0.5	0.06

* The MDLs were calculated as 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* = 3.00 for eight replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

water blank fortified at a concentration of 3–5 times the estimated instrument detection limit.[12] The use of PCR addition and UV detection allows quantification of bromate down to 0.5 µg/L, without compromising the detection limits obtained with suppressed conductivity detection for the other anions of interest. Electronic smoothing (Olympic, 25 points, 5 sec, 1 iteration) of the UV signal was used to improve the calculated MDL for bromate.[13]

Figures 3–6 illustrate the method's performance for the determination of inorganic oxyhalide DBP anions and bromide in drinking water and bottled water samples. Figure 3 shows the chromatograms from a direct injection of drinking water (from Sunnyvale, CA). The top trace (A) was obtained with the conductivity detector and the bottom trace (B) was obtained with the UV/Vis absorbance detector after postcolumn reaction with acidified KI. Chlorite, bromate, bromide, and chlorate were all observed in the drinking water sample. The target analyte anions were well resolved from the sample matrix. The bromide was probably present in the source water. During ozonation, some of the bromide can convert to bromate. Chlorate can enter the water both as a source water contaminant and as a disinfection byproduct from the use of hypochlorite. Chlorite is a residual from treatment with chlorine dioxide.

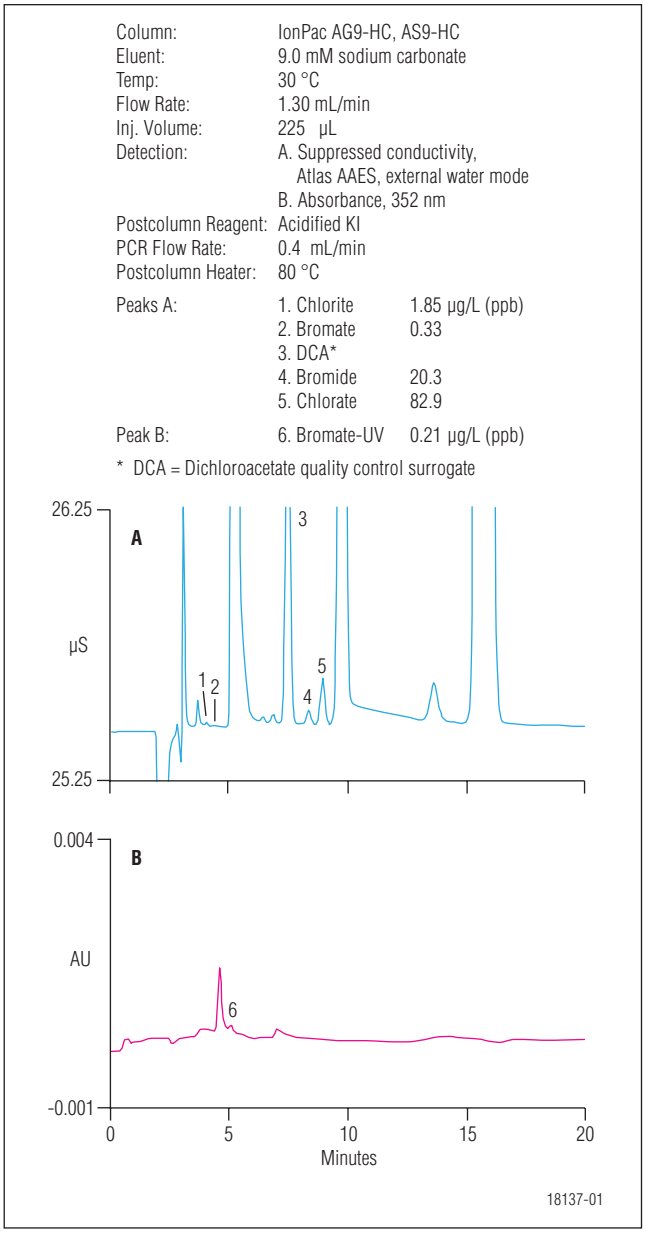


Figure 3. Determination of DBP anions in Sunnyvale, CA drinking water; (A) suppressed conductivity detection and (B) UV absorbance detection after PCR with acidified KI.

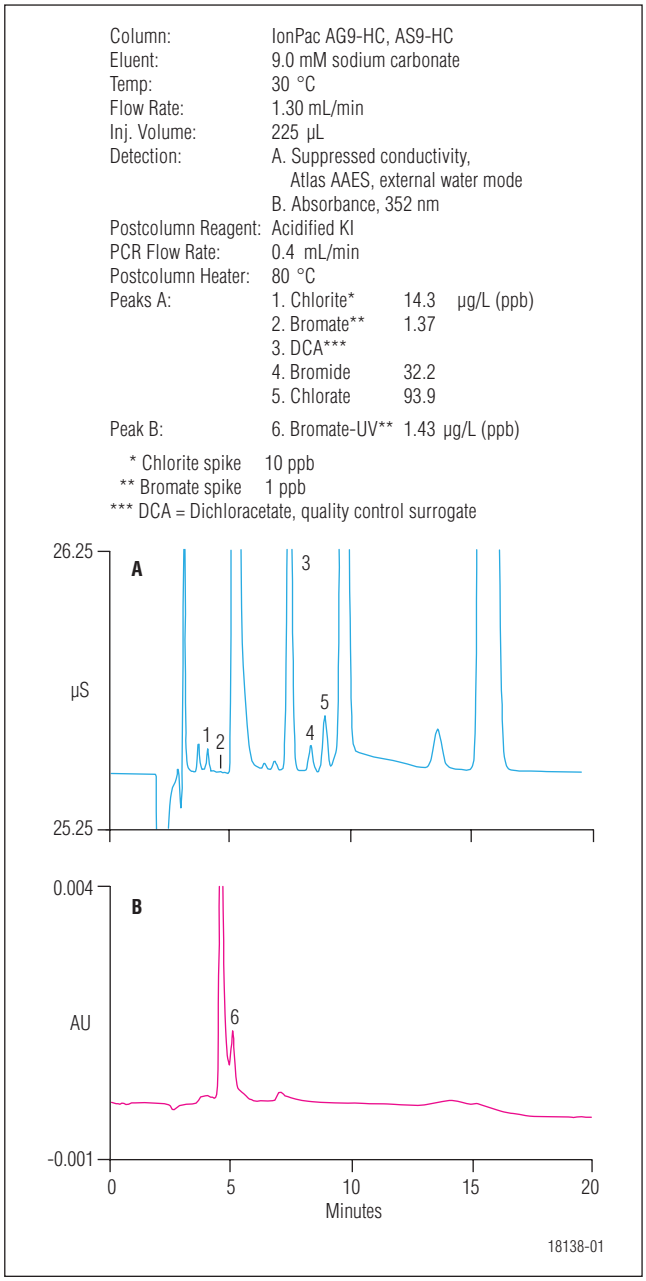


Figure 4. Determination of DBP anions in spiked Sunnyvale, CA drinking water; (A) suppressed conductivity detection and (B) UV absorbance detection after PCR with acidified KI.

Figure 4 shows chromatograms of the same drinking water sample spiked with bromate at 1 µg/L, and with chlorite, bromide, and chlorate at 10 µg/L. The top trace (A) was obtained with the conductivity detector and the bottom trace (B) was obtained with the UV/Vis absorbance detector after postcolumn reaction with acidified KI. The benefits of PCR with UV detection for bromate determination can clearly be seen in Figure 4 (B), where the bromate peak response is significantly enhanced compared to the conductivity detector. No response is observed for the large chloride peak that elutes immediately after bromate. Table 3 shows that quantitative recoveries were obtained for the oxyhalide anions and the bromide spiked into drinking water. In addition, quantitative recoveries were obtained for the oxyhalide anions and bromide spiked into the simulated high-ionic-strength water that contained elevated levels of the common matrix anions: chloride, carbonate, sulfate, nitrate, and phosphate. The use of PCR with UV/Vis detection allows the quantification of bromate down to 0.5 µg/L in the presence of 100 mg/L chloride (a 200,000 fold excess) with no sample pretreatment.

Table 3. Anion Recoveries for Spiked Water Samples

	Tap Water		High-Ionic-Strength Water	
Anion*	**Amount Added (µg/L)**	**Recovery**	**Amount Added (µg/L)**	**Recovery**
Chlorite	10	114%	100	97%
Bromate-conductivity	1	107%	10	98%
Bromide	10	98%	100	105%
Chlorate	10	113%	100	99%
Bromate-UV	1	124%	10	65%***
Bromate-UV**			1.0	106%

*Data were obtained from multianalyte spikes into Sunnyvale, CA tapwater and high-ionic-strength water (HIW) containing 100 mg/L chloride, 100 mg/L carbonate, 100 mg/L sulfate, 10 mg/L nitrate-N, and 10 mg/L phosphate-P.

** Bromate only (1.0 µg/L) was added to an HIW sample to determine low-level recovery for this anion using UV detection.

*** Bromate recovery was reduced by chlorite interference.

Column:	IonPac AG9-HC, AS9-HC
Eluent:	9.0 mM sodium carbonate
Temp:	30 °C
Flow Rate:	1.30 mL/min
Inj. Volume:	225 µL
Detection:	A. Suppressed conductivity, Atlas AAES, external water mode
	B. Absorbance, 352 nm
Postcolumn Reagent:	Acidified KI
PCR Flow Rate:	0.4 mL/min
Postcolumn Heater:	80 °C

Peaks A:	1. Chlorite	n.d.	µg/L (ppb)
	2. Bromate	1.52	
	3. DCA*		
	4. Bromide	1.12	
	5. Chlorate	1.08	
Peak B:	6. Bromate-UV	1.84	

* DCA = Dichloroacetate, quality control surrogate

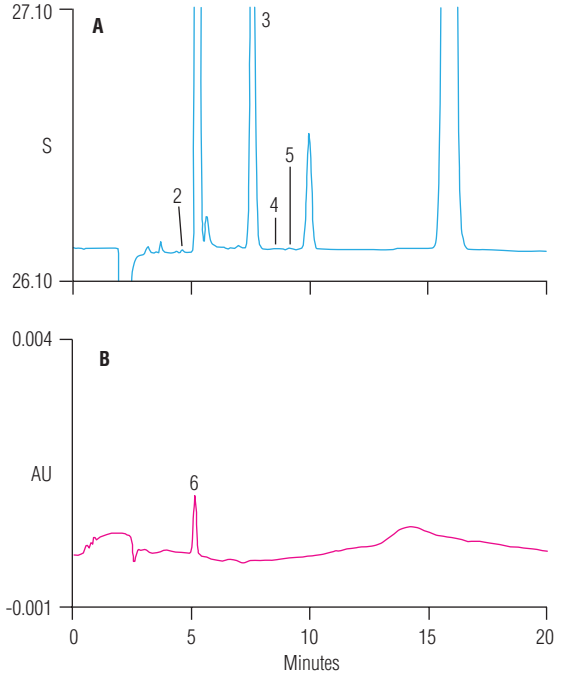


Figure 5. Determination of DBP anions in bottled water; (A) suppressed conductivity detection and (B) UV absorbance detection after PCR with acidified KI.

Figure 5 shows the chromatograms from a direct injection of bottled water. The top trace (A) was obtained with the conductivity detector, and the bottom trace (B) was obtained with the UV/Vis absorbance detector. The bottle label read: "Prepared using filtration, reverse osmosis, deionization, and ozonation". The DBP precursor bromide and the DBP bromate were both observed in the bottled water sample.

Column:	IonPac AG9-HC, AS9-HC
Eluent:	9.0 mM sodium carbonate
Temp:	30 °C
Flow Rate:	1.30 mL/min
Inj. Volume:	225 µL
Detection:	A. Suppressed conductivity, Atlas AAES, external water mode
	B. Absorbance, 352 nm
Postcolumn Reagent:	Acidified KI
PCR Flow Rate:	0.4 mL/min
Postcolumn Heater:	80 °C

Peaks A:	1. Chlorite	10.8	µg/L (ppb)
	2. Bromate**	2.80	
	3. DCA*		
	4. Bromide	12.7	
	5. Chlorate	12.6	
Peak B:	6. Bromate-UV**	2.85	

* DCA = Dichloroacetate, quality control surrogate
** Bromate spike 1 ppb

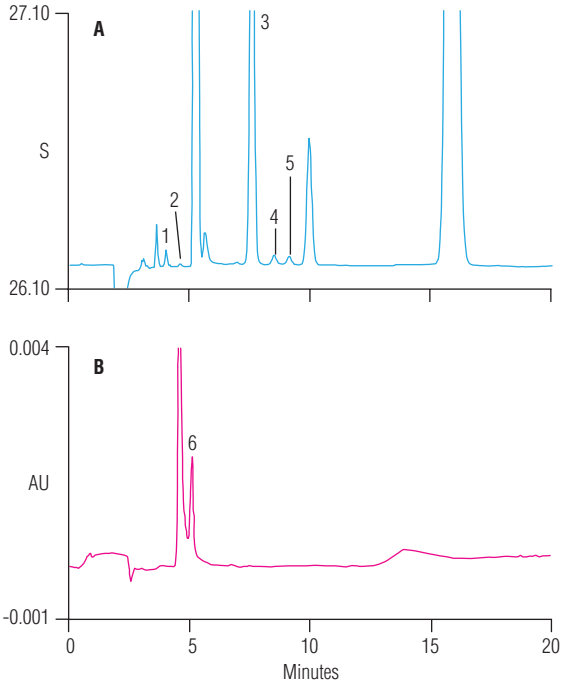


Figure 6. Determination of DBP anions in spiked bottled water; (A) suppressed conductivity detection and (B) UV absorbance detection after PCR with acidified KI.

Figure 6 shows the chromatograms of the same bottled water sample spiked with bromate at 1.0 µg/L, and with chlorite, bromide, and chlorate at 10 µg/L. The top trace (A) was obtained with the conductivity detector, and the bottom trace (B) was obtained with the UV/Vis absorbance detector after postcolumn reaction with acidified KI. Quantitative recoveries were obtained for all the added oxyhalide anions and bromide.

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 acidified KI and produces a response at 352 nm. High chlorite levels can interfere with quantification of bromate at low concentrations. The interference from chlorite can be minimized by reducing the chlorite with ferrous sulfate, as described in the "Sample Preparation" section. To evaluate the ferrous sulfate treatment, we analyzed a series of simulated chlorine dioxide-treated tap waters (STWs) spiked with varying levels of bromate. After determining the bromate level in each STW, we prepared the corresponding laboratory-fortified matrices (LFMs) by spiking each STW sample with an amount of bromate equal to 50–100% of the observed level. We then treated each STW and its corresponding LFM with ferrous sulfate and reanalyzed. The results, summarized in Table 4 and Figure 7, 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 uses an IonPac AS9-HC column and suppressed conductivity detection, followed by postcolumn addition of acidified KI with UV detection, specifically for enhanced bromate response to determine all key oxyhalide anions and bromide at low- µg/L levels in drinking and bottled waters. The postcolumn addition and UV detection allows quantification of bromate at 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 at 15–50 µg/L.

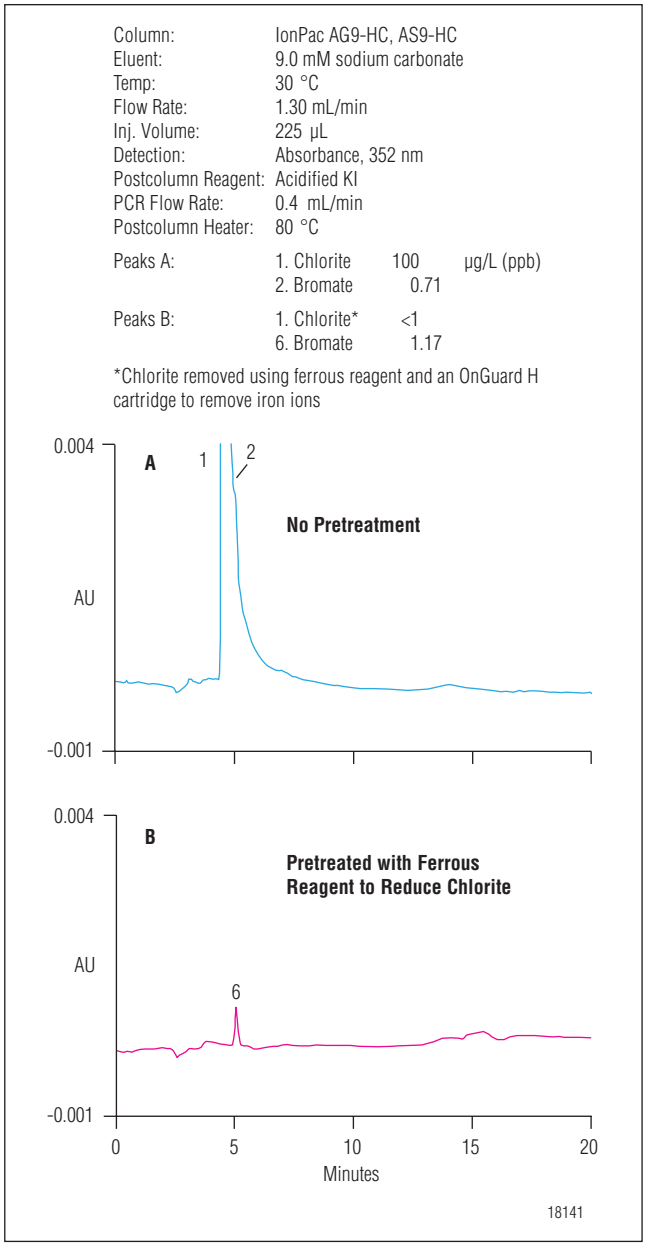


Figure 7. Determination of DBP anions in simulated chlorine dioxide-treated water (STW). (A) Untreated STW, UV absorbance detection after PCR with acidified KI, and (B) STW after treatment with ferrous sulfate to remove chlorite, UV absorbance detection after PCR with acidified KI.

Table 4. Bromate Recovery from Simulated Chlorine Dioxide-Treated Waters (STW)*						
	Spiked STW Fe (II) Treated			Laboratory Fortified Matrix Fe (II) Treated		
Sample	Amount Added (µg/L)	Amount Found (µg/L)	Recovery	Amount Added (µg/L)	Amount Found (µg/L)	Recovery
STW	0	0.19		0.5	0.61	84%
STW-1	0.5	0.70	102%	0.5	1.20	100%
STW-2	1.0	1.17	98%	1.0	2.24	107%
STW-3	2.0	2.18	100%	2.0	4.33	108%
STW-4	5.0	5.22	101%	5.0	10.24	100%

* Chlorite present at 100 µg/L.

REFERENCES

1. Wagner, H. P.; Pepich, B. V.; Hautman, D. P.; Munch, D.J. *J. Chromatogr. A,* **1999,** *850,* 119.
2. Kruithof, J. C.; Meijers, R. T. *Water Supply,* **1995,** *13,* 117.
3. *Fed. Reg.*, 59 (145), **1994,** 38709.
4. *Fed. Reg.*, 63 (241), **1998,** 69389.
5. *U.S. EPA Method 300.1,* U.S. Environmental Protection Agency: Cincinnati, OH, 1997.
6. *U.S. EPA Method 317.0,* U.S. Environmental Protection Agency: Cincinnati, OH, 2000.
7. *U.S. EPA Method 321.8,* U.S. Environmental Protection Agency: Cincinnati, OH, 2000.
8. *U.S. EPA Method 326.0,* U.S. Environmental Protection Agency: Cincinnati, OH, 2002.
9. Sahli, E.; Von Gunten, U. *Wat. Res.* **1999,** *15,* 3229.
10. Hautman, D. P.; Bolyard, M. *J. Chromatogr. A* **1992,** *602,* 65.
11. Wagner, H. P.; Pepich, B. V.; Hautman, D. P.; Munch, D. J. *J. Chromatogr. A* **2000,** *882,* 309.
12. Glaser, J. A. ; Foerst, D. L.; McKee, G. D.; Quave, S. A.; Budde, W. L. *Environ. Sci. Technol.* **1981,** *15,* 1426.
13. Schibler, J. A. *Am. Lab.* **1997,** 63.
14. Method 4500-C102.C. In *Standard Methods for the Examination of Water and Wastewater,* 18th Ed.; Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D. (Eds.); APHA: Washington, DC, 1992.

SUPPLIERS

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

Alfa Products, 30 Bond St., Ward Hill, MA 01835 USA, Tel.: 800-343-0660, info@alfa.com.

EM Science, P.O. Box 70, Gibbstown, NJ 08027 USA, Tel: 800-222-0342, www.emscience.com.

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

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

J. T. Baker, 222 Red School Lane, Phillipsburg, NJ 08865 USA. Tel.: 800-582-2537, www.jtbaker.com (order from VWR).

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-LAB-SPEX, www.spexcsp.com (order from Fisher).

ULTRA Scientific (order from VWR).

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

Determination of Inorganic Anions in Environmental Waters Using a Hydroxide-Selective Column

INTRODUCTION

Ion chromatography (IC) is now a well-established and accepted technique for the monitoring of inorganic anions in environmental waters, such as surface, ground, and drinking waters. In the U.S., water quality is legislated through the Safe Drinking Water Act (SDWA) and the Clean Water Act (CWA). The goal of the CWA is to reduce the discharge of pollutants into waters, whereas the SDWA ensures the integrity and safety of drinking waters.[1,2] Primary and secondary drinking water standards have been adopted in the U.S. for certain inorganic anions. The U.S. National Primary Drinking Water Standards (NPDWS) include fluoride, nitrite, and nitrate. A maximum contaminant level for each of these anions is specified in the NPDWS as the regulatory standard for minimizing potential health effects arising from their ingestion in drinking water.[3] Other common inorganic anions, such as fluoride, chloride, and sulfate, are considered secondary contaminants and are regulated under the U.S. National Secondary Drinking Water Standards, which are guidelines regarding taste, color, odor, and certain aesthetic effects.[4]

IC has been approved for the compliance monitoring of primary and secondary inorganic anions in drinking water since the mid-1980s, as described in U.S. EPA Method 300.0.[5] In 1992, the U.S. EPA-EMSL (Cincinnati) laboratory recommended promulgation of U.S. EPA Method 300.0 for compliance monitoring in all U.S. EPA regions for the analysis of inorganic anions in wastewater under the National Pollution Discharge Elimination System program.[2]

Many other industrialized countries have similar health and environmental standards and a considerable number of regulatory IC methods have been published worldwide (e.g., in Germany, France, Italy, and Japan) for the analysis of anions in drinking water. In addition, many standard organizations, including ISO, ASTM, and AWWA, have validated and published IC methods for the analysis of inorganic anions in drinking water, groundwater, and wastewater.[6,7]

U.S. EPA Method 300.0 (Part A) describes the use of a Dionex IonPac® AS4A anion-exchange column using a carbonate/bicarbonate eluent and suppressed conductivity detection for the determination of inorganic anions in environmental waters, such as drinking water, wastewater (mixed domestic and industrial), groundwater, and aqueous solid extracts. However, the method allows for alternative columns, eluents, suppression devices, and detectors to be used—provided that equivalent or better performance for the method is obtained and that the quality assurance requirements are met, including an initial demonstration of capability.

Traditionally, columns designed for use with carbonate/bicarbonate eluents have been used for determining inorganic anions in environmental samples. Columns that use hydroxide eluents (i.e., hydroxide-selective columns) have not been as widely used for routine analysis of inorganic anions in environmental waters due to the lack of appropriate selectivity and difficulty in preparing contaminant-free hydroxide eluents. The introduction of automated, electrolytic eluent generation has eliminated the difficulty in preparing hydroxide eluents. A hydroxide-selective column, the Dionex IonPac AS18, was developed to determine inorganic anions in environmental waters. In this application note, we describe the use of automated eluent generation, combined with a high-capacity, hydroxide-selective, anion-exchange column—the IonPac AS18—for the determination of inorganic anions in environmental waters. The linear range, method detection limits, and recovery of fortified sample matrices are described.

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 Degas
- EluGen EGC-KOH Cartridge (Dionex P/N 058900)
- CR-ATC (Dionex P/N 060477)

AS50 Autosampler

Chromeleon® 6.5 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)

CONDITIONS

Columns:	IonPac AS18 Analytical, (4 × 250 mm P/N 060549) IonPac AG18 Guard, (4 × 50 mm P/N 060551)
Eluent:	22–40 mM KOH from 7–8 min
Eluent Source:	ICS-2000 with CR-ATC
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection:	25 µL
Detection:	Suppressed conductivity, ASRS® ULTRA, 4 mm (P/N 053947) AutoSuppression® Recycle Mode 100 mA current
System Backpressure:	~2500 psi
Run Time:	20 min

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Standard Solutions

Stock Anion Standard Solutions (1000 mg/L)

For several of the analytes of interest, 1000 mg/L standard solutions are available from Dionex and 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 1000 mL of deionized water according to Table 1. Stock standard solutions for nitrite and nitrate were prepared in concentration units as nitrite-*N* and nitrate-*N*, whereas phosphate was prepared in concentration units as phosphate-*P* as specified in U.S. EPA Method 300.0. Standards are stable for at least one month when stored at 4 °C.

Working Standard Solutions

Composite working standard solutions at lower analyte concentrations are prepared from the 1000 mg/L stock solutions. Working standards containing less than 100 mg/L anions should be prepared daily. Seven levels of calibration standards were used in this study to cover the expected concentrations found in environmental samples. Table 2 shows the anion standard concentrations used to calculate the method detection limits (MDLs) and the concentration of the quality control standard (QCS) used to determine retention time stability and peak area precision. Table 3 shows the linear concentration range investigated for each inorganic anion.

SAMPLE PREPARATION

All samples were filtered through an appropriate 0.45-µm syringe filter, discarding the first 300 µL of the effluent. The only exception was the domestic wastewater, which was filtered through a 0.20-µm syringe filter before injection into the IC. However, to prolong column lifetimes, some domestic wastewater samples may require pretreatment with a C18 cartridge to remove hydrophobic organic material.[8,9]

Table 1. Preparation of Stock Standard Solution

Anion	Compound	Amount (g)
Fluoride	Sodium fluoride (NaF)	2.210
Chloride	Sodium chloride (NaCl)	1.648
Nitrite	Sodium nitrite ($NaNO_2$-*N*)	4.926
Bromide	Sodium bromide (NaBr)	1.288
Nitrate	Sodium nitrate ($NaNO_3$-*N*)	6.068
Phosphate	Potassium phosphate, monobasic (KH_2PO_4-*P*)	4.394
Sulfate	Sodium sulfate (Na_2SO_4)	1.479

Table 2. Concentration of MDLs and QCS Standards

Analyte	MDL Calculation Standard (µg/L)	QCS Used for RSD Calculation (mg/L)
Fluoride	10	2
Chloride	10	20
Nitrite-*N*	6.1 (20 as NO_2)	2
Bromide	25	2
Nitrate-*N*	6.8 (30 as NO_3)	10
Phosphate-*P*	23 (70 as PO_4)	2
Sulfate	20	60

RESULTS AND DISCUSSION

Although U.S. EPA Method 300.0 (Part A) specifies the use of an IonPac AS4A column, section 6.2.2.1 states that, "An optional column may be used if comparable resolution of peaks is obtained and the requirements of Section 9.2 can be met." Section 9.4.6 further states that, "In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns and/or eluents to improve the separations or lower the cost of measurements." Each time such modifications

to the method are made, the analyst is required to repeat the procedure in Section 9.2 of the method. Section 9.2 discusses the quality control parameters, including the initial demonstration of performance, linear calibration range, quality control samples, and determination of MDLs. Based on this information, the analyst may substitute a column, such as the IonPac AS18, in place of the AS4A, as well as the use of a different eluent, such as hydroxide in place of carbonate/bicarbonate, as in the case of the AS18 hydroxide-selective column.

The IonPac AS18 is a latex agglomerated column with a 7.5-µm-diameter macroporous resin bead consisting of ethylvinylbenzene (EVB) cross-linked with 55% divinylbenzene (DVB), which makes the column 100% solvent compatible. The outer layer consists of 65 nm latex functionalized with alkanol quaternary ammonium groups. The net result is a column with a high-capacity, improved efficiency, and greater selectivity toward hydroxide eluents than the AS4A column.

The IonPac AS18 has a significantly higher capacity (285 µeq/column compared to 20 µeq/column for the AS4A). This higher capacity allows improved resolution between chloride and nitrite and the ability to better tolerate high-ionic-strength matrices without column overloading, which is important in the environmental industry—particularly for the analysis of wastewater samples. Comparison of chromatograms (Figure 1) obtained with the AS18 and AS4A columns reveals noticeable differences in selectivities. Hydroxide-selective stationary phases typically give a greater retention of weakly retained analytes, such as fluoride and acetate, and only moderate retention of divalent hydrophilic anions, such as sulfate.[10] This greater retention is evident from the separation using the AS18 column (Figure 1B) where fluoride is well resolved from the void volume, whereas fluoride is not completely resolved from the void volume using the AS4A column (Figure 1A). Additionally, sulfate elutes between bromide and nitrate on the AS18, whereas on the AS4A sulfate is the last eluting peak, which is typical for a column using carbonate eluents. Finally, phosphate elutes after sulfate on the AS18 column when using the hydroxide eluent conditions in Figure 1B. The higher eluent pH, compared to the AS4A, results in a greater charge on the polyprotic acid species, therefore increasing its retention.

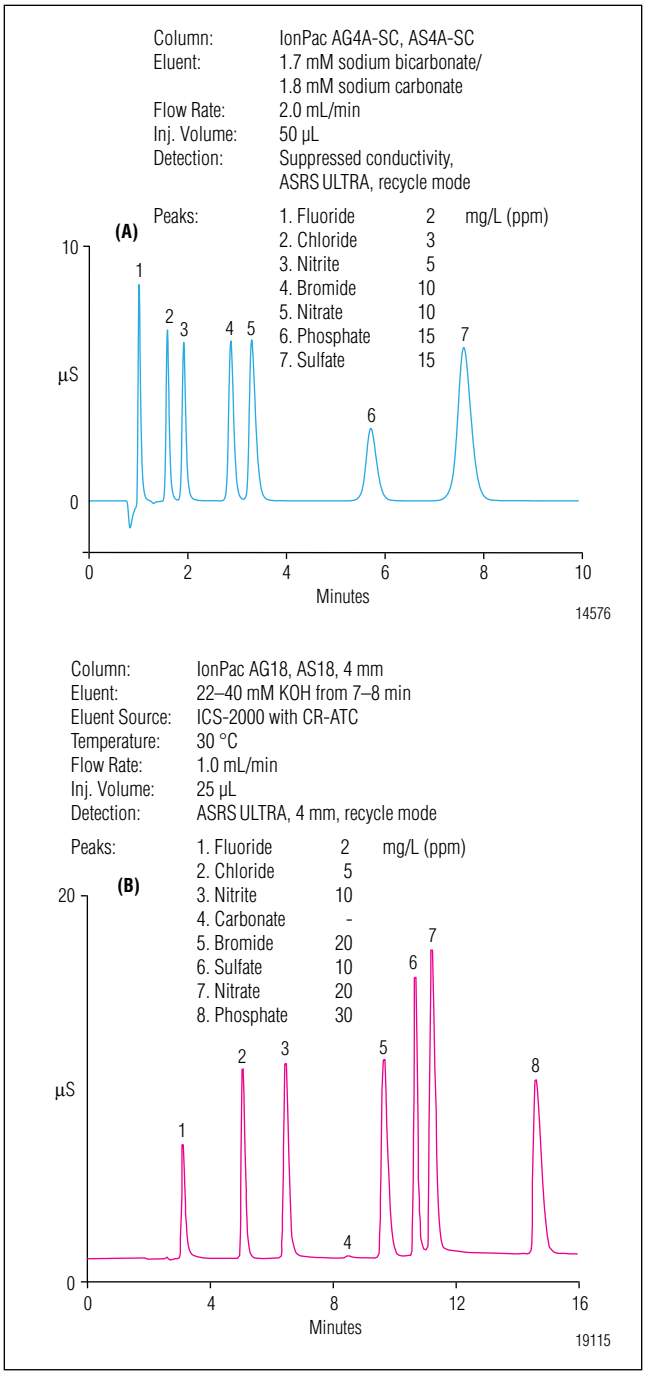


Figure 1. Separation of common inorganic anions using the IonPac AS4A-SC column (A) and the IonPac AS18 column (B).

Traditionally, common inorganic anions have not been determined using hydroxide eluents due to the lack of a suitable hydroxide-selective column and the difficulty in preparing contaminant-free hydroxide eluents. Additional precautions must be taken when

Table 3. Linearity, MDLs, and Retention Time and Peak Area Precisions Obtained Using the IonPac AS18 Column[a]

Analyte	Range (mg/L)	Linearity (r^2)	Calculated MDL[b] (µg/L)	Retention Time Precision (RSD[c]) %	Peak Area Precision (RSD) %
Fluoride	0.1–100	0.9991	2.3	0.13	0.27
Chloride	0.2–200	0.9999	2.5	0.09	0.19
Nitrite-*N*	0.1–100	0.9992	1.6 (5.3 as NO_2)	0.06	0.25
Bromide	0.1–100	0.9999	5.7	<0.05	0.73
Nitrate-*N*	0.1–100	0.9999	1.6 (7.1 as NO_3)	<0.05	0.19
Phosphate-*P*	0.1–100	0.9999	5.3 (16.3 as PO_4)	<0.05	0.63
Sulfate	0.2–200	0.9998	5.1	<0.05	0.19

[a] Dionex ICS-2000 Reagent-Free IC System

[b] MDL = $\sigma t_{S,99}$ where $t_{S,99}$ = 3.14 for n = 7

[c] RSD = Relative Standard Deviation, n = 7

preparing hydroxide eluents to minimize contamination by carbonate, which can cause a significant baseline shift during a hydroxide gradient and variation in retention times.[11] Therefore, eluents are best prepared from fresh 50% (w/w) sodium hydroxide aqueous solution rather than pellets, because the pellets are normally coated with a layer of carbonate formed when CO_2 from the atmosphere is absorbed onto the pellet surface. The hydroxide solution should be weighed and quickly transferred to a container with an appropriate volume of degassed water and then pressurized with helium. Use of an anion-exchange trap column can reduce carbonate contamination in the eluent. However, a moderate baseline rise is still observed during hydroxide gradient analysis.

To overcome the difficulties typically encountered when preparing hydroxide eluents, an electrolytic eluent generation device has been developed that automates the production of high-purity, carbonate-free potassium hydroxide eluents. This device essentially eliminates the adsorption of carbon dioxide in the hydroxide eluent that can result in undesirable baseline shifts and irreproducible retention times, and therefore compromise the integrity of the analytical results. The replacement of a conventional anion-exchange trap column with a Continuously Regenerated Anion Trap Column (CR-ATC)—for removal of carbonate and other anionic contaminants from the source water—is strongly recommended when using hydroxide eluents. The CR-ATC minimizes baseline shifts, improves retention time stability, and improves detection limits.[12] In addition, the CR-ATC offers several advantages over conventional anion trap columns. The CR-ATC eliminates the need for off-line chemical regeneration of the trap, allowing continuous operation and fast IC system start-up after shutdown.

The quality control section of U.S. EPA Method 300.0 (Section 9.0) requires a demonstration of linearity, MDLs, and acceptable instrument performance by the analysis of a QCS prior to performing analyses using the method. The method linearity using the IonPac AS18 was determined over a seven-point calibration range. MDLs for each of the anions in U.S. EPA Method 300.0 Part A were determined by performing seven replicate injections of deionized water, fortified at a concentration of three to five times the estimated instrument detection limits. Table 2 shows the standards used to calculate the MDLs and concentrations of the QCS. Table 3 shows the linear concentration ranges investigated, the coefficients of determination (r^2), and calculated MDLs for each target anion that was performed on the IonPac AS18 column using electrolytic generation of potassium hydroxide with an ICS-2000 system. Retention time and peak area precisions were determined from seven replicate injections of a QCS prepared in deionized water (Table 3). The high retention time stability can be attributed to the consistent generation of high-purity potassium hydroxide using the ICS-2000 system.

The data in Table 3 represent the typical results expected when using the IonPac AS18 for routine analyses of common inorganic anions with U.S. EPA Method 300.0. These results demonstrate that the IonPac

Table 4. Anion Recoveries for Spiked Water Samples Obtained Using the IonPac AS18 Column						
	Drinking Water		**Raw Water**		**Surface Water**	
Anion	**Amount Added (mg/L)**	**Recovery (%)**	**Amount Added (m/gL)**	**Recovery (%)**	**Amount Added (mg/L)**	**Recovery (%)**
Fluoride	1	115.5	1	99.2	1	103.4
Chloride	40	96.9	30	93.8	30	100.3
Nitrite-*N*	1	103.8	2	106.4	2	115.1
Bromide	1	102.2	2	105.3	2	100.3
Nitrate-*N*	5	107.7	5	94.9	5	101.5
Phosphate-*P*	5	102.8	10	92.5	10	93.4
Sulfate	60	97.0	40	98.8	80	97.0
	Domestic Wastewater		**Industrial Wastewater**		**Well Water**	
Anion	**Amount Added (mg/L)**	**Recovery (%)**	**Amount Added (mg/L)**	**Recovery (%)**	**Amount Added (mg/L)**	**Recovery (%)**
Fluoride	1	114.5	1	103.1	1	96.9
Chloride	60	101.1	30	94.8	40	99.0
Nitrite-*N*	2	119.9	2	103.5	2	101.1
Bromide	2	106.0	2	104.7	2	102.5
Nitrate-*N*	5	101.8	5	95.1	5	95.0
Phosphate-*P*	20	101.4	5	91.9	5	88.1
Sulfate	56	101.0	80	94.9	50	103.3

AS18 and electrolytically generated hydroxide eluent "improves the separations" as required in Section 9.4.6. The routine use of hydroxide eluents has the potential to further improve the performance of other existing IC methods and applications where carbonate/bicarbonate eluents have commonly been used. The advantages of using hydroxide eluents for IC are improved linearity, lower background conductivity, and improved MDLs when compared to "conventional" IC columns such as the IonPac AS4A that use carbonate/bicarbonate eluents. The use of electrolytically generated potassium hydroxide eluent further increases method automation. Water is the only solution required to operate the system because the hydroxide eluent is electrolytically generated on-line, the CR-ATC requires no off-line regeneration using chemical reagents, and the ASRS electrolytically generates the hydronium ion used for suppression.

The performance of the AS18 was also evaluated through a single-operator precision and bias study using spiked water samples of various origins. Table 4 shows typical recovery results for single-operator data obtained using the IonPac AS18 column for common inorganic anions spiked into drinking water, raw (unfinished) drinking water, and other environmental water matrices.

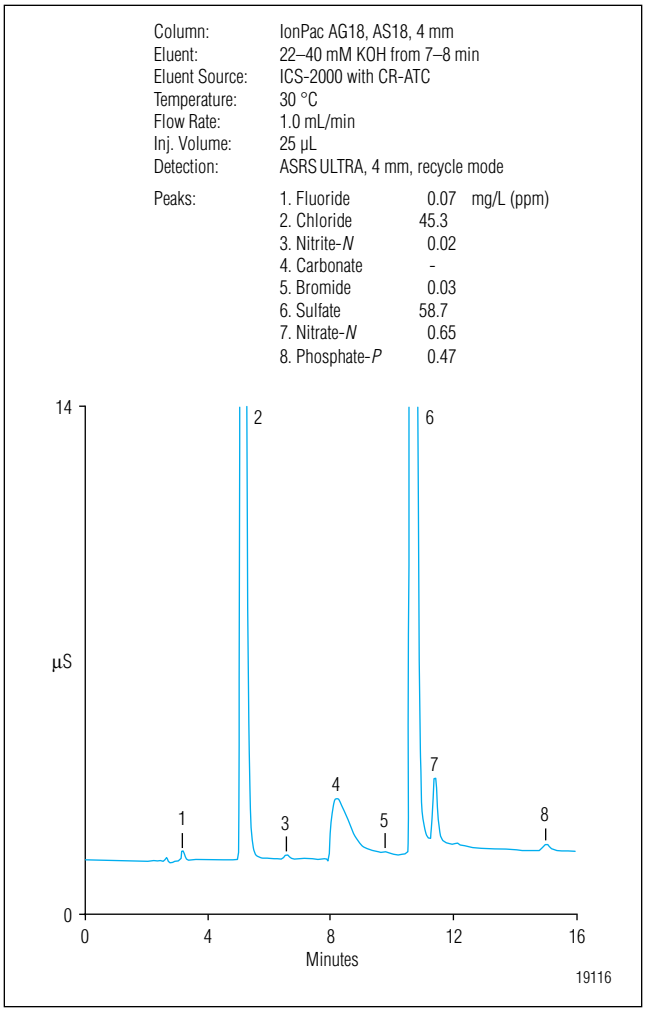


Figure 2. Determination of inorganic anions in Sunnyvale, CA, drinking water using the IonPac AS18 column.

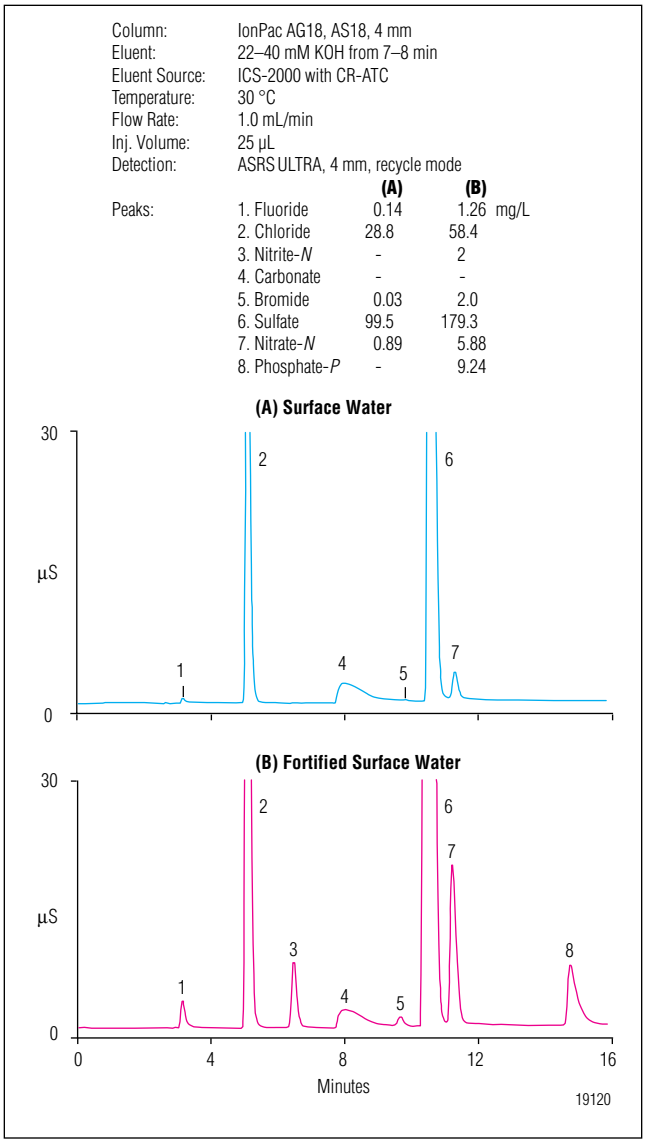


Figure 3. Determination of inorganic anions in (A) surface water and (B) fortified surface water using the IonPac AS18 column.

Figure 2 shows a chromatogram of inorganic anions in Sunnyvale, California, drinking water. As Table 4 shows, all inorganic anions demonstrated acceptable recoveries (i.e., 80–120%) using the criteria outlined in U.S. EPA Method 300.0. Figure 3A shows a chromatogram of surface water obtained from a lake in Northern California. Figure 3B shows the same surface water sample spiked with 1–80 mg/L of the target inorganic anions. All peaks were well resolved and recoveries were within the method's required limits (see Table 4, surface water percent recovery). Despite the high concentration of sulfate present, there was no interference with the relatively low concentration of nitrate.

Figure 4 shows a chromatogram of inorganic anions in a more complex matrix, a domestic wastewater sample obtained from a septic sewage system. This chromatogram demonstrates that a high concentration of sulfate (>200 mg/L) can be accurately quantified with excellent peak efficiency and no column overloading. In fact, U.S. EPA Method 300.0 recommends a maximum calibration concentration point of 95 mg/L sulfate and diluting the sample into the working range if the concentration exceeds 95 mg/L. Therefore, the improved linearity obtained by using hydroxide eluents, and the higher capacity of the AS18 column with a calibration range of 0.2–200 mg/L (see Table 3) for sulfate, can improve sample throughput by reducing the need to dilute and reanalyze high-ionic-strength samples.

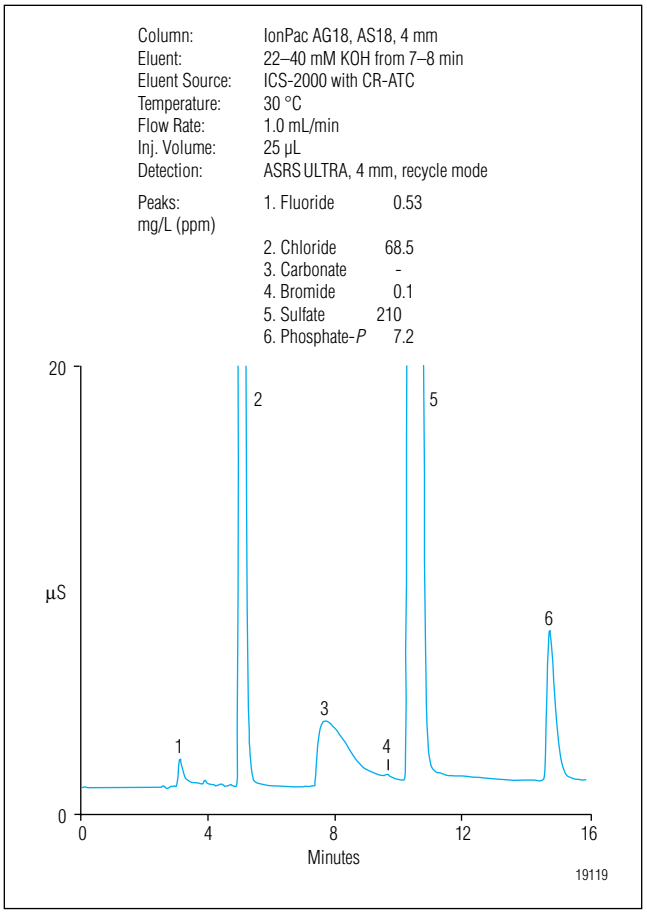


Figure 4. Determination of inorganic anions in domestic wastewater using the IonPac AS18 column.

SUMMARY

The use of a Reagent-Free ion chromatograph with an IonPac AS18 column and electrolytic eluent generation is an improved approach to the routine determination of inorganic anions in environmental waters. The AS18 provides improved retention for fluoride from the column void volume, overall improved selectivity, and a significantly higher capacity compared to the AS4A column specified in U.S. EPA Method 300.0. Quantitative recoveries were obtained for all common inorganic anions spiked into typical environmental waters using the AS18 column. In addition, electrolytic generation of potassium hydroxide eliminates the need to manually prepare eluents, increasing the level of automation, ease of use of the IC system, and data reproducibility. This approach to U.S. EPA Method 300.0 allows improved method performance for resolution, linearity, precision, and MDLs. The use of hydroxide eluents in U.S. EPA Method 300.0 and 300.1 has been determined by the U.S. EPA Office of Water to be acceptable for compliance monitoring under the CWA and SDWA.[13]

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA, 15275-1126 USA, Tel: 1-800-766-7000, www.fishersci.com

VWR Scientific, 1310 Goshen Parkway, West Chester, PA 19380 USA, Tel: 1-800-932-5000, www.vwrsp.com.

REFERENCES

1. *Fed. Regist.* **1999**; Vol. 64, No. 230.
2. *Fed. Regist*. **1995**; Vol. 60, No. 201.
3. *Fed. Regist.* **1998**; Vol. 63, No. 170.
4. *Fed. Regist.* **1994**; Vol. 59, No. 145.
5. *The Determination of Inorganic Anions in Water by Ion Chromatography;* Method 300.0; U.S. Environmental Protection Agency; Cincinnati, Ohio, 1993.
6. Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D., Eds.; *Standard Methods for the Examination of Water and Wastewater,* 18th ed.; Am. Public Health Assoc.; Washington, DC, 1992.
7. *Standard Test Methods for Anions in Water by Chemically Suppressed Ion Chromatography;* D4327-97, Vol. 11.01; American Society for Testing and Materials; West Conshohocken, Pennsylvania, 1999; p. 420–427.
8. Dionex Corporation. Application Note 133; Sunnyvale, CA.
9. Dionex Corporation. Application Note 135; Sunnyvale, CA.
10. Jackson, P. E.; Pohl, C. A. Advances in Stationary Phase Development in Suppressed Ion Chromatography. *Trends Anal. Chem.* **1997**, *16,* 393–400.
11. Liu, Y.; Avdalovic, N.; Pohl, C.; Matt, R.; Dhillon, H.; Kiser, R. An On-Line, High Purity Acid and Base Eluent Generation System for Ion Chromatography. *Am. Lab.*, **1998**, November, 48C–54C.
12. Srinivasan, K.; Lin, R.; Saini, S.; Pohl, C.; Avdalovic, N. A New Continuously Regenerated Trap Column for Ion Chromatography. Presented at the International Ion Chromatography Symposium October 2002; Baltimore, MD.
13. U.S. Environmental Protection Agency, Office of Water; November 19, 2002.

Determination of Trace Concentrations of Disinfection By-Product Anions and Bromide in Drinking Water Using Reagent-Free Ion Chromatography Followed by Postcolumn Addition of *o*-Dianisidine for Trace Bromate Analysis

INTRODUCTION

To ensure that public water systems (PWSs) are free from potentially dangerous microbes, the water is often disinfected before entering a community's distribution system.[1] The most common disinfectants are chlorine, chloramine, chlorine dioxide, and ozone. Many PWSs have converted from using chlorination to chloramination because chlorine treatment produces potential human carcinogens, such as trihalomethanes, that pose human health risks. However, chloramine can produce the by-product chlorate, whereas chlorine dioxide disinfection can generate both chlorite and chlorate.[2] Ozone is a particularly effective disinfection treatment that can alleviate most of the taste and odor issues often present in chlorinated water. However, ozonation of source water containing naturally occurring bromide can produce the disinfection by-product (DBP) bromate, a suspected human carcinogen. The World Health Organization (WHO) has estimated an excess lifetime cancer risk of 10^{-5} for drinking water containing 3 µg/L bromate.[3] In the U.S., the lifetime cancer risk was estimated to be 10^{-4} for drinking water containing 5 µg/L bromate with a potential 10^{-5} risk at 0.5 µg/L.[4]

The U.S. Environmental Protection Agency (EPA) requires 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 water.[5] In 1998, the EPA established a maximum contaminant level (MCL) of 10 µg/L bromate and 1000 µg/L chlorite in drinking water under the Stage 1 Disinfectants/Disinfection By-Products (D/DBP) Rule.[6] The European Union also reduced their regulatory value for bromate from 50 to 10 µg/L[7], and the WHO recently established the same provisional guideline of 10 µg/L bromate as technological advances allowed the determination of lower bromate concentrations.[8]

Traditionally, ion chromatography (IC) has been used to determine bromate and other oxyhalides in environmental waters as described in U.S. EPA Method 300.0 Part B.[9] This method uses an IonPac AS9-SC column with a reported method detection limit (MDL) of 20 µg/L bromate. EPA Method 300.1 was promulgated under the Stage 1 D/DBP Rule as an update to Method 300.0 to further reduce the bromate MDL to 1.4 µg/L. Method 300.1 uses an IonPac AS9-HC column, a high-capacity anion-exchange column, with a carbonate eluent and a large-loop injection followed by suppressed conductivity detection.[10] The MDL for bromate can be reduced to <1 µg/L using sample pretreatment followed by preconcentration.[11,12] However, this approach adds considerable complexity and cost to the analysis.

The U.S. EPA proposed the Stage 2 D/DBP Rule in 2003. Although no changes were made to the MCLs for bromate or chlorite, two postcolumn derivatization methods were promulgated to improve the selectivity and sensitivity for bromate.[13] U.S. EPA Method 317.0 is an extension of Method 300.1 B that combines suppressed conductivity detection and postcolumn addition of o-diansidine (ODA) followed by visible detection to achieve a bromate MDL of 0.1 µg/L with a practical quantitation limit (PQL) of 0.5 µg/L.[4,14] Bromate has also been detected by postcolumn reaction (PCR) with excess iodide under acidic conditions, as described in Method 326.0. The formation of the triiodide ion is detected spectrophotometrically at 352 nm, allowing an MDL <0.2 µg/L bromate using a large-injection volume.[15] IC coupled to mass spectrometry or inductively coupled plasma mass spectrometry has also been used to determine bromate in environmental waters, permitting bromate MDLs of 0.5 and 0.8 µg/L, respectively.[16,17]

Most promulgated EPA methods reported using an IonPac AS9-HC column with a carbonate eluent to determine trace bromate and other disinfection by-product anions in drinking waters. However, hydroxide eluents provide considerably lower suppressed background conductivity, lower noise, and therefore lower detection limits than carbonate eluents. An electrolytically generated hydroxide eluent combined with a hydroxide-selective IonPac AS19 column reduced the bromate MDL by more than 50% compared to using a carbonate eluent.[18] In this application note, we demonstrate the performance of the hydroxide-selective AS19 column for U.S. EPA Method 317.0. This method combines the advantages of a hydroxide eluent using suppressed conductivity detection with postcolumn addition to further improve the quantification of sub-µg/L bromate. The linearity, method detection limits, and the quantification of chlorite, bromate, chlorate, and bromide in municipal and bottled drinking waters are discussed.

EQUIPMENT

A Dionex ICS-2500 Reagent-Free Ion Chromatography system (RFIC™) consisting of:

- GP50 Gradient Pump with vacuum degas option
- CD25A Conductivity Detector
- AD25 UV-Vis Absorbance Detector with 10-mm cell
- AS50 Thermal Compartment with conductivity cell
- AS50 Autosampler
- EG50 Eluent Generator

EluGen® EGC-KOH Cartridge (Dionex P/N 058900)

Continuously Regenerated Anion Trap Column, CR-ATC (Dionex P/N 060477)

PC10 Pneumatic Postcolumn Delivery Module (Dionex P/N 050601)

PCH-2 Postcolumn Reaction Heater (Dionex P/N 039348)

Knitted Reaction Coil, 500 µL, potted (for PCH-2) (Dionex P/N 039349)

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

Chromeleon® Chromatography Workstation

REAGENTS AND STANDARDS

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

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

Ethylenediamine (EDA, Sigma-Aldrich E-1521)

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

Methanol (spectrophotometric grade, Sigma-Aldrich M-3641)

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

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

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

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

Sodium Chlorate ($NaClO_3$, Fluka 71370)

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 0–10 min, 10–45 mM from 10–25 min*
Eluent Source:	EG50 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® external water mode, 130 mA current
Background Conductance:	<1 µS
System Backpressure:	~2200 psi
Run Time:	30 min

PCR

Detection:	Absorbance at 450 nm (tungsten lamp)
Postcolumn Reagent Flow:	0.54 mL/min
Postcolumn Heater Temperature:	60 °C

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

PREPARATION OF SOLUTIONS AND REAGENTS

Postcolumn Reagent

Add 40 mL of 70% nitric acid to about 300 mL of deionized (DI) water in a 500-mL volumetric flask. Add 2.5 g potassium bromide and stir to dissolve. Dissolve 250 mg *o*-dianisidine • 2HCl in 100 mL methanol, add to the nitric acid/KBr solution, and bring to volume with DI water. Allow the solution to stand overnight until the slight champagne color fades. Then filter through a 0.45-µm filter before use.

Stock Standard Solutions

Prepare 1000 mg/L stock standard solutions by dissolving the corresponding mass of the salt in 100 mL DI water (Table 1). Stock standards for most anions listed in Table 1 are stable for at least 6 months when stored at <6 °C. Chlorite is only stable for two weeks when stored at <6 °C and protected from light.

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

Analyte	Compound	Amount (g)
Chlorite	Sodium chlorite ($NaClO_2$), 80%	0.1676
Bromate	Sodium bromate ($NaBrO_3$)	0.1180
Chlorate	Sodium chlorate ($NaClO_3$)	0.1275
Bromide	Sodium bromide (NaBr)	0.1288

Prepare a secondary stock standard containing 5 mg/L each of chlorite, chlorate, and bromide by combining 0.5 mL of each anion in a 100-mL volumetric flask and dilute to volume with DI water. Prepare a separate secondary stock standard containing bromate only at 1 mg/L by adding 0.1 mL of the 1000-mg/L bromate stock to a 100-mL volumetric flask and dilute to volume with DI water.

Working Standard Solutions

Prepare dilute working standards by performing appropriate dilutions of the secondary stock solutions as necessary. Dilute working standards should be prepared monthly, except those that contain chlorite, which must be prepared every two weeks or sooner if degradation is indicated by repeated quality check failures.

Preservation Solution

Dilute 2.8 mL of 99% ethylenediamine (EDA) to 25 mL with DI water according to Section 7.4 in EPA Method 317.0 to prepare a 100-mg/mL EDA solution. Use 50 µL of 100-mg/mL EDA per 100 mL of standard or sample so the final EDA concentration is 50 mg/L. Prepare fresh monthly.

SAMPLE PREPARATION

Filter samples, as necessary, through single-use 0.45-µm syringe filters, 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.

SYSTEM PREPARATION AND SETUP

Prepare the ASRS ULTRA II for use by hydrating the suppressor. Use a disposable plastic syringe and push approximately 3 mL of degassed DI water through the "Eluent Out" port and 5 mL of degassed DI water through the "Regen In" port. Allow the suppressor to stand for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS ULTRA II for use in the external water mode by connecting the "Regen Out" of the suppressor to the "Regen In" of the CR-ATC. The "Regen In" of the suppressor should connect directly to the external water source. The "Regen Out" of the CR-ATC is then connected to the "SRS Waste In" of the EG50 degasser. This configuration allows the eluent out of the analytical column to be connected to the mixing tee of the PCR system.

Install the EGC II KOH cartridge in the EG50 and configure it with the CR-ATC according to the *CR-TC Quickstart* (Document No. 031911). Use the Chromeleon system configuration to set up the EGC II KOH cartridge with the software. Condition the cartridge as directed by the EGC II Quickstart (Document No. 031909) with 50 mM KOH at 1 mL/min for 30 min. Install a 4 × 50 mm AG19 and 4 × 250 mm AS19 column. Make sure the pressure displayed by the pump is at an optimal pressure of ~2300 psi when 45 mM KOH is delivered at 1 mL/min. This setting allows the EG50 degas assembly to effectively remove hydrolysis gases from the eluent. If necessary, install additional backpressure tubing to adjust the pressure to 2300 ± 200 psi.

Configure the ICS-2500 with the PCR system as shown in Figure 1. To maintain a 1-mL/min analytical flow rate, the PCR flow rate was determined based on the analytical to PCR flow rate ratio provided in EPA Method 317.0. For the ICS-2500 system, this ratio resulted in the use of 0.54 mL/min PCR flow rate. Set the temperature on the PCH-2 to 60 °C and the wavelength on the AD25 to 450 nm. Measure the PCR flow rate at the operating parameters by collecting the combined effluent from the IC pump and PCR system in a 10-mL graduated cylinder for at least 5 min. The PCR flow rate is the difference between the total flow rate and

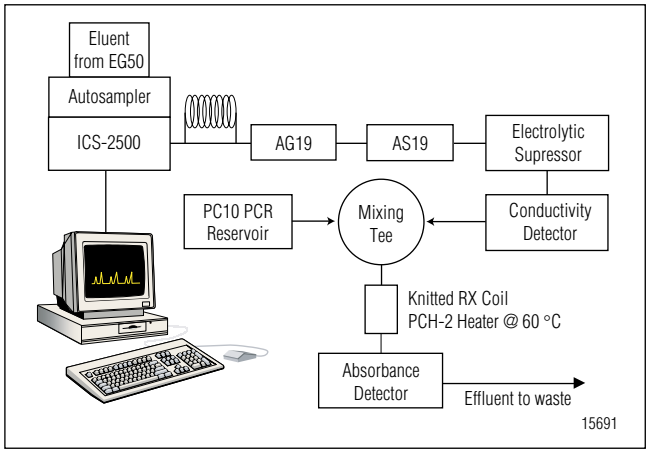


Figure 1. System Configuration for EPA Method 317.0

that of the IC pump divided by the amount of time used for collection (e.g., 5 min). Adjust the pressure of the postcolumn delivery module (PC10) and measure the flow rate again until the correct flow rate of 0.54 mL/min is achieved. Confirm the flow rate daily, whenever the PCR is changed, and if the quality control standard deviates from the EPA's acceptance criteria. Allow both the suppressed conductivity and visible detection baselines to equilibrate. Prior to analyzing any samples, inject 250 µL of DI water using the described method. This is the method blank. No peaks should elute at the same retention time as the target analytes. An equilibrated system has a suppressed background conductance <1 µS and peak-to-peak noise of ~1–2 nS/min.

RESULTS AND DISCUSSION

U.S. EPA Method 317.0 specifies the use of an IonPac AS9-HC column with a 9 mM sodium carbonate eluent for the determination of chlorite, chlorate, and bromide by suppressed conductivity detection and bromate by suppressed conductivity and visible detection after postcolumn reaction with o-dianisidine (ODA).[14] This method reports a bromate detection limit of 0.71 µg/L for a 225-µL injection by suppressed conductivity and 0.12 µg/L by visible absorbance (225-µL injection). Previously, we demonstrated that the bromate detection limit can be reduced further to 0.34 µg/L using an electrolytically generated hydroxide eluent with a novel hydroxide-selective IonPac AS19 column and detection by suppressed conductivity.[18] In this application note, we examine the feasibility of using the IonPac AS19 column with the combination of suppressed conductivity detection and a postcolumn reaction system for visible absorbance detection. The use of a

suitable hydroxide-selective column for this application allows for lower detection limits for the target disinfection by-product anions by suppressed conductivity detection while still providing the improved sensitivity and selectivity for bromate obtained by the PCR.

A calibration curve was established for determining the target analytes, chlorite, bromate, chlorate, and bromide by conductivity detection. In this application, chlorite, chlorate, and bromide were calibrated from 5–500 µg/L, as suggested by Method 317.0. This calibration range is expected to cover the concentrations found in typical environmental samples. However, in field samples, bromate is usually present at significantly lower concentrations than other inorganic DBP anions. The improved sensitivity obtained using an electrolytically generated hydroxide eluent allowed a lower PQL of 1 µg/L bromate compared to 5 µg/L using the AS9-HC column with a carbonate eluent. Therefore, bromate was calibrated from 1–40 µg/L, which is expected to cover concentrations found in most environmental samples. According to Method 317.0, the linear range should not cover more than two orders of magnitude in concentration. Because our linear range extended two orders of magnitude in concentration, seven calibration standards were used. Bromate was calibrated over the range 0.5–15 µg/L with the PCR system. Although this calibration is less than two orders of magnitude, Method 317.0 still recommends using at least five calibration standards for the absorbance detector. Table 2 summarizes the calibration data and method detection limits (MDLs) obtained for the DBP anions and bromide using the AS19 column. The MDLs for the target analytes were determined by performing seven replicate injections of reagent water fortified at a concentration of three to five times the estimated instrument detection limit.[14] The use of a PCR system and visible detection with a hydroxide-selective column provides a bromate PQL of 0.5 µg/L, comparable to that reported in Method 317.0 using the AS9-HC column. Also, the addition of a PCR system did not compromise the sensitivity obtained by suppressed conductivity detection using a hydroxide eluent. Figure 2 shows chromatograms of the target DBP anions containing 5 µg/L bromate and 10 µg/L each of chlorite, chlorate, and bromide using suppressed conductivity (Figure 2A) and visible detection (Figure 2B) following postcolumn addition of ODA. Notice the enhanced response for bromate on the absorbance detector compared to the conductivity detector.

Table 2. Linearity and MDLs for Disinfection By-Products, Anions, and Bromide

Analyte	Range (µg/L)	Linearity (r^2)	MDL Standard (µg/L)	Calculated MDL (µg/L)
Chlorite	5–500	0.9982	1.0	0.26
Bromate (conductivity)	1–40	0.9997	1.5	0.32
Bromate (Vis)	0.5–15	0.9996	0.5	0.14
Chlorate	5–500	0.9999	1.3	0.38
Bromide	5–500	0.9997	2.0	0.52

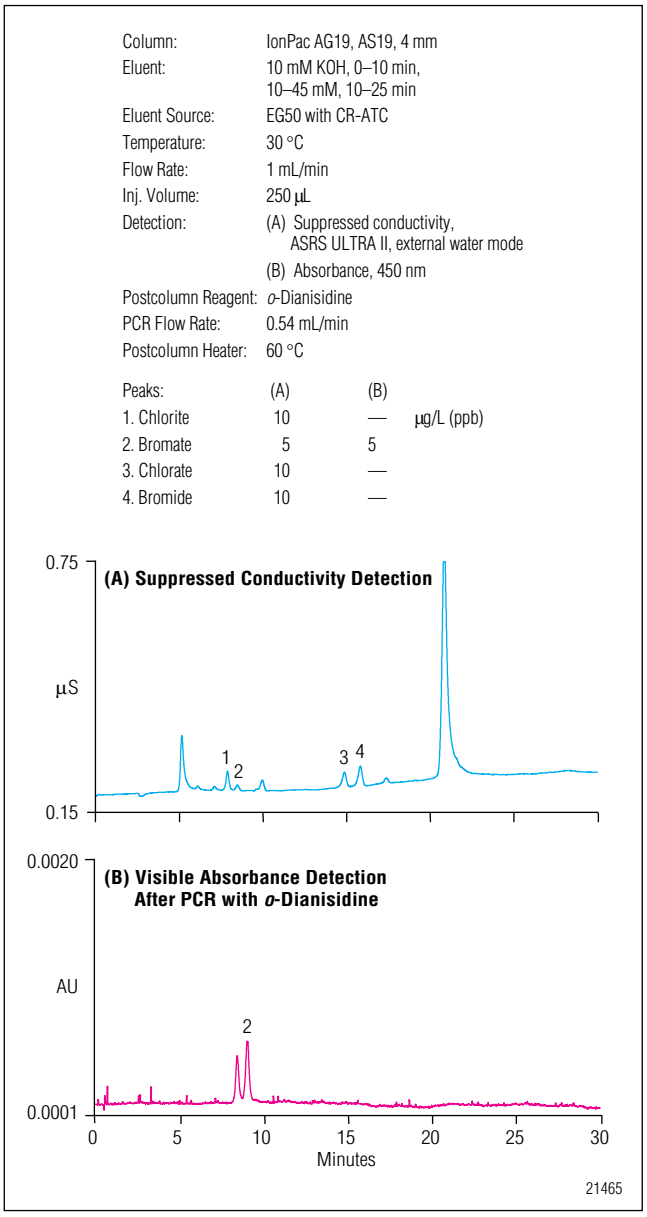


Figure 2. Separation of low ppb DBP anions and bromide using the IonPac AS19 column

Table 3. Recoveries of Trace DBP Anions and Bromide Spiked into Water Samples												
	Tap water			Bottled water A			Bottled water B			Surface water		
Analyte	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	5.0	97.0	<MDL	5.0	94.6	<MDL	5.0	97.5	<MDL	5.0	104.5
Bromate (conductivity)	2.5	3.0	103.3	10.0	10.0	95.7	<MDL	1.0	110.5	<MDL	1.0	103.4
Bromate (Vis)	2.2	3.0	96.3	10.1	10.0	102.8	<MDL	1.0	106.9	<MDL	1.0	97.4
Chlorate	64.0	73.0	94.2	<MDL	5.0	99.0	1.6	5.0	104.0	<MDL	5.0	103.7
Bromide	19.0	20.0	98.1	18.0	20.0	97.5	0.9	5.0	111.5	<MDL	5.0	102.0

EPA Method 317.0 requires an initial demonstration of capability to characterize the instrument and laboratory performance of the method prior to performing sample analyses, as described in Section 9.2.[14] An initial demonstration of precision, accuracy, and analysis of a quality control sample (QCS) are part of the criteria used for this characterization. For evaluating the precision and accuracy of the conductivity detector, Method 317.0 recommends using 20 μg/L each of the four target DBP anions. However, because the use of an electrolytically generated hydroxide eluent improves the overall sensitivity of the method, we determined that the use of 5 μg/L bromate and 10 μg/L each of chlorite, chlorate, and bromide was suitable for characterizing the instrument and laboratory performance. For the absorbance detector, a recommended concentration of 2 μg/L bromate was used. EPA Method 317.0 considers a %RSD <20% and an average recovery of ±15% to be acceptable performance. The precision of our replicate analyses was <4.5% and the recovery was 94–103%, well within EPA's acceptance criteria. A QCS should be analyzed after the calibration curves are initially established, on a quarterly basis, or as required to meet data quality objectives. All QCS analyses in our experiments met the EPA's ±20% recovery criteria.

The analyte recoveries for the target DBP anions and bromide were assessed by fortifying known amounts of the anions into the field samples. The concentrations were fortified at concentrations equal to or greater than the native concentrations. Table 3 summarizes the recovery data for the analysis of drinking water, surface water, and bottled drinking water samples. As shown, analyte recoveries were in the range of 94–112%, well within the 75–125% acceptance criteria of Method 317.0. Figures 3–6 illustrate the performance for the determination of DBP anions and bromide in municipal tap water and bottled drinking water using the IonPac AS19 column. Figure 3 shows chromatograms of a 250-μL injection of tap water using suppressed conductivity and visible detection at 450 nm after postcolumn addition of ODA. In this sample, bromate, chlorate, and bromide were detected in the tap water. Bromate is clearly visible at about 2 μg/L with the absorbance detector. However, this bromate concentration can also be easily determined using suppressed conductivity detection with the method parameters described in this application document. Figure 4 shows the same tap water sample spiked with chlorite, bromate, chlorate, and bromide at concentrations ranging from 3–73 μg/L. Analyte recoveries for this sample ranged from 94–103%.

Figure 5 shows chromatograms of bottled drinking water B using suppressed conductivity and visible detection after postcolumn reaction with ODA. The conductivity detector observed only trace amounts of chlorate and bromide in the sample. However, no bromate was found with either of the detection methods, which is in agreement with the manufacturer who did not report using any ozonation as a disinfection treatment for this bottled water product. Figure 6 shows the same sample spiked with 1–5 μg/L of the target DBP anions. The average recoveries of the spiked sample were 97–112%, well within the acceptance criteria.

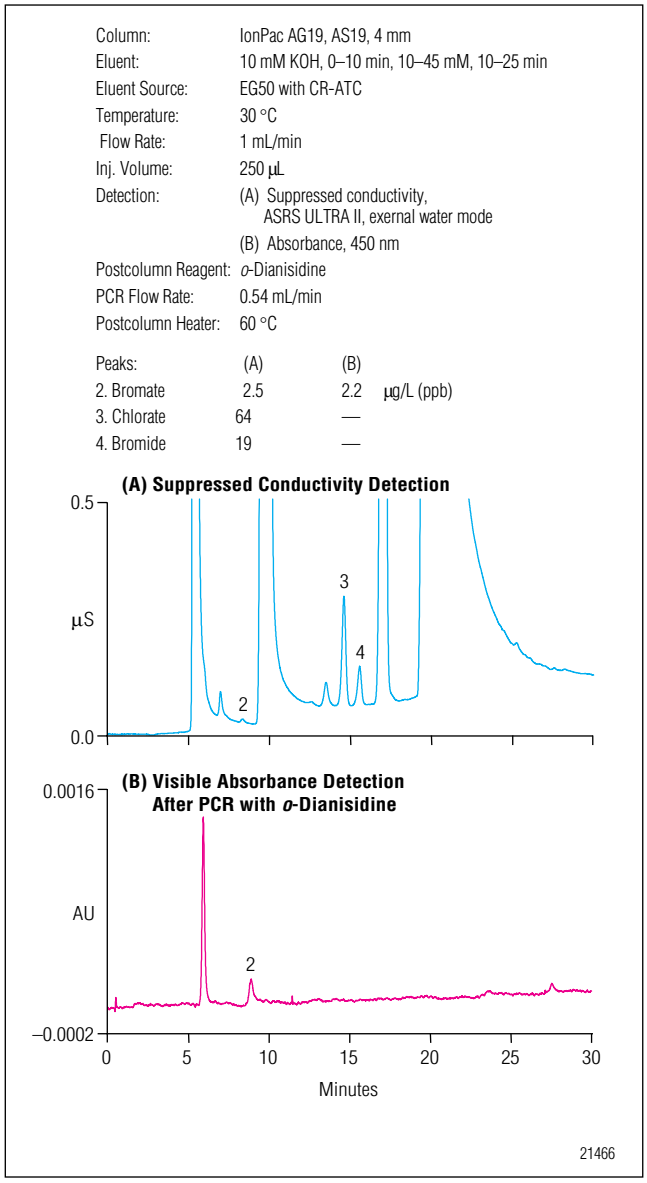


Figure 3. Determination of trace DBP anions and bromide in tap water.

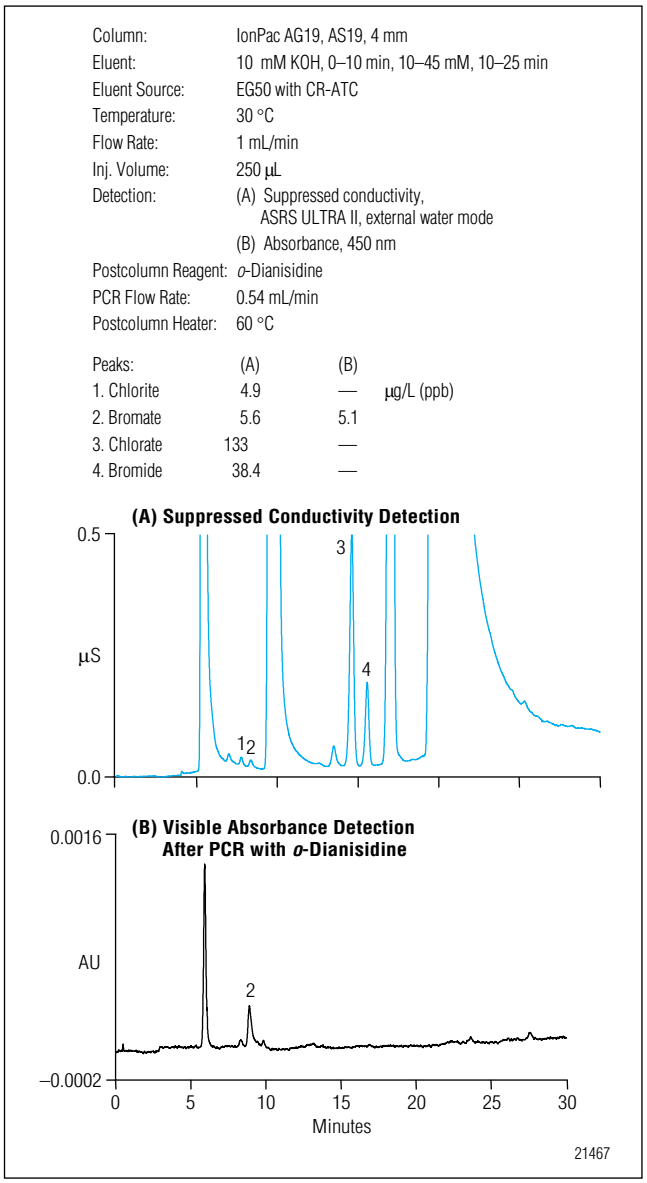


Figure 4. Determination of trace DBP anions and bromide in spiked tap water.

CONCLUSION

This application note described an IC method that used an electrolytically generated potassium hydroxide eluent combined with a hydroxide-selective IonPac AS19 column for the determination of trace DBP anions and bromide. This method used suppressed conductivity detection followed by postcolumn addition of ODA with visible detection to improve the selectivity and sensitivity for the determination of bromate in environmental waters. The use of a hydroxide eluent improved the sensitivity for bromate using suppressed conductivity, compared to a 9 mM carbonate eluent used with the AS9-HC column, as described in Method 317.0.

However, comparable sensitivities for both the AS19 and AS9-HC columns were observed using postcolumn addition of ODA and visible detection. The use of postcolumn addition and visible detection with the AS19 column allowed quantification of bromate from 0.5–15 µg/L without compromising the suppressed conductivity detection of chlorite, bromate, chlorate, and bromide. This application document demonstrates that the hydroxide-selective AS19 column combined with a hydroxide eluent can be successfully used in place of the AS9-HC column for compliance monitoring by U.S. EPA Method 317.0.

Column:	IonPac AG19, AS19, 4 mm
Eluent:	10 mM KOH, 0–10 min, 10–45 mM, 10–25 min
Eluent Source:	EG50 with CR-ATC
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	250 µL
Detection:	(A) Suppressed conductivity, ASRS ULTRA II, external water mode
	(B) Absorbance, 450 nm
Postcolumn Reagent:	*o*-Dianisidine
PCR Flow Rate:	0.54 mL/min
Postcolumn Heater:	60 °C

Peaks:	(A)	(B)	
3. Chlorate	1.6	—	µg/L (ppb)
4. Bromide	0.9	—	

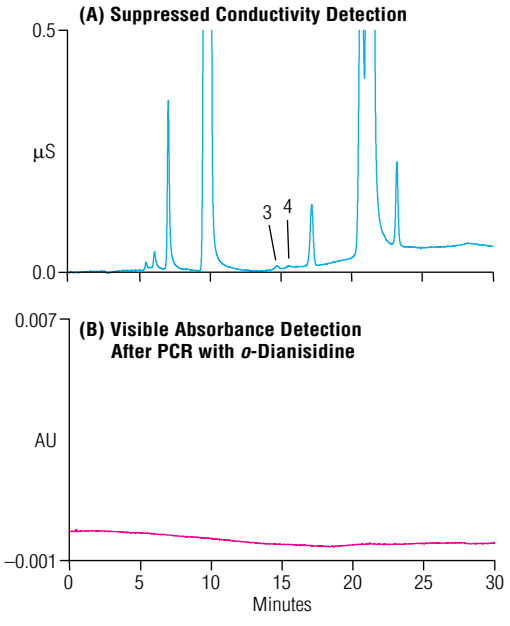


Figure 5. Determination of trace DBP anions and bromide in bottled drinking water B.

Column:	IonPac AG19, AS19, 4 mm
Eluent:	10 mM KOH, 0–10 min, 10–45 mM, 10–25 min
Eluent Source:	EG50 with CR-ATC
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	250 µL
Detection:	(A) Suppressed conductivity, ASRS ULTRA II, external water mode
	(B) Absorbance, 450 nm
Postcolumn Reagent:	*o*-Dianisidine
PCR Flow Rate:	0.54 mL/min
Postcolumn Heater:	60 °C

Peaks:	(A)	(B)	
1. Chlorite	5.1	—	µg/L (ppb)
2. Bromate	1.0	1.1	
3. Chlorate	5.0	—	
4. Bromide	5.0	—	

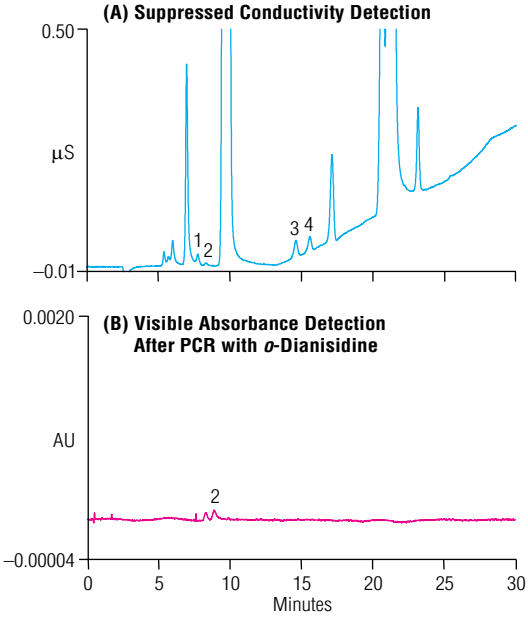


Figure 6. Determination of trace DBP anions and bromide in spiked bottled drinking water B.

REFERENCES

1. U.S. Environmental Protection Agency. *Drinking Water Treatment*; EPA Document No. 810-F-99-013; 1999.
2. World Health Organization. *Disinfectants and Disinfection By-Products*; International Programme on Chemical Safety-Environmental Health Criteria 216; Geneva, Switzerland, 2000.
3. World Health Organization. *Draft Guideline for Drinking Water Quality*; 3rd ed.; 2003.
4. Wagner, H. P.; Pepich, B. V.; Hautman, D. P.; Munch, D. J. *J. Chromatogr.*, *A* **1999**, *850*, 119–129.
5. *Fed. Reg*., **1996**, 61 (94), 24354.
6. *Fed. Reg*., **1998**, 63 (241), 69389.
7. European Parliament and Council. *Quality of Water Intended for Human Consumption*; Directive No. 98/83/EC; 1998.
8. World Health Organization. *Draft Guideline for Drinking Water Quality*; 3rd ed., 2003.
9. U.S. EPA Method 300.0; U.S. Environmental Protection Agency, Cincinnati, OH, 1993.
10. U.S. EPA Method 300.1; U.S. Environmental Protection Agency, Cincinnati, OH, 1997.
11. Joyce, R. J.; Dhillon, H. P. *J. Chromatogr., A* **1994**, *671*, 165–171.
12. Weinberg, H. *J. Chromatogr., A* **1994**, *671*, 141–149.
13. *Fed. Reg.* **2003**, 68 (159), 49647.
14. U.S. EPA Method 317.0; U.S. Environmental Protection Agency, Cincinnati, OH, 2000.
15. U.S. EPA Method 326.0; U.S. Environmental Protection Agency, Cincinnati, OH, 2002.
16. Roehl, R.; Slingsby, R.; Avdalovic, N.; Jackson, P. E. *J. Chromatogr., A* **2002**, *956*, 245–254.
17. U.S. EPA Method 321.8; U.S. Environmental Protection Agency, Cincinnati, OH, 1997.
18. Dionex Corporation. Application Note 167; Sunnyvale, CA.

SUPPLIERS

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

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

Determination of Disinfection By-Product Anions and Bromide in Drinking Water Using a Reagent-Free™ Ion Chromatography System Followed by Postcolumn Addition of an Acidified On-Line Generated Reagent for Trace Bromate Analysis

INTRODUCTION

Public drinking water municipalities routinely disinfect their water supplies to protect the public from potentially dangerous microorganisms. Chlorine dioxide, chloramine, and ozone are common disinfection treatments used to treat public water supplies.[1] These treatments produce by-products that expose the public to potentially harmful chemicals. For example, the use of chlorine dioxide for disinfection treatment can generate the oxyhalide disinfection by-products (DBPs) chlorite and chlorate, whereas the use of chloramine can produce the by-product chlorate.[2] Although ozonation of water supplies is a particularly effective disinfection treatment, bromate may be generated if the source water contains elevated levels of naturally occurring bromide. Bromate has been identified as an animal carcinogen and a potential human carcinogen by the International Agency for Research on Cancer.[3] The U.S. EPA has estimated a potential cancer risk of 1 in 10^4 for a lifetime exposure to drinking water containing 5 µg/L bromate and a potential risk of 1 in 10^5 for 0.5 µg/L bromate.[4]

The U.S. EPA promulgated the Stage 1 Disinfectants/Disinfection By-Products (D/DBP) Rule in 1998 that established a maximum contaminant level (MCL) for bromate at 10 µg/L and an MCL for chlorite of 1000 µg/L.[5] At the same time, the U.S. EPA set a maximum contaminant goal of zero for bromate. In an EU (European Union) directive, the EU also proposed a regulatory value of 10 µg/L bromate that must be met within 10 years after entry into the EU.[6] The World Health Organization has reduced their bromate guideline from 25 µg/L to a provisional value of 10 µg/L.[7]

Considerable efforts have focused on developing improved analytical methods for determining trace concentrations of inorganic DBPs in drinking water to meet current regulatory requirements. Traditionally, ion chromatography (IC) with suppressed conductivity detection has been used to determine chlorite, bromate, and chlorate in environmental waters, as described in Method 300.0 (B).[8] This method describes the use of an IonPac® AS9-SC column with a reported method detection limit (MDL) of 20 µg/L bromate. Method 300.1 (B) was published in the Stage 1 D/DBP Rule as an update to Method 300.0 to further reduce the bromate MDL from 20 to 1.4 µg/L.[9] Method 300.1 describes the use of an IonPac AS9-HC column with a carbonate eluent and a large volume injection followed by suppressed conductivity detection. The bromate detection limit can be reduced to <1 µg/L by using preconcentration after sample pretreatment.[10,11]

Postcolumn derivatization methods can also be used to quantify bromate at sub-µg/L concentrations. The Stage 2 D/DBP Rule published two methods that combine Method 300.1 (B) with a postcolumn reagent (PCR) to further improve the sensitivity of bromate determinations in environmental waters.[12] EPA Method 317.0 combines suppressed conductivity and the postcolumn addition of *o*-dianisidine (ODA) followed by visible detection to achieve a bromate MDL of 0.1 µg/L with a practical quantitation limit (PQL) of 0.5 µg/L.[3,13] However, the ODA PCR is a potential human carcinogen.[14] Therefore, EPA Method 326.0 was developed as an alternative to Method 317.0. Method 326.0 uses a postcolumn reaction that generates hydroiodic acid (HI) in situ, from an excess of potassium iodide (KI), that combines with bromate from the column effluent to form the triiodide anion (I_3^-) that is detected by absorbance at 352 nm.[15]

Most published EPA methods specify the use of an IonPac AS9-HC column and a 9 mM sodium carbonate eluent for determining DBP anions in drinking water. The use of a hydroxide-selective column has not been used for this application due to the lack of a suitably selective column for the target DBP anions, chlorite, bromate, and chlorate. The introduction of the IonPac AS19 column, a hydroxide-selective column, not only improved the selectivity for disinfection by-products, but also provided the typical advantages observed when using a hydroxide eluent for trace applications, such as lower baseline noise and improved sensitivity. For example, the use of the AS19 column combined with an electrolytically-generated potassium hydroxide eluent resulted in a bromate MDL that is approximately three times less than with the AS9-HC column and a carbonate eluent.[16,17] The AS19 can also be used by combining suppressed conductivity detection with postcolumn addition of ODA to further improve the sensitivity of bromate.[18] In this application note, we demonstrate the performance of the AS19 column for EPA Method 326.0. This method allows quantification of bromate to 1 µg/L by suppressed conductivity detection with a hydroxide eluent and 0.5 µg/L using postcolumn reaction with UV detection. The linearity, method detection limits, and quantification of the target DBP anions and bromide in municipal and bottled drinking waters are discussed.

EQUIPMENT

A Dionex ICS-2500 Reagent-Free Ion Chromatography System (RFIC™) consisting of:
- GP50 Gradient Pump with Vacuum Degas Option
- CD25A Conductivity Detector
- AD25 UV/Vis Absorbance Detector with 10-mm cell
- AS50 Thermal Compartment with Conductivity Cell
- AS50 Autosampler
- EG50 Eluent Generator

EluGen® EGC-KOH Cartridge (Dionex P/N 058900)

Continuously-Regenerated Anion Trap Column, CR-ATC (Dionex P/N 060477)

PC10 Postcolumn Pneumatic Delivery Module (Dionex P/N 050601)

PCH-2 Postcolumn Reaction Heater (Dionex P/N 039348)

Knitted Reaction Coil, 500 µL, potted (for PCH-2) (Dionex P/N 039349)

Four 4-L plastic bottle assemblies
- Two bottles for external water mode of suppression
- Two bottles for the AMMS III

Chromeleon® Chromatography Management Software

REAGENTS AND STANDARDS

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

Potassium iodide (KI)

Ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}•4H_2O]$ (Aldrich, 22,136-6)

Ethylenediamine (EDA) (Sigma-Aldrich, E-1521)

Sulfuric acid, 36 N (J.T. Baker INSTRA-ANALYZED 9673-00)

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

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

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

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

Sodium chlorate ($NaClO_3$) (Fluka 71370)

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 0–10 min, 10–45 mM from 10–25 min*
Eluent Source:	EG50 with CR-ATC
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Inj. Volume:	250 µL
Detection:	Suppressed conductivity, ASRS-ULTRA II, 4 mm (Dionex P/N 061561) AutoSuppression® external water mode 130 mA current
Background Conductance:	<1 µS
System Backpressure:	~2200 psi
Run Time:	30 min

PCR

Detection:	Absorbance at 352 nm (deuterium lamp)
Postcolumn Reagent Flow:	0.26 M potassium iodide at 0.3 mL/min
AMMS III:	0.3 N sulfuric acid at 2.5 mL/min
Postcolumn Heater Temp:	80 °C

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

PREPARATION OF SOLUTIONS AND REAGENTS

Ethylenediamine (EDA) Preservation Solution

Dilute 2.8 mL of 99% EDA to 25 mL with DI water according to Section 7.1.3 in EPA Method 326.0 to prepare a 100 mg/mL solution. Use 50 µL of 100 mg/mL EDA per 100 mL of standard or sample so the final EDA concentration is 50 mg/L. Prepare fresh monthly.

Sulfuric Acid Solution (0.3 N)

Dilute 33.3 mL of concentrated sulfuric acid to 4,000 mL of DI water.

Ammonium Molybdate Solution (2.0 mM)

Add 0.247 g of ammonium molybdate $[(NH_4)_6Mo_7O_{24}•4H_2O]$ to about 50 mL DI water in a 100-mL volumetric flask according to Section 7.1.4 in EPA Method 326.0. Dissolve and bring to volume with DI water. This solution is stored in an opaque plastic bottle and prepared fresh monthly.

Postcolumn Reagent (PCR)

The PCR is prepared by adding 43.1 g of potassium iodide (KI) to a 1-L volumetric flask containing about 500 mL DI water and mixing to completely dissolve the solid. Add 215 µL of 2.0 mM ammonium molybdate solution, dilute to volume with DI water, and mix. The PCR is either sparged with helium to remove dissolved gases or sonicated under vacuum for 20 min. Immediately place the solution in the PC-10 reagent delivery vessel and pressurize with helium. Protect the PC-10 from light by covering with aluminum foil. If properly protected from light, this reagent is stable for 24 h.

Stock Standard Solutions

Prepare 1000 mg/L stock standard solutions by dissolving the corresponding mass of the salt in 100 mL DI water (Table 1). Stock standards for most anions listed in Table 1 are stable for at least six months when stored at <6 °C. Chlorite is only stable for two weeks when stored at <6 °C and protected from light.

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

Analyte	Compound	Amount (g)
Chlorite	Sodium chlorite ($NaClO_2$), 80%	0.1676
Bromate	Sodium bromate ($NaBrO_3$)	0.1180
Chlorate	Sodium chlorate ($NaClO_3$)	0.1275
Bromide	Sodium bromide (NaBr)	0.1288

Prepare a secondary stock standard containing 5 mg/L each of chlorite, chlorate, and bromide by combining 0.5 mL of each anion in a 100-mL volumetric flask and diluting to volume with DI water. Prepare a separate secondary stock standard containing 1 mg/L of bromate only by adding 0.1 mL of the 1000 mg/L bromate stock to a 100-mL volumetric flask and dilute to volume with DI water.

Working Standard Solutions

Prepare dilute working standards by performing appropriate dilutions of the secondary stock solutions as necessary. Dilute working standards should be prepared monthly, except those that contain chlorite which must be prepared every two weeks, or earlier if evidence of degradation is indicated by repeated QC failures as discussed in Method 326. Store all working standard solutions containing EDA at <6 °C.

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. If a sample contains an excess amount of chlorite then the chlorite removal procedure described in Section 11.1.4.1 in Method 326.0 must be followed and the sample must then be reanalyzed for bromate. The holding time for preserved samples stored at <6 °C is 28 days for bromate, chlorate, and bromide and 14 days for chlorite.

SYSTEM PREPARATION AND SETUP

Prepare the ASRS® ULTRA II for use by hydrating the suppressor. Use a disposable plastic syringe and push approximately 3 mL of degassed DI water through the "Eluent Out" port and 5 mL of degassed DI water through the "Regen In" port. Allow the suppressor to stand for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS ULTRA II for use in the external water mode by connecting the "Regen Out" of the suppressor to the "Regen In" of the CR-ATC. The "Regen In" of the suppressor should connect directly to the external water source. The "Regen Out" of the CR-ATC is then connected to the "SRS Waste In" of the EG50 degasser. This configuration allows the eluent out of the analytical column to be connected to the mixing tee of the PCR system.

Prepare the AMMS III (P/N 56750) for use by hydrating the suppressor. Use a disposable plastic syringe and push approximately 3 mL of 200 mN sulfuric acid through the "Eluent Out" port and 5 mL of 200 mN sulfuric acid through the "Regen In" port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the suppressor in the chemical regeneration mode. Adjust the head pressure on the 0.3 N sulfuric acid reservoir to deliver a flow rate of 2–3 mL/min (~10–15 psi if an approximately 45 cm piece of 0.01-in i.d. PEEK tubing is connected to the AMMS III "Regen Out" port).

Install the EGC II KOH cartridge in the EG50 and configure it with the CR-ATC according to the *CR-TC Quickstart* (LPN 031911). Use the Chromeleon system configuration to set up the EGC II KOH cartridge with the software. Condition the cartridge as directed by the *EGC II Quickstart* (LPN 031909) with 50 mM KOH at 1 mL/min for 30 min. Install a 4 x 50 mm AG19 and 4 × 250 mm AS19 column. Make sure the pressure displayed by the pump is at an optimal pressure of ~2300 psi when 45 mM KOH is delivered at 1 mL/min. This allows the EG50 degas assembly to effectively remove hydrolysis gases from the eluent. If necessary, install additional backpressure tubing to adjust the pressure to 2300 ± 200 psi.

Configure the ICS-2500 with the PCR system as shown in Figure 1. The PCR flow rate for this application was determined based on the analytical to PCR flow rate ratio provided in EPA Method 326.0. For our system, this resulted in the use of 0.3 mL/min PCR flow rate. Set the temperature on the PCH-2 to 80 °C and the wavelength on the AD25 to 352 nm. Allow both the suppressed conductivity and visible detection baselines to stabilize. Measure the PCR flow rate by collecting the combined effluent from the IC pump and PCR system in a 10-mL graduated cylinder for at least 5 min under the operating method parameters. The PCR flow rate is the difference between the total flow rate and that of the IC pump divided by the amount of time used for collection (e.g., 5 min). Adjust the pressure of the postcolumn delivery module (PC10)

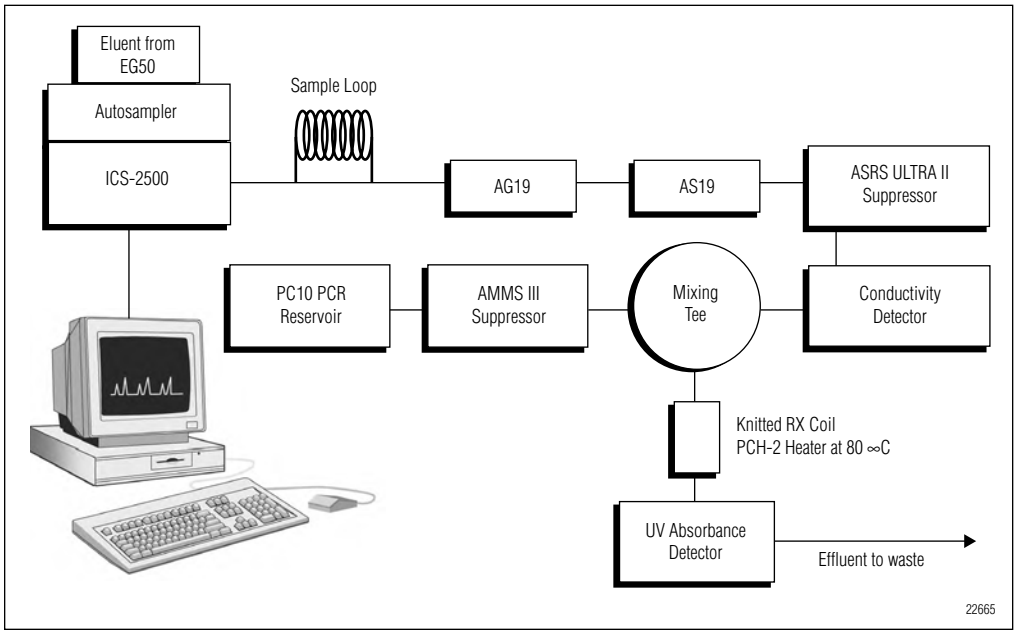


Figure 1. IC system configuration for EPA Method 326.0.

and measure the flow rate again until the correct flow rate of 0.3 mL/min is achieved. Confirm the flow rate daily, whenever the PCR is changed, or if the quality control standard deviates from the EPA's acceptance criteria. Prior to analyzing any samples, inject 250 µL of DI water using the described method. This is the method blank. No peaks should elute at the same retention times as the target analytes. An equilibrated system has a suppressed background conductance <1 µS and peak-to-peak noise of ~1–2 nS per min.

RESULTS AND DISCUSSION

U.S. EPA Method 326.0 specifies the use of an IonPac AS9-HC column with a 9 mM sodium carbonate eluent for the determination of chlorite, chlorate, and bromide by suppressed conductivity detection and bromate by suppressed conductivity and UV absorbance detection after postcolumn reaction with acidified potassium iodide.[15] Method 326.0 reports a bromate detection limit of 1.2 µg/L for a 225-µL injection by suppressed conductivity and 0.17 µg/L by UV absorbance (225-µL injection). Previously, we demonstrated that the bromate detection limit by suppressed conductivity can be reduced further to 0.34 µg/L using an electrolytically generated hydroxide eluent and a novel hydroxide-selective IonPac AS19 column.[16] Furthermore, we demonstrated that suppressed conductivity detection and postcolumn reaction with *o*-dianisidine may be used with an electrolytically generated hydroxide eluent and the AS19 column to achieve an bromate detection limit by visible detection equivalent to that reported in Method 317.0.[13] In this application note, we examine the feasibility of using the IonPac AS19 column with the combination of suppressed conductivity detection and a postcolumn reaction system for UV absorbance detection. The use of a suitable hydroxide-selective column for this application allows for lower detection limits for the target disinfection by-product anions by suppressed conductivity detection while still providing the improved sensitivity and selectivity for bromate obtained by the postcolumn reaction system.

Figure 2 shows chromatograms of 5 µg/L bromate and 10 µg/L each of chlorite, chlorate, and bromide. The top chromatogram shows the response obtained using suppressed conductivity detection and the bottom chromatogram was obtained using UV detection after postcolumn reaction with acidified KI. Bromate is well-resolved from chlorite. Although bromate is easily detected at this concentration using suppressed conductivity detection, an enhanced response for bromate is observed after postcolumn reaction with acidified KI followed by UV detection.

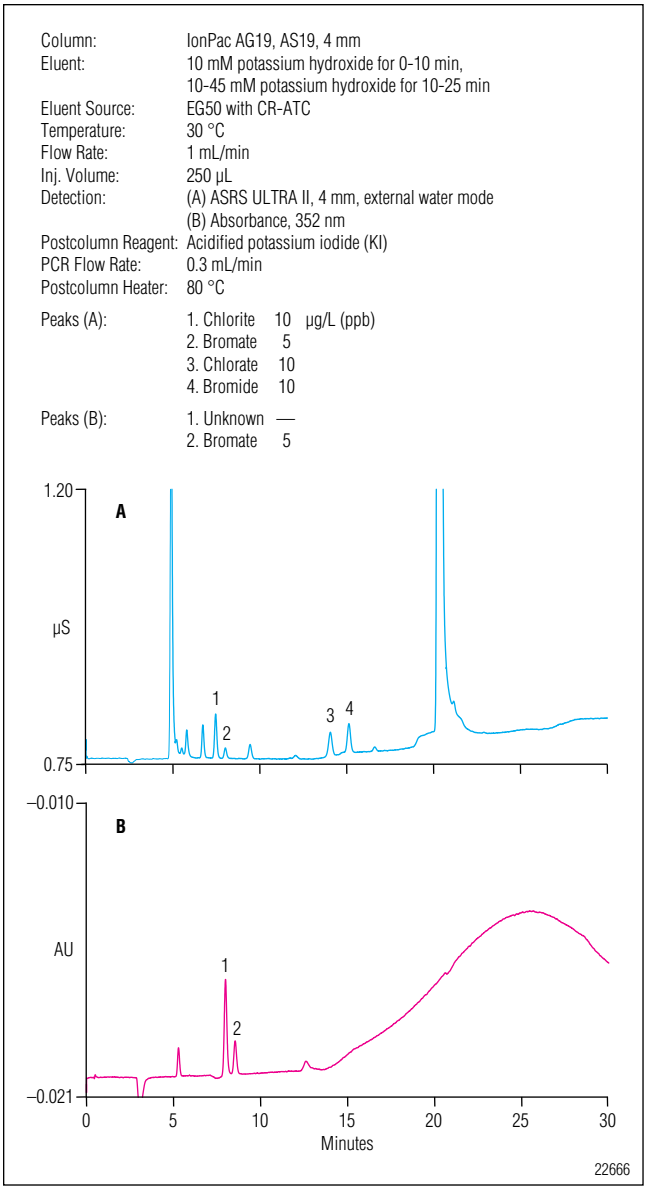


Figure 2. Separation of low ppb DBP anions and bromide on the IonPac AS19 column using suppressed conductivity detection and UV absorbance after PCR with acidified potassium iodide.

Table 2 summarizes the calibration data and method detection limits (MDLs) obtained for the DBP anions and bromide using the AS19 column and an electrolytically generated hydroxide eluent with suppressed conductivity and UV detections. The MDLs for the target analytes were determined by performing seven replicate injections of reagent water fortified at a concentration of three to five times the estimated instrument detection limit.[15] The calculated MDLs for bromate using suppressed conductivity detection followed by postcolumn reaction and UV

Table 2. Linearity and MDLs for Disinfection By-Product Anions and Bromide

Analyte	Range (µg/L)	Linearity (r^2)	MDL Standard (µg/L)	Calculated MDL (µg/L)
Chlorite	5–500	0.9996	1.0	0.17
Bromate (conductivity)	1–40	0.9999	1.0	0.29
Bromate (UV)	0.5–15	1.0000	0.2	0.04
Chlorate	5–500	0.9991	1.2	0.37
Bromide	5–500	0.9998	2.0	0.45

detection were 0.29 µg/L and 0.04 µg/L, respectively. These detection limits are approximately four times less than reported in Method 326.0 using an IonPac AS9-HC column with a carbonate eluent. This method allows quantification of bromate to 1 µg/L using suppressed conductivity and 0.5 µg/L with UV detection using an AS19 column with an electrolytically generated potassium hydroxide eluent. Therefore, bromate was calibrated from 1–40 µg/L with suppressed conductivity and 0.5–15 µg/L using UV detection. Chlorite, chlorate, and bromide were each calibrated from 5–500 µg/L. These calibration ranges are expected to cover the typical concentrations found in environmental samples.

EPA Method 326.0 requires an initial demonstration of capability to characterize the instrument and laboratory performance of the method prior to performing sample analyses, as described in Section 9.2.[15] An initial demonstration of precision, accuracy, and analysis of a quality control sample (QCS) are part of the criteria used for this characterization. For evaluating the precision and accuracy of the conductivity detector, Method 326.0 recommends using 20 µg/L each of the four target DBP anions. However, because an electrolytically generated hydroxide eluent improves the overall sensitivity of the method, we determined that 5 µg/L bromate and 10 µg/L each of chlorite, chlorate, and bromide standards were suitable for characterizing the instrument and laboratory performance. For the absorbance detector, 2 µg/L bromate was used. EPA Method 326.0 considers an RSD ≤20% and an average recovery of ±15% to be acceptable performance. The precision of our replicate analyses was <3.5% RSD and

the accuracy was 93–109%, well within EPA's acceptance criteria. A QCS should be analyzed after the calibration curves are initially established, on a quarterly basis, or as required to meet data quality needs. All QCS analyses in our experiments met the EPA's ±15% recovery criteria.

Table 3 summarizes the method's performance for the determination of trace DBP anions and bromide in municipal and bottled drinking water samples. For samples fortified with low concentrations of the target analytes, recoveries ranged from 90–112%, well within the 75–125% acceptance criteria of EPA Method 326.0. Figures 3–6 illustrate the performance for the determination of DBP anions and bromide in municipal tap waters and bottled drinking waters using the IonPac AS19 column. Figure 3 shows chromatograms of a 250-µL injection of Tap Water B using suppressed conductivity and UV detection at 352 nm after postcolumn reaction with acidified KI. Bromate, chlorate, and bromide were detected in the tap water. Bromide was not completely resolved from the earlier eluting unknown analyte. However, fortification of the sample with 20 µg/L bromide still produced good recovery of 92%. Bromate is clearly visible at about 3 µg/L with the absorbance detector; however, this bromate concentration was also easily determined using suppressed conductivity detection with the AS19 column. Figure 4 shows the same tap water sample spiked with chlorite, bromate, chlorate, and bromide at concentrations ranging from 3–70 µg/L. Analyte recoveries for this sample ranged from 92–108%.

Bottled Water A-2 is the same brand of bottled water product as A-1, except it was purchased approximately seven months later. The initial bromate concentration detected in A-1 was at the current EPA regulatory limit of 10 µg/L. However, the bromate concentration found in the second purchase (A-2) was ~8.7 µg/L, slightly below the regulatory limit. Figure 5 shows chromatograms of the ozonated bottled drinking water A-2 containing 8.7 µg/L bromate and 3.2 µg/L bromide. The top chromatogram (Figure 5A) shows the response of the target analytes obtained by suppressed conductivity detection and the bottom chromatogram (Figure 5B) was obtained by UV detection after postcolumn reaction with acidified KI. The bromate response is easily observed on both detector channels; however, the response using UV detection is enhanced compared to the conductivity detector. Figure 6 shows the same bottled drinking water sample spiked with 6–10 µg/L of the target DBP anions and bromide.

Table 3. Recoveries of Trace DBP Anions Spiked into Water Samples

Analyte	Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Tap Water A			
Chlorite	4.6	6.9	95.9
Bromate (conductivity)	0.32	1.0	95.5
Bromate (UV/Vis)	0.35	1.0	98.1
Chlorate	74.7	80.1	97.5
Bromide	34.6	39.9	95.4
Tap Water B			
Chlorite	< MDL	4.6	108.0
Bromate (conductivity)	2.4	3.0	102.8
Bromate (UV/Vis)	2.8	3.0	94.7
Chlorate	62.4	69.7	96.7
Bromide	17.5	19.9	92.3
Bottled Water A-1			
Chlorite	< MDL	4.9	105.3
Bromate (conductivity)	9.5	9.7	101.1
Bromate (UV/Vis)	10.8	9.7	97.3
Chlorate	< MDL	6.2	99.8
Bromide	19.0	19.9	95.0
Bottled Water A-2			
Chlorite	<MDL	6.4	95.9
Bromate (conductivity)	8.7	9.7	95.7
Bromate (UV/Vis)	8.5	9.7	98.4
Chlorate	< MDL	6.4	107.6
Bromide	3.2	6.4	111.8
Bottled Water B			
Chlorite	< MDL	4.9	108.3
Bromate (conductivity)	< MDL	1.0	102.4
Bromate (UV/Vis)	< MDL	1.0	104.5
Chlorate	< MDL	5.2	101.5
Bromide	10.4	9.9	90.8

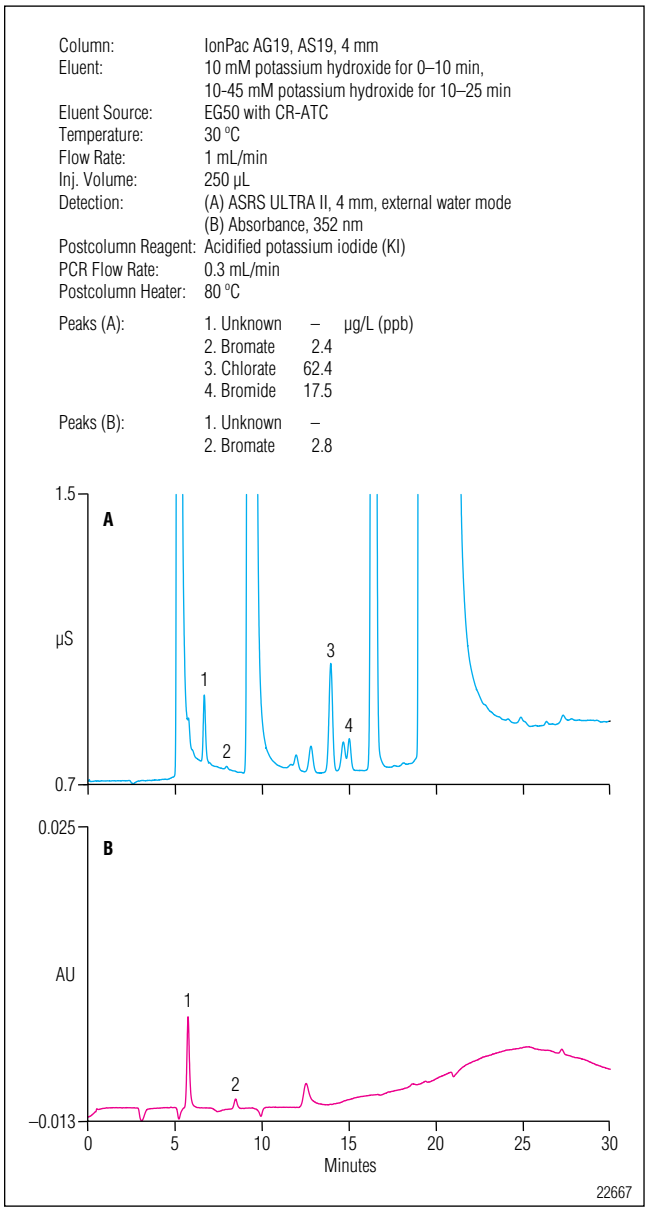


Figure 3. Determination of trace DBP anions and bromide in tap water B using suppressed conductivity detection and UV absorbance after PCR with acidified potassium iodide.

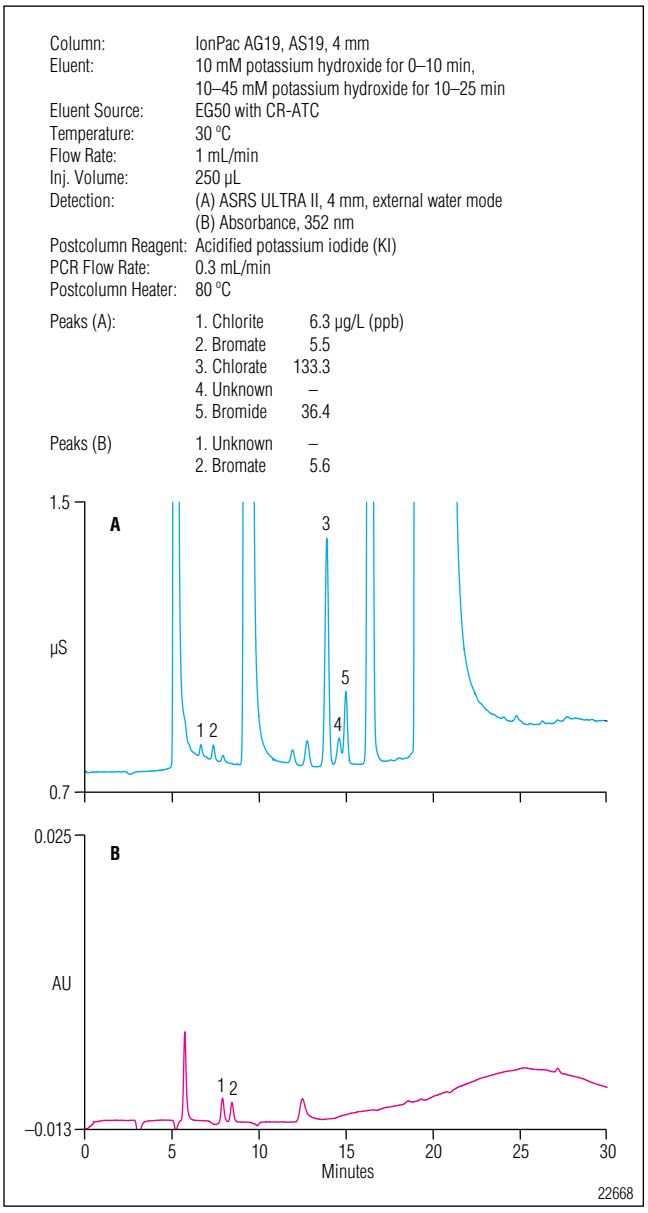


Figure 4. Determination of DBP anions spiked into tap water B using suppressed conductivity detection and UV absorbance after PCR with acidified potassium iodide.

CONCLUSION

This application note described an IC method using an electrolytically generated potassium hydroxide eluent combined with a hydroxide-selective IonPac AS19 column for the determination of trace DBP anions and bromide using suppressed conductivity detection followed by postcolumn addition of acidified KI with UV detection. The postcolumn reaction improves the selectivity and sensitivity for the determination of bromate in environmental waters. The use of a hydroxide eluent improved the sensitivity for bromate using suppressed conductivity and UV detection compared to using a 9 mM carbonate eluent with the AS9-HC column, as described in Method 326.0. Furthermore, the use of postcolumn addition and

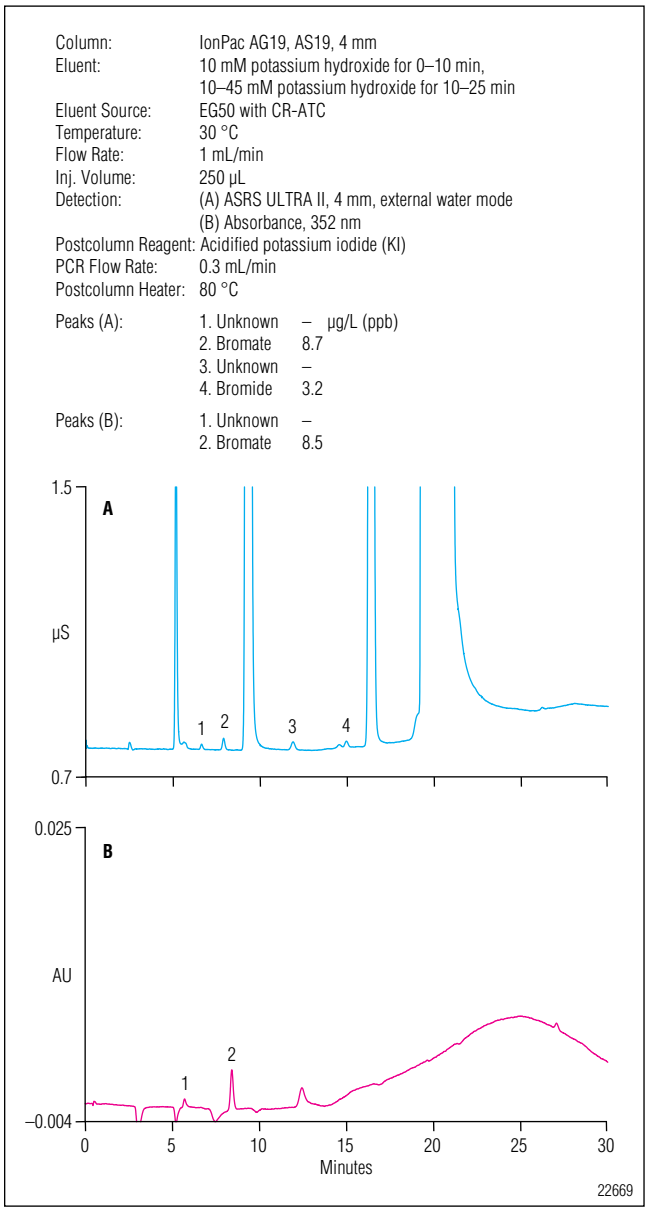


Figure 5. Determination of DBP anions and bromide in bottled water A-2 using suppressed conductivity detection and UV absorbance after PCR with acidified potassium iodide.

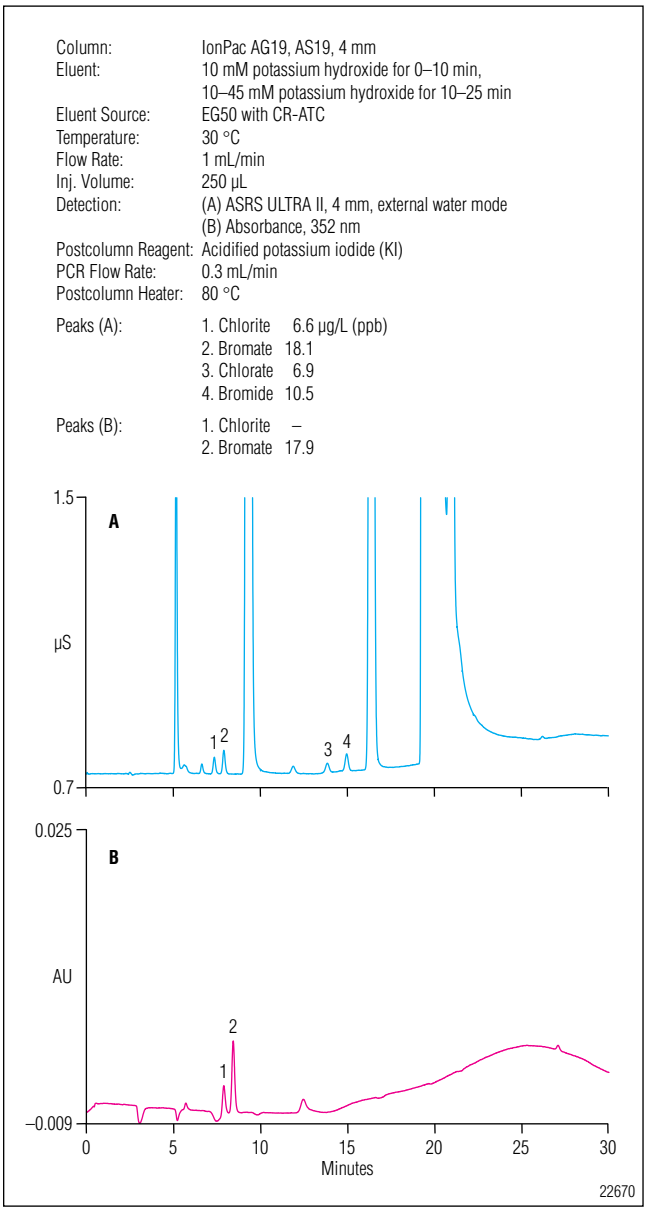


Figure 6. Determination of DBP anions bromide in spiked bottled water A-2 using suppressed conductivity detection and UV absorbance after PCR with acidified potassium iodide.

UV detection with the AS19 column allowed quantification of bromate from 0.5–15 µg/L without compromising the suppressed conductivity detection of chlorite, bromate, chlorate, and bromide. However, the significant improvement in bromate detection by suppressed conductivity with an electrolytically generated hydroxide eluent may eliminate the need for postcolumnn reaction for some environmental samples. Finally, this application document demonstrates that the hydroxide-selective AS19 column combined with a hydroxide eluent can be successfully used in place of the AS9-HC column for compliance monitoring by U.S. EPA Method 326.0.

REFERENCES

1. *Drinking Water Treatment*. EPA-810/F-99/013; U.S. Environmental Protection Agency, U.S. Government Printing Office: Washington, DC, 1999.
2. *Disinfectants and Disinfection By-Products*; World Health Organization, International Programme on Chemical Safety–Environmental Health Criteria 216: Geneva, Switzerland, 2000.
3. Wagner, H. P.; Pepich, B. V.; Hautman, D. P.; Munch, D. J. *J. Chromatogr., A.* **1999,** *850,* 119–129.
4. *Fed. Regist.* **1994,** *59* (145), 38709.
5. *Fed. Regist.* **1996,** *61* (94), 24354.
6. *Quality of Water Intended for Human Consumption.* European Parliament and Council Directive No. 98/83/EC, 1998.
7. *Draft Guideline for Drinking Water Quality.* World Health Organization, WHO Technical Report; 3rd edition, 2003.
8. U.S. EPA Method 300.0, U.S. Environmental Protection Agency, Cincinnati, OH, 1993.
9. U.S. EPA Method 300.1, U.S. Environmental Protection Agency, Cincinnati, OH, 1997.
10. Joyce, R. J.; Dhillon, H. P. *J. Chromatogr., A.* **1994,** *671,* 165–171.
11. Weinberg, H. *J. Chromatogr., A.* **1994,** *671,* 141–149.
12. *Fed. Regist.* **2003,** *68* (159), 49647.
13. U.S. EPA Method 317.0, U.S. Environmental Protection Agency, Cincinnati, OH, 2000.
14. Delcomyn, C. A.; Weinberg, H. S.; Singer, P. C. *J. Chromatogr., A,* **2001,** *920,* 213-219.
15. U.S. EPA Method 326.0, U.S. Environmental Protection Agency, Cincinnati, OH, 2002.
16. *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.* Application Note 167; Dionex Corporation, Sunnyvale, CA, 2004.
17. De Borba, B. M.; Rohrer, J. S.; Pohl, C. A.; Saini, C. Determination of Trace Concentrations of Bromate in Municipal and Bottled Drinking Waters Using a Hydroxide-Selective Column with Ion Chromatography. *J. Chromatogr., A.* **2005,** *1085,* 23–32.
18. *Determination of Trace Concentrations of Disinfection By-Product Anions and Bromide in Drinking Water Using Reagent-Free™ Ion Chromatography Followed by Postcolumn Addition of* o-*Dianisidine for Trace Bromate Analysis.* Application Note 168; Dionex Corporation, Sunnyvale, CA, 2005.

SUPPLIERS

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

Fluka Biochemika, 1001 West St. Paul Avenue, P.O. Box 2060, Milwaukee, WI 53201, USA. Tel: 800-558-9160. www.sigma-aldrich.com

Improved Determination of Trace Concentrations of Perchlorate in Drinking Water Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection

INTRODUCTION

The multiple pathways from which perchlorate may be ingested into the body and its associated health risks has increased the interest in the determination of low concentrations of perchlorate. Perchlorate inhibits the normal uptake of iodide by the thyroid gland which results in reduced thyroid hormone production. Low thyroide hormone production results in improper metabolic regulation and can potentially lead to the development of thyroid tumors in adults.[1,2] The fetuses of pregnant women with hypothyroidism are particularly at higher risk because reduced thyroid hormone production can cause impaired mental development, and in some cases birth defects.[1,3] In 2005, the National Academy of Sciences recommended a reference dose of 0.7 µg/kg/day from all available sources which it believes should not threaten the health of even the most sensitive populations.[1]

Perchlorate has been detected at nearly 400 sites across the United States where most contamination appears to be confined to the western and southwestern regions.[4] It is estimated that over 11 million people have perchlorate in their drinking water supplies at a concentration of 4 µg/L (ppb) or greater.[1] Evidence also suggests that perchlorate can be taken up by plants through contaminated irrigation water and soil.[5] In addition, a recent study reported the detection of perchlorate in food items, such as milk and lettuce.[4,6]

Currently, there are no federal drinking water regulations for perchlorate. However, several states have adopted their own advisory levels that range in concentration from 1 to 18 ppb perchlorate. In 2004, the California Office of Environmental Hazard Assessment established a public health goal of 6 ppb perchlorate.[7] While Massachusetts, Maryland, and New Mexico have established lower perchlorate advisory levels of 1 ppb.[8] The U.S. EPA identified perchlorate as a contaminant of potential concern with its Contaminant Candidate List (CCL) publication in 1998. Following this publication, the EPA proposed the Unregulated Contaminant Monitoring Rule (UCMR).[2] EPA Method 314.0 was developed in conjunction with this publication to determine trace concentrations of perchlorate in drinking water.[9] This method describes the use of a 4-mm IonPac® AS16 column and a 1-mL direct injection with suppressed conductivity detection to determine perchlorate at concentrations of 4 ppb or greater. Although, significant improvements have been made to reduce the method reporting limit (MRL) from 4 to 1 ppb, the determination of trace perchlorate in high-ionic-strength matrices is still a challenging problem.[10] Typically, this requires the use of sample pretreatment cartridges to remove the common anions chloride, sulfate, and carbonate from the matrix. This sample treatment procedure can be a very time consuming and laborious process.

Recently, the US EPA published Method 314.1 as an update to 314.0 to improve the sensitivity for perchlorate in high ionic strength matrices. This method requires the concentration of a 2-mL sample on an IonPac Cryptand C1 preconcentration column followed by matrix elimination with 1 mL of 10 mM sodium hydroxide.[11,12] Perchlorate is then separated using a 2-mm IonPac AS16 column in the primary method. If perchlorate is positively identified with this method then the sample must be reanalyzed on a confirmatory column, the IonPac AS20, to verify the presence of perchlorate and thereby reduce the likelihood of a false positive.

Alternatively, a two-dimensional ion chromatographic approach can be used to resolve perchlorate from high concentrations of common matrix ions.[13] The first dimension uses a 4-mm IonPac AS20 column to divert the matrix ions while 5 mL of the suppressed effluent containing perchlorate is trapped on a TAC-ULP1 concentrator column and then separated on a 2-mm IonPac AS16 column in the second dimension for quantitative analysis. This method provides several advantages, such as the ability to inject large sample volumes, the ability to focus the perchlorate that is partially resolved in the first dimension onto a concentrator column and separate it in the second dimension, and the ability to combine two different column chemistries to enhance the selectivity and reduce the possibility of a false positive. This application note demonstrates this approach for determining trace concentrations of perchlorate in environmental waters using the same criteria specified in EPA Method 314.1.

EQUIPMENT

Dionex® ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) system consisting of:

- DP Dual Pump module
- EG Eluent Generator module
- DC Detector/Chromatography module (single or dual temperature zone configuration)
- AS Autosampler with a 5-mL syringe (P/N 053915), 8.2-mL sampling needle assembly (P/N 061267), and sequential injection option (P/N 063294)

Two EluGen® EGC II KOH cartridges (P/N 058900)

Two Continuously-Regenerated Anion Trap Columns, CR-ATC (P/N 060477)

Carbonate Removal Device (CRD), 2 mm (P/N 062986) and 4 mm (P/N 062983)

Four 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Management Software

REAGENTS AND STANDARDS

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

Sodium Perchlorate, $NaClO_4$ (Aldrich 41,024-1)

Sodium Chloride, NaCl (J.T. Baker; VWR P/N JT3625-1)

Sodium Sulfate, Na_2SO_4 (Aldrich 29,931-3)

Sodium Bicarbonate, $NaHCO_3$ (EM Science SX0320-1)

CONDITIONS

First Dimension

Columns:	IonPac AS20 Analytical, 4 × 250 mm (P/N 063148) IonPac AG20 Guard, 4 × 50 mm (P/N 063154)
Eluent:	35 mM potassium hydroxide 0–30 min, step to 60 mM at 30.1 min*, 60 mM 30.1–40 min, step to 35 mM at 40.1 min, 35 mM 40.1–45 min
Eluent Source:	ICS-3000 EG
Flow Rate:	1 mL/min
Temperature:	30 ˚C (lower compartment) 30 ˚C (upper compartment)
Inj. Volume:	4000 µL
Detection:	Suppressed conductivity, ASRS® ULTRA II (4 mm), Autosuppression external water mode (flow rate: 3–5 mL/min) Power setting – 150 mA
CRD:	4-mm format (P/N 062983)
System Backpressure:	~2500 psi
Background Conductance:	~0.2-0.3 µS
Noise:	~1-2 nS/min peak-to-peak
Run Time:	45 min

*The step change described here should occur after the valve on system #2 has switched from the load to inject position.

Second Dimension

Columns:	IonPac AS16 Analytical, 2 × 250 mm (P/N 055378) IonPac AG16 Guard, 2 × 50 mm (P/N 055379)
Eluent:	65 mM potassium hydroxide
Eluent Source:	ICS-3000 EG
Flow Rate:	0.25 mL/min
Temperature:	30 ˚C (lower compartment) 30 ˚C (upper compartment)
Inj. Volume:	5 mL (on the concentrator column from first dimension)
Concentrator	TAC-ULP1, 5 x 23 mm (P/N 061400)
Detection:	Suppressed conductivity, ASRS ULTRA II (2 mm), Autosuppression external water mode (flow rate: 1–3 mL/min) Power setting—41 mA
CRD:	2-mm format (P/N 062986)
System Backpressure:	~2500 psi
Background Conductance:	~0.7–0.8 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	45 min

PREPARATION OF SOLUTIONS AND STANDARDS

Stock Perchlorate Standard Solution

Dissolve 0.1231 g sodium perchlorate in 100 mL of deionized water for a 1000 mg/L standard solution. When stored in an opaque, plastic storage bottle, this stock solution may be stable for up to one year.

Perchlorate Primary Dilution Standard

Prepare 10 mg/L perchlorate solution by adding 1 mL of the 1000 mg/L stock standard in a 100-mL volumetric flask and dilute to volume with deionized water. When stored in an opaque plastic storage bottle, the resulting solution is stable for at least one month.

Perchlorate Secondary Dilution Standard

Prepare a 1 mg/L perchlorate solution by adding 10 mL of the primary dilution solution to a 100-mL volumetric flask and dilute to volume with deionized water. When stored in an opaque, plastic storage bottle, the resulting solution is stable for at least one month.

Perchlorate Calibration Standards

Prepare perchlorate calibration standards at 0.3, 0.5, 1, 3, 5, and 10 µg/L by adding the appropriate volumes of the perchlorate secondary dilution solution to separate 100-mL volumetric flasks.

Common Anion Stock Solution

Prepare 25 mg/mL (25,000 mg/L) each of chloride, sulfate, and bicarbonate. Dissolve 4.121 g sodium chloride in deionized water and dilute to 100 mL. Dissolve 3.696 g sodium sulfate in deionized water and dilute to 100 mL. Dissolve 3.442 g sodium bicarbonate in deionized water and dilute to 100 mL.

Sample Preparation

All samples must be sterile filtered with a 0.2 µm syringe filter (Corning 26-mm surfactant-free cellulose acetate, Fisher 09-754-13) to remove any potential microorganisms. Perchlorate is susceptible to microbiological degradation by anaerobic bacteria.[7] A disposable sterile syringe (Henke Sass Wolf, 20 mL luer lock, Fisher 14-817-33) is used to draw up ~20 mL of the sample followed by attaching a sterile syringe filter. Discard the first 3–5 mL of sample, and then filter the remaining sample in a 125–mL sterile sample container (high density polyethylene, HDPE, I-Chem, Fisher N411-0125). Discard the syringe and filter after each use.

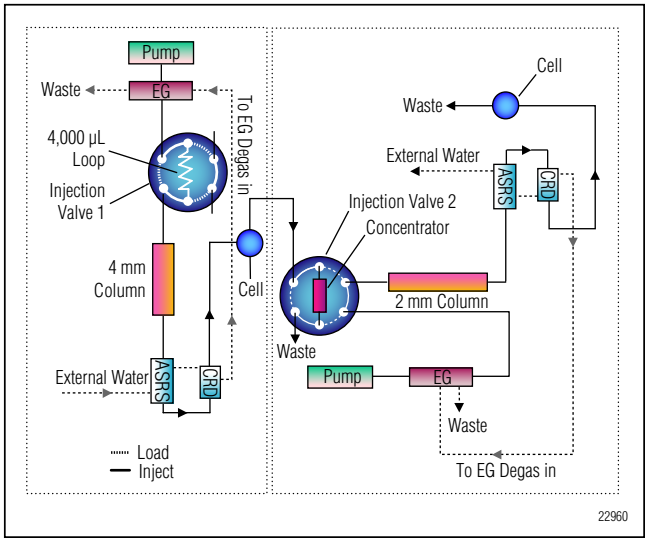


Figure 1. Schematic diagram of two-dimensional system for the determination of trace concentrations of perchlorate.

SYSTEM PREPARATION AND SETUP

Install and configure the EG by first installing backpressure tubing in place of the columns on both system channels to produce a total backpressure of ~2000–2500 psi at a flow rate of 1 mL/min. Install an EGC II KOH cartridge for each system channel. Condition the cartridges by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After completing the conditioning process, disconnect the backpressure tubing temporarily installed in place of the column set. Install a CR-ATC between the EGC II KOH cartridge and the EGC degas. Hydrate the CR-ATC prior to use by following the instructions outlined in the EluGen Cartridge Quickstart Guide (Document No. 065037-02). Figure 1 shows a schematic diagram of the system setup.

Install and configure the AS autosampler. The most accurate and precise sample injections with the AS autosampler are made with a calibrated sample loop, flushed with about four to five times the loop volume. Because this application requires large sample injection volumes, a minimum sample syringe size of 5 mL (P/N 053915) should be installed. To accommodate the larger volume, an 8.2-mL sampling needle assembly

(P/N 061267) is also required for operation. To inject 4000 µL, select the normal mode from the front panel of the autosampler and set the injection loop size to 4000 µL. Prepare a 4000 µL sample loop by measuring approximately 345.5 in. of 0.030-in. i.d. tubing. Verify the volume of the loop by first weighing the empty tubing, fill the tube with DI water, then reweigh the filled tube and calculate the volume. The total sample volume should be ~4000 µL ± 5%. Install the sample loop on injection valve 1 of the DC-3000. To allow independent control of the DC-3000 injection valves, the DC settings in the Chromeleon system configuration must be changed. To modify this configuration, go to the DC high pressure valves tab in the system configuration, double-click InjectValve_2, and change controlled by AS to DC.

Install the IonPac AG20 (4 × 50 mm) and the IonPac AS20 (4 × 250 mm) columns on system #1 in the lower compartment of the DC. Install the IonPac AG16 (2 × 50 mm) and the IonPac AS16 (2 x 250 mm) columns on system #2. Connect a piece of 0.01-in. i.d. tubing from the cell out on system #1 to the sample inlet port on injection valve #2. The length of this tubing should be kept to a minimum. Install a TAC-ULP1 (5 × 23 mm) concentrator in place of the sample loop on system #2. The direction of sample loading should be in the opposite direction of the analytical flow. Make sure the pressure for both systems is ~2200–2500 psi using the operating conditions described earlier to allow the degas assembly to effectively remove electrolysis gases from the eluent. If necessary, install additional backpressure tubing between the degas assembly and the injection valve to achieve the recommended pressure setting. Monitor the pressure periodically as it can gradually rise over time. To reduce pressure, trim the backpressure tubing.

Hydrate the ASRS ULTRA II suppressor prior to installation by using a disposable plastic syringe and push ~3 mL of degassed deionized water through the Eluent Out port and ~5 mL of degassed deionized water through the Regen In port. Allow the suppressor to stand for ~20 min to fully hydrate the suppressor screens and membranes. Hydrate the CRD according to the instructions in the operating manual. Prior to installing the suppressor, rinse the analytical column with 65 mM KOH while diverting to waste. Install the ASRS ULTRA II for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the CRD and connect the Regen In of the suppressor to the external water source. The Regen Out of the CRD is connected to the Regen In of the CR-ATC, while the Regen Out of the CR-ATC connects to the Regen In of the EG degasser.

Equilibrate the AS20 with 35 mM KOH and the AS16 with 65 mM KOH at their respective flow rates shown in the conditions section for approximately 60 min. Analyze a matrix blank by injecting deionized water. An equilibrated system has a background conductance of <0.3 µS and <0.8 µS for the AS20 and AS16 columns, respectively. Determine the cut time (preconcentration time) for the second dimension, as described in the next section, before analyzing perchlorate.

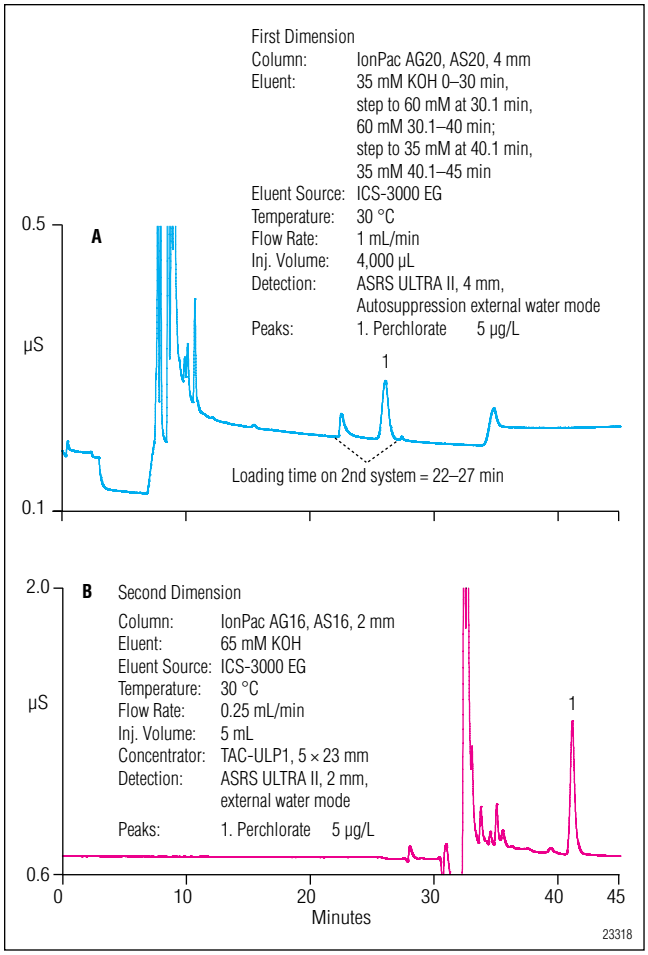


Figure 2. Separation of a 5 µg/L perchlorate standard with the (A) IonPac AS20 column in the first dimension and (B) IonPac AS16 column in the second dimension.

DETERMINING THE CUT TIME FOR THE SECOND DIMENSION

Because there may be slight variations in system plumbing, column capacity, and tubing lengths, individual laboratories should first determine the optimum cut time (from the first dimension) before determining perchlorate in the second dimension. The injection of 4000 µL of sample or standard will increase the retention time of perchlorate on the AS20 column compared to the provided quality assurance report of the column. Therefore, we recommend performing duplicate 4000-µL injections of 5 ppb perchlorate to determine the average perchlorate retention time on the AS20 column. *It is important to verify the retention time of perchlorate on the AS20 column weekly to ensure good trapping efficiency on the TAC-ULP1 concentrator.* In our experiments, the perchlorate retention time (t_{ClO_4}) was approximately 26 min. Therefore, valve #2 in the second dimension was placed in the load position at 22 min ($t_{ClO_4^-}$ 4 min) and then switched to the inject position at 27 min ($t_{ClO_4^+}$ 1 min). In this configuration, perchlorate eluted in ~41 min from the AS16 column. Figure 2 shows example chromatograms of 5 ppb perchlorate separated on the AS20 and AS16 columns.

RESULTS AND DISCUSSION

The second system was calibrated by injecting one blank and duplicate injections of six calibration standards to cover the desired concentration range. Because this two-dimensional (2-D) approach was found to be slightly more sensitive than Method 314.1, the system was calibrated from 0.3 µg/L instead of 0.5 µg/L.[12] However, the minimum reporting level (MRL) remained at 0.5 µg/L for the 2-D method to compare with data generated by Method 314.1. The peak area response generated by the calibration standards was tabulated against the perchlorate concentration using a quadratic regression curve. Table 1 summarizes the calibration data obtained from injecting standards in the range of 0.3–10 µg/L perchlorate. This calibration curve produced a correlation coefficient of 0.9998 with the 2-mm IonPac AS16 column in the second dimension. We verified the accuracy of the calibration curve by injecting a 5 ppb perchlorate standard from a second source. This produced a calculated recovery of 103.3%, well within the ±25% required by Method 314.1.

Table 1. Calibration Data and Method Detection Limits for Perchlorate

Analyte	Range (µg/L)	Linearity (r^2)*	MDL Standard (µg/L)	SD (µg/L)	Calculated MDL (µg/L)
Perchlorate	0.3–10	0.9998	0.06	0.005	0.016

**Quadratic fit*

Section 9.2.7 of Method 314.1 states that the determination of the detection limit is not a specific requirement of this method. However, some laboratories may require this determination due to the various regulatory bodies associated with compliance monitoring. The limit of detection (LOD) was determined for perchlorate using the 2-D method by performing seven replicate injections of deionized water fortified with 0.06 ppb perchlorate. The LOD was calculated using the following equation:

$$LOD = St_{(n-1,\ 1-\alpha = 0.99)}$$

where:

$t_{(n-1,\ 1-\alpha = 0.99)}$ = students' t-value for a 99% confidence level with n-1 (t = 3.14 for seven replicate injections)

n = number of replicates

S = standard deviation of replicate analyses

The results from this equation produced a calculated LOD of 16 ng/L, slightly less than the 23–26 ng/L previously determined with Method 314.1. Table 1 summarizes the results of this calculation using the 2-D method.

To confirm that 0.5 µg/L perchlorate is an appropriate MRL, seven replicates at this concentration were analyzed. The mean and standard deviation of the replicate analyses were then calculated. Section 9.2.4 describes equations used to determine the upper and lower limits for the Prediction Interval of Results (PIR). The results of these equations produced lower and upper limits for the PIR at 86.9% and 107.5%, respectively. These recovery limits are well within the ±50% requirement of Method 314.1. Therefore, 0.5 µg/L perchlorate is an acceptable MRL for this application. Figure 3 shows a chromatogram of 0.5 µg/L perchlorate standard separated on the AS16 column.

Samples containing high concentrations of the common anions chloride, sulfate, and carbonate influence the integrity of perchlorate and dramatically reduce the likelihood of obtaining meaningful results. To overcome this challenge, Method 314.0 required the use of OnGuard® sample preparation cartridges. However, this time-consuming and laborious procedure still may not yield the desired results depending on the ionic strength of the sample being analyzed. This procedure was overcome with the development of Method 314.1 that allowed the direct analysis of high-ionic-strength matrices with lower limits of detection. The performance of the 2-D method described in this application note was evaluated by preparing the Laboratory Fortified Sample Matrices (LFSM) used in Method 314.1. This was accomplished by adding known quantities of perchlorate to each matrix and calculating the percent recovery. We evaluated the recovery of perchlorate by analyzing six matrices, including reagent water, a synthetic high ionic strength inorganic water (HIW), and four drinking waters from different sources. Each sample was fortified with 0.5 and 5 µg/L perchlorate. To ensure the accuracy of the calibration curve, quality control standards prepared at 0.5, 5, and 10 µg/L perchlorate were analyzed at the beginning, middle, and end of each sample analysis batch.

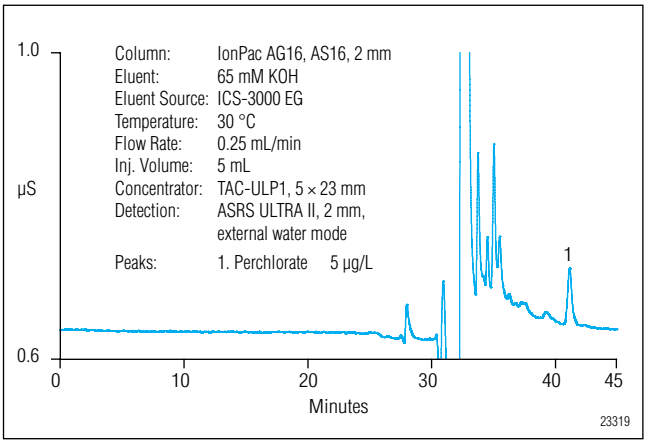


Figure 3. Chromatogram of a 0.5 µg/L perchlorate standard with the IonPac AS16 column in the second dimension.

Table 2. Perchlorate Recoveries from Laboratory Fortified Sample Matrices (LFSM)

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Peak Area Precision (%RSD)	Recovery (%)
Reagent water	—	0.5	7	2.66	97.1
		5.0	7	0.79	101.0
HIW[1]	—	0.5	7	2.08	95.8
		5.0	7	0.22	99.7
Drinking water A	0.060	0.5	7	1.41	95.9
		5.0	7	0.76	98.9
Drinking water B	0.085	0.5	7	1.80	102.0
		5.0	7	0.97	99.0
Drinking water C	0.055	0.5	7	1.56	100.8
		5.0	7	1.00	100.9
Drinking water D	<MDL[2]	0.5	7	1.53	97.1
		5.0	7	0.99	100.9

[1]HIW = high inorganic water contains 1000 mg/L each of chloride, sulfate, and bicarbonate

[2]<MDL = less than the method detection limit

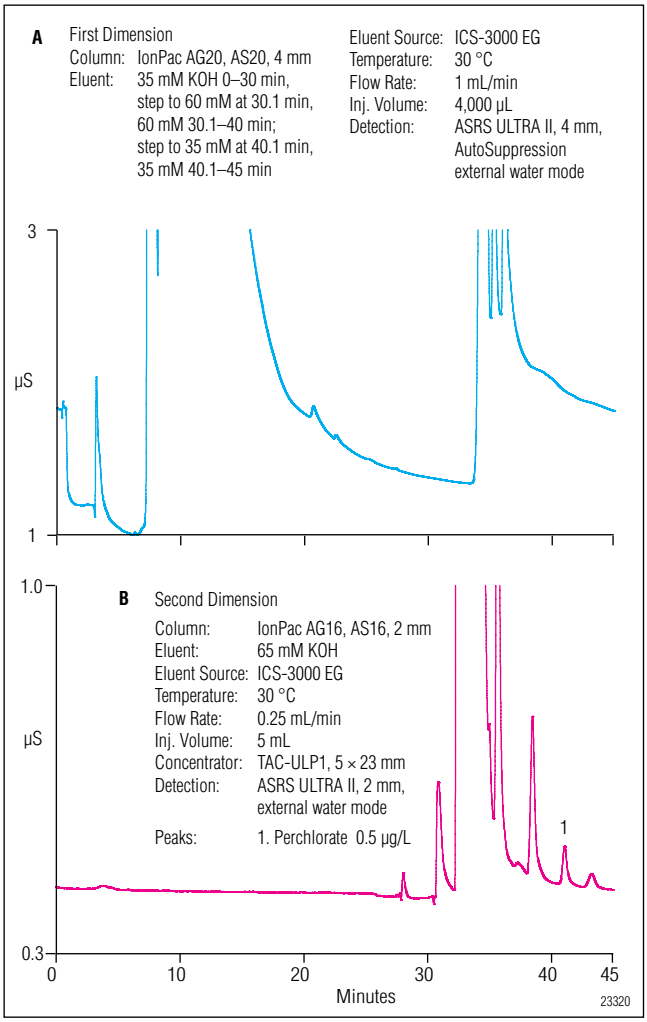


Figure 4. Chromatogram of drinking water D fortified with 0.5 µg/L perchlorate in the (A) first dimension and (B) second dimension.

Table 2 summarizes the performance of the method for determining low concentrations of perchlorate using a two-dimensional ion chromatography method. Calculated recoveries for samples fortified with 0.5 µg/L perchlorate were in the range of 96–102%, well within the ±50% specification of Method 314.1. Similarly, samples fortified with 5 µg/L perchlorate produced recoveries from 99–101% which were within ±25% requirement. Figure 4 shows chromatograms of unfortified and fortified drinking water D using the combined IonPac AS20/AS16 two-dimensional approach. As shown, perchlorate is well resolved from any potential matrix interference, and therefore produces an excellent recovery of 97% when fortified with 0.5 µg/L perchlorate. Previously, an unknown interferent was observed that coeluted with perchlorate in this sample using the IonPac AS20 with EPA Method 314.1.[12] Consequently, this interferent is eliminated by first separating perchlorate on the IonPac AS20 and then trapping a 5-mL portion of the effluent containing the perchlorate peak on a TAC-ULP1 concentrator and, finally, separating the perchlorate on an IonPac AS16 column. By using this two-dimensional approach no interfering peaks were observed in the sample matrices examined in this study. Also, some samples may contain significantly higher concentrations of the common anions typically found in most drinking water samples. To demonstrate applicability of these sample types, a synthetic high inorganic water (HIW) was prepared and analyzed by this method. Figure 5 shows an example of a synthetic HIW sample fortified with 0.5 µg/L perchlorate. As shown, nearly the entire sample matrix is eliminated and, therefore, resulted in excellent recovery of the perchlorate peak.

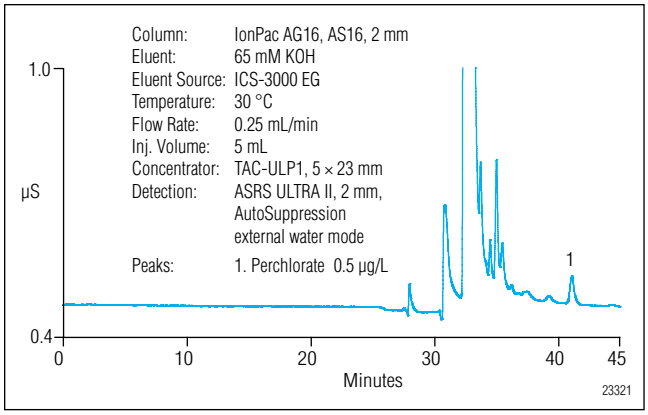


Figure 5. Chromatogram of synthetic high inorganic water fortified with 0.5 µg/L perchlorate in the second dimension.

CONCLUSION

This application note describes a two-dimensional system for determining trace concentrations of perchlorate in environmental waters. The method resulted in an improvement to the existing EPA Methods 314.0 and 314.1 by providing lower detection limits and improved precision and recovery of perchlorate fortified in different sample matrices. In addition, samples can be injected directly without the need for sample preparation, a sample rinse step with sodium hydroxide, and the addition of matrix ions to the standards and samples. These characteristics enhance the method's ease-of-use and can provide improved results between analysts and laboratories. Also, the method is further expanded by combining two different analytical columns with slightly different selectivities to allow for the determination of low concentrations of perchlorate in a wide range of sample matrices.

REFERENCES

1. *Health Implications of Perchlorate Ingestion.* National Research Council of the National Academies, National Academies Press; Washington, D.C., **2005**. http://darwin.nap.edu/books/0309095689/html/R1.html.
2. Wagner, H. P. Suarez, F. X. Pepich, B. V. Hautman, D. P. Munch, D. J. *J. Chromatogr. A,* **2004**, *1039*, 97.
3. Haddow, J. E. Klein, R. Z. Mitchell, M. *New England J. Med.* **1999**, *341*, 2017.
4. *Perchlorate: A System to Track Sampling and Cleanup Results is Needed*. Document GAO-05-462, United States Government Accountability Office; Washington, D.C., May **2005**.
5. Yu, L. Cañas, J.E. Cobb, G.P. Jackson, W.A. Anderson, T.A. *Ecotoxicol. Environ. Saf.* **2004**, *58*, 44.
6. Kirk, A. B. Smith, E. E. Tian, K., Anderson, T. A. Dasgupta, P. K. *Environ. Sci. Technol.* **2003**, 4979.
7. *Perchlorate in California Drinking Water: Overview and Links.* Updated January, **2006**, California Department of Health Services: www.dhs.ca.gov/ps/ddwem/chemicals/perchl/perchlindex.htm.
8. *State Perchlorate Advisory Levels.* U.S. Environmental Protection Agency: Federal Facilities Restoration and Reuse Office, Updated April, **2005**. http://www.epa.gov/fedfac/pdf/stateadvisorylevels.pdf
9. Determination of Perchlorate in Drinking Water Using Ion Chromatography. Method 314.0; U.S. Environmental Protection Agency; Cincinnati, Ohio, 1999.
10. *Determination of Perchlorate in Drinking Water Using Reagent-Free Ion Chromatography.* Application Update 148, Dionex Corporation, Sunnyvale, CA.
11. *Determination of Perchlorate in Drinking Water Using Inline Column Concentration/Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection: Method 314.1*. U.S. Environmental Protection Agency, Cincinnati, Ohio, **2005.**
12. *An Improved Method for Determining Sub-ppb Perchlorate in Drinking Water Using Preconcentration/Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection By U.S. EPA Method 314.1.* Application Note 176, in press. Dionex Corporation, Sunnyvale, CA.
13. Lin, R. De Borba, B. Srinivasan, K. Woodruff, A. Pohl, *C. Anal. Chim. Acta,* **2006**, *567*, 135.

Direct Determination of Cyanide in Drinking Water by Ion Chromatography with Pulsed Amperometric Detection (PAD)

INTRODUCTION

The toxicity of cyanide is well known. Cyanide occurs naturally in many foods (cassava, sorghum, African lima beans, bamboo shoots, bitter almonds, and apricot, cherry, and peach pits) and is naturally generated by microorganisms.[1] Cyanide is used in many industries (e.g., plating and mining) and it can be released into the air from burning coal and plastics. In the U.S., drinking water contamination with cyanide is typically from an industrial source or leached from waste sites.

The U.S. government classifies cyanide as a regulated inorganic contaminant in drinking water (U.S. National Primary Drinking Water Regulations, 40CFR 141.62).[2] These regulations are enforced by the U.S. EPA and state EPA agencies. Bottled water is classified separately as a food, and is regulated by the FDA Center for Food Safety and Applied Nutrition (CFSAN) division. For community water systems, non-transient non-community water systems (defined as a temporary water system for ≥25 people used for less than six months), and drinking water, the maximum contaminant level (MCL) is 200 µg/L cyanide as free cyanide. Typical free cyanide levels are much lower. A 1978 U.S. EPA survey showed that only 7% of drinking water had cyanide concentrations >10 µg/L.[3]

Cyanide is determined as total cyanide (EPA 335.2),[4] disassociated cyanide, and free (amenable) cyanide. Total cyanide is determined by distillation with acid and an oxidizing agent to generate hydrogen cyanide gas that is captured in a pH 13 sodium hydroxide solution, and then determined by a colorimetric or titration method.

The EPA approved free cyanide methods use spectophotometry (335.1),[5] colorimetry (335.3),[6] and ion-selective electrode detection (Standard Methods SM-4500-CN-F).[7] The colorimetric and spectrophotometric methods require distillation and have many interferences, including difficulty with high-pH solutions, oxidizers, and sulfur-bearing compounds. The ion-selective electrode method does not require distillation, but it is very matrix sensitive. Ion chromatography (IC) methods for cyanide use DC amperometric detection[8] or pulsed amperometric detection (PAD).[9–11] The DC amperometry method exhibits electrode fouling problems over time. The reported PAD detection based methods did not determine cyanide in drinking water samples.

This Application Note demonstrates fast, accurate determinations of free cyanide in drinking water samples using IC-PAD with a waveform optimized for cyanide, and use with a disposable silver working electrodes. This method is compatible with the basic solutions used to preserve drinking water samples for cyanide analysis and is unaffected by other compounds typically found in drinking water.

EXPERIMENTAL EQUIPMENT

Dionex ICS-3000 system consisting of:

- Single Gradient Pump (SP) or Dual Gradient Pump (DP) module with degas option and gradient mixer (Dionex GM-4, P/N 049135)
- Detector and Chromatography Module (DC) with a single temperature zone and one injection valve
- Electrochemical Detector ED (P/N 061718) with an electrochemical cell containing a combination pH-Ag/AgCl reference electrode (cell and reference electrode, P/N 061756, reference electrode P/N 071879) and a Certified Disposable Silver (Ag) working electrode (Package of 6 electrodes, P/N 063003)
- AS Autosampler with Sample Tray Temperature Controlling option and 1.5 mL sample tray
- Chromeleon® Chromatography Workstation with Chromeleon 6.7
- Filter unit, 0.2-µm nylon (Nalgene Media-Plus with 90-mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter
- Vacuum pump
- 1.5-mL polypropylene sample vials, with caps and slit septa (Dionex vial kit, P/N 079812)
- Disposable polystyrene 10-mL and 25-mL graduated pipettes
- Micropipettor and tips for preparing samples, standards, and pipetting samples into vials
- Dionex OnGuard® II H cartridges (2.5 cc, package of 48, P/N 057086)
- Black PEEK (0.254-mm or 0.010-in. i.d.) tubing, used for eluent connections to cell, Pump 1, and columns (5 ft, P/N 052306)
- Red PEEK (0.127-mm or 0.005-in. i.d.) tubing, installed in DC heat exchanger (5 ft, P/N 052310)
- Green PEEK (0.76-mm or 0.030-in. i.d.) tubing, installed in AS Autosampler (5 ft, P/N 052305)

REAGENTS AND STANDARDS

Use only ACS reagent grade chemicals for all reagents and standards.

Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity or better, freshly degassed by vacuum filtration

Sodium cyanide, anhydrous (Aldrich, P/N 20,522-2)

Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

pH 7 (yellow) and pH 10 (blue) buffer solutions (VWR International, P/N 34170-130, 34170-133)

Used for experiments that determined retention times and possible interferences:

Copper reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SC194-100)

Iron reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SI124-100)

Nickel reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SN70-100)

Sodium bromide, anhydrous (Aldrich, P/N 310506)

Sodium iodide, anhydrous (Aldrich, P/N 383112)

Sodium sulfide, nonahydrate, >99.99% (Aldrich, P/N 431648)

Sodium sulfite, anhydrous (Aldrich, P/N 239321)

Sodium thiocyanate, (Aldrich, P/N 251410)

Sodium thiosulfate, pentahydrate (Aldrich, P/N 2929)

SAMPLES:

City of Sunnyvale (sampled on multiple days), City of San Jose, and Twain Harte Valley, CA drinking water samples

The sources (flumes) of Twain Harte Valley (an old gold mining region) drinking water and Alamitos Creek in Almaden region (an old mercury mining region) of San Jose, CA

ELECTROCHEMICAL DETECTOR (ED)

The ICS-3000 electrochemical detector is composed of an ED module with the electronics and an amperometric cell containing working, reference, and counter electrodes. The ED is a "plug and play" module and easily installs into the ICS-3000 Detector/Chromatography (DC) upper chamber and the cell mounts on the ED.

In this application, the working electrode is a disposable silver working electrode. When used with a recommended waveform, the disposable silver working electrodes have a background specification of -45 to +55 nC against the reference electrode in AgCl mode. Typically, the background will rise or fall to the equilibrium background within 10 min. The waveform was optimized for cyanide but it can also detect sulfide, bromide, and thiosulfate (Table 1).[11]

Calibration, handling, and installation tips for the reference electrode and Certified Disposable Silver working electrodes are thoroughly described in the System Preparation and Setup section of this application note, the Dionex ICS-3000 Operator's Manual,[12] and the Dionex Product Manual for Gold and Silver Disposable Electrodes.[13]

Table 1. Cyanide Waveform				
Time (sec)	Potential vs Ag/AgCl (V)	Gain Region	Integration	Ramp
0.00	–0.10	Off	Off	On
0.20	–0.10	On	On (Start)	On
0.90	–0.10	On	Off (End)	On
0.91	–1.00	On	Off	On
0.93	–0.30	Off	Off	On
1.00	–0.30	Off	Off	On

CONDITIONS

Columns: IonPac® AS15 Analytical, 2 × 250 mm (P/N 053941)
IonPac AG15 Guard, 2 × 50 mm (P/N 053943)

Flow Rate: 0.25 mL/min

Eluent: 63 mM Sodium hydroxide (31.5% Eluent B, 200 mM sodium hydroxide)

Column Temp: 30 ºC

Tray Temp: 10 ºC

Inj. Volume: 10 µL (PEEK sample loop, P/N 042949), full-loop injection

Detection: Pulsed Amperometric Detection (PAD)

Waveform: See Table 1

Electrodes: Reference: pH-Ag/AgCl electrode (P/N 061879) in AgCl mode

Working: Certified disposable Ag working electrode

Background: 3–13 nC versus Ag/AgCl[a]

Backpressure: ~1100 psi

Noise: <7 pC

Run Time: 25 min

Syringe Speed: 4

Flush Volume: 250 µL

[a] The disposable silver electrodes have a background specification of -45 to + 55 nC versus Ag/AgCl with a recommended waveform.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Preparation

It is essential to use high quality Type 1 water (>18.2 MΩ-cm) containing as little dissolved carbon dioxide as possible. Degas the deionized water before eluent preparation. It is also essential to use high quality 50% (w/w) sodium hydroxide solution for eluent and diluent preparation. Sodium hydroxide pellets are coated with sodium carbonate and, therefore, are not acceptable for this application. Eluent preparation is thoroughly discussed in the AminoPac® PA10 and *AAA-Direct*™ Product Manuals.[14]

Eluent A (degassed deionized water)

To prepare degassed deionized Type 1 water (Eluent A), degas 2-L of Type 1 deionized water using ultrasonic agitation and applied vacuum to aid in removing the gas bubbles. Pour the degassed deionized water into a 2-L precleaned eluent bottle. Connect the eluent bottle to the Eluent A line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Prime the pump with the new eluent.

Eluent B (200 mM Sodium Hydroxide)

Add 2000.0 g of degassed Type 1 deionized water into a 2-L precleaned eluent bottle. This is measured on a top loader balance that is accurate to ±0.01 g. Rinse a 25-mL graduated plastic pipette several times with deionized water and shake out the excess water. Using the pipette, remove 21.0 g of deionized water from the 2-L eluent bottle and discard it. Shake out or blow out with a pipette bulb the last remaining drops in the pipette. Using the same pipette, add 32.0 g (~21.0 mL) of 50% (w/w) sodium hydroxide solution into the 2-L eluent bottle. Connect the eluent bottle to the Eluent B line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new eluent.

100 mM Sodium Hydroxide Diluent Solution

All of the cyanide standards were prepared gravimetrically in 100 mM sodium hydroxide diluent. To prepare the 100 mM sodium hydroxide solution, add 1000.0 g of degassed deionized water into a 2-L precleaned eluent bottle. Rinse a 10-mL graduated, plastic pipette several times with deionized water and shake

out the excess water. Using the pipette, remove 5.2 g of deionized water from the 2-L eluent bottle and discard it. Shake out or blow out with a pipette bulb the last remaining drops in the pipette. Using the same 10-mL pipette, add 8.0 g (~5.25 mL) of 50% (w/w) sodium hydroxide solution into the 2-L eluent bottle. Place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the diluent.

AS Autosampler Flush Solution

Prepare the degassed deionized water in the same manner described in Eluent A.

STANDARD PREPARATION

Warning: Cyanide is a poison by inhalation, contact, and ingestion. It generates the poisonous hydrogen cyanide gas at neutral or acidic pH. Solutions containing cyanide must be stabilized with base. Read and follow the material safety data sheet (MSDS) instructions for personnel handling, exposure, and disposal information. Also consult local safety personnel for regulations concerning the proper disposal of cyanide.

Cyanide Standards

To prepare a 1000 mg/L stock solution, weigh 0.0377 g of reagent grade, sodium cyanide into a 20-mL polyethylene bottle. Add 100 mM sodium hydroxide diluent to a total weight of 20.00 g. Prepare an intermediate standard solution of 1.0 mg/L cyanide by pipetting 20 µL of the 1000 mg/L stock solution into a 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to a final weight of 20.00 g.

To prepare 2.0, 3.0, 5.0, 10.0, 50.0, 100 µg/L working standards of cyanide from the 1.0 mg/L intermediate standard, pipette 40, 60, 100, 200, 1000, and 2000 µL, respectively, of the intermediate standard into 20-mL polyethylene bottles. Dilute these working standards with 100 mM sodium hydroxide to 20.00 g total weight. The stock solution and the intermediate standard are stable for more than a month when refrigerated. The working standards should be prepared daily.

Table 2. Amount of Compound Used to Prepare 20.00 g (~ 20 mL) of Individual 1000 mg/L Stock Solutions

Anion	Compound	Mass (g)
Bromide	Sodium bromide (NaBr)	0.0258
Iodide	Sodium iodide (NaI)	0.0362
Sulfide	Sodium sulfide, nonahydrate ($Na_2S \cdot 9H_2O$)	0.1498
Sulfite	Sodium sulfite (Na_2SO_3)	0.0315
Thiocyanate	Sodium thiocyanate (NaSCN)	0.0279
Thiosulfate	Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	0.0440

Standards for Interference and Retention Time Determination Experiments

To prepare individual 1000 mg/L stock solutions of the ions (bromide, iodide, sulfide, sulfite, thiocyanate, and thiosulfate) for the interference experiments, dissolve the amount of reagent grade compound (Table 2) in deionized water in a 20-mL polyethylene bottle and dilute to 20.00 g with deionized water. These stock standards will be diluted to 1.0 mg/L concentration for interference and retention time determination experiments.

Prepare separate intermediate standards of 1.0 mg/L from each of the 1000 mg/L stock solutions. Pipette 20 µL of the individual stock solution into a 20-mL polyethylene bottle and dilute with deionized water to 20.00 g total weight. To prepare 10 µg/L individual standards of bromide, iodide, sulfide, sulfite, thiocyanate, and thiosulfate, pipette 200 µL of the intermediate standard into a separate 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to 20.00 g total weight. These standards were used to determine anion retention times.

To prepare 5 µg/L and 10 µg/L of separate cyanide standards spiked with 10 µg/L of the individual interference ion (bromide, iodide, sulfide, sulfite, thiocyanate, or thiosulfate), pipette 100 µL and 200 µL, respectively, of the 1.0 mg/L cyanide intermediate standard into individual 20-mL polyethylene bottles. Pipette 200 µL of the 1.0 mg/L intermediate standard of the interference ion (bromide, iodide, sulfide, sulfite, thiocyanate, or thiosulfate) into the 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to 20.00 g total weight. These standards were used to evaluate possible interferences with cyanide determinations.
Note: Sodium sulfide solutions degrade quickly. They should only be prepared from a new bottle of sodium

sulfide, nonahydrate solid. Once exposed to air, sulfide rapidly breaks down to sulfite. All the sulfide solutions, including the 1000 mg/L, are unstable even when refrigerated. Low-level sulfide standards must be prepared every two days and tested at 10 ºC. Intermediate sulfide standards must be prepared every 2 weeks. The 1000 mg/L sulfide solution showed long term stability only when it was preserved by freezing at -10 ºC.

STANDARDS FOR METAL INTERFERENCE EXPERIMENTS

To prepare 650 µg/L iron, and separate 300 µg/L of copper and nickel working standards from the 1000 mg/L iron, copper, and nickel reference standards, pipette 65 µL of the iron reference standard, and 30 µL each of the copper, and nickel reference standards, into individual 125-mL polypropylene bottles. Dilute these working standards with deionized water to 100.00 g total weight. These standards were used to evaluate the interference of dissolved metals on free cyanide determinations.

PROCEDURE TO REMOVE DISSOLVED METALS

To remove the dissolved metals, treat the dissolved metal solutions with 2.5-cc capacity, Dionex OnGuard II H cartridges per the product manual instructions.[15] These cartridges are designed to remove alkali and alkaline earth metals, and cationic transition metals. Fill a 5-mL disposable syringe with the dissolved metal solution, attach a new 2.5-cc OnGuard II H cartridge, and dispense the solution through the cartridge, at ~1 mL/min, and into a 10-mL graduated cylinder. Fill the syringe again and dispense through the same cartridge until the graduated cylinder contains 6 mL of filtrate. Discard this initial filtrate. Tare a 10-mL sample vial and dispense the filtrate into the sample vial. Continue to refill the syringe and dispense the filtrate until 7.80 g of filtrate is dispensed in the vial. Add ~2 drops of 50% sodium hydroxide to the filtrate. Mix thoroughly, and spike the solution with 100 µL of 1.0 mg/L of cyanide, resulting in 10 µg/L cyanide in ~100 mM sodium hydroxide. It is critical that the sodium hydroxide is added and mixed into the solution prior to adding the cyanide. As a control, we treated 10 µg/L of cyanide in 100 mM sodium hydroxide with an OnGuard II cartridge in the same manner as the dissolved metal solutions, except that no additional sodium hydroxide or cyanide was added. A new cartridge was used for each dissolved copper, iron, and nickel solution.

To prepare 10 mL each of the combined cyanide and metal solutions, spike the metal solutions with ~2 drops of 50% sodium hydroxide, mix thoroughly, and spike with 100-µL of 1.0 mg/L of cyanide to a final concentration of 10 µg/L cyanide. These samples were tested immediately.

SAMPLE PREPARATION

Cyanide is reactive and unstable, therefore drinking water samples should be stabilized as soon as possible and free cyanide determined as soon as possible. Oxidizing agents decompose cyanide. Also any free cyanide present at neutral pH will volatilize to hydrogen cyanide.

Because of these issues, the drinking and surface water samples were stabilized as soon as practical. Sunnyvale and San Jose municipal drinking water samples were stabilized within one hour of sampling by addition of a 50% (w/w) sodium hydroxide solution. The procedure is as follows: rinse the 10-mL graduated plastic pipette several times with deionized water and shake out the remaining drops. Using the same pipette, add 2.00 g (~1.5 mL) of 50% (w/w) sodium hydroxide solution into a 250-mL polypropylene bottle, add the municipal drinking water to 250.00 g total weight, then mix thoroughly.

Alamitos Creek (in the Almaden mining area of San Jose) and Twain Harte Valley were located farther away from the laboratory so sampling kits were prepared for the Alamitos Creek surface water, and the Twain Harte Valley drinking and surface water samples. The kit contained disposable vinyl gloves for chemical handling, 100-mL wide-mouth specimen containers to collect the samples, and the 250-mL sampling bottles to store and stabilize the samples, all placed into a large resealable bag. The 250-mL sampling bottles were prepared in a similar manner as those for the municipal drinking samples. At the sampling site, 250 mL of the drinking and surface water samples were collected and measured with the 100-mL specimen containers, transferred to the 250-mL sampling bottles, and then thoroughly mixed with 50% sodium hydroxide, as described above.

Spike recovery samples of 5 and 10 µg/L cyanide from both municipal drinking water samples and the Alamitos Creek surface water were prepared by pipetting 100 µL and 200 µL, respectively, of 1.0 mg/L cyanide standard into 20-mL polyethylene bottles, and diluting with the base-treated water sample to a total weight of 20.00 g.

To remove potentially interfering dissolved metals, portions of both municipal drinking waters, and Twain Harte drinking and surface water samples were treated with the OnGuard II H cartridges. These samples were prepared in the same manner as the dissolved metals samples, though without further sodium hydroxide additions. To prepare 5 and 10 µg/L cyanide spike recovery samples of the drinking and surface water samples and the cartridge-treated drinking and surface water samples, pipette 50 µL and 100 µL, respectively, of 1.0 mg/L cyanide in individual 20-mL polyethylene bottles, and dilute with water sample or treated water sample to 10.00 g total weight.

SYSTEM PREPARATION AND SETUP

The setup for the individual modules, components, and system is thoroughly described in the ICS-3000 Operator's Manual,[16] and ICS-3000 Installation Manual,[17] and the Chromeleon "Help" menus.

PLUMBING THE CHROMATOGRAPHY SYSTEM

Connect black PEEK (0.254-mm or 0.010-in. i.d.) tubing from Pump 1 to the gradient mixer (GM-4) and from the gradient mixer to position "P" on Injection Valve 1 inside the DC module. Connect the red PEEK (0.127-mm or 0.005-in. i.d.) tubing from Injection Valve 1, position "C" to the heat exchanger. Install the IonPac AS15 column set according to the IonPac AS15 Product Manual.[18] Connect the red PEEK tubing exiting the System 1 heat exchanger to the column set. The free end will be installed into the ED cell. Install a 10-µL loop in DC Injection Valve 1 in both "L" positions. Connect the AS Autosampler injection port tubing and the green PEEK (0.76-mm or 0.030-in. i.d.) tubing waste line to DC Injection Valve 1 positions "S" and "W", respectively.

CONFIGURING THE AS AUTOSAMPLER

Configure the AS Autosampler and connect the sample prep and sample syringes according the AS Autosampler Operator's Manual.[19] Enter the loop size (10 µL) in loop size V1, on the AS front panel, under Menu and Plumbing Configuration. Select the syringe sizes of the sample prep and the sample syringe from the pull down menus, under Menu and System Parameters. Also select "Normal" sample mode and "Enable" Wait function, under Menu and System Parameters.

CONFIGURING THE SYSTEM

With all the power on and the Chromeleon monitor program running, open the Chromeleon Server Configuration program. To configure the system, first create a cyanide timebase and then add the devices: ICS-3000 DP or SP pump module, the DC module, and AS Autosampler. If a SP single pump is used, assign the Pump 1 to the cyanide timebase, (right click on SP module, select properties, select Devices tab, and select the cyanide timebase on pull-down menu for the pump). If a DP dual pump is used, select Devices tab, click off the share boxes ("share eluent bottles", and "share waste bottle"). Also insert a "pump off" command in the program for Pump 2. Verify that the AS device has the same options (e.g., sample preparation, temperature tray control, etc.,) as listed on the AS Autosampler module (Installed Options under Module Setup Menu). Save and check the configuration before leaving the program.

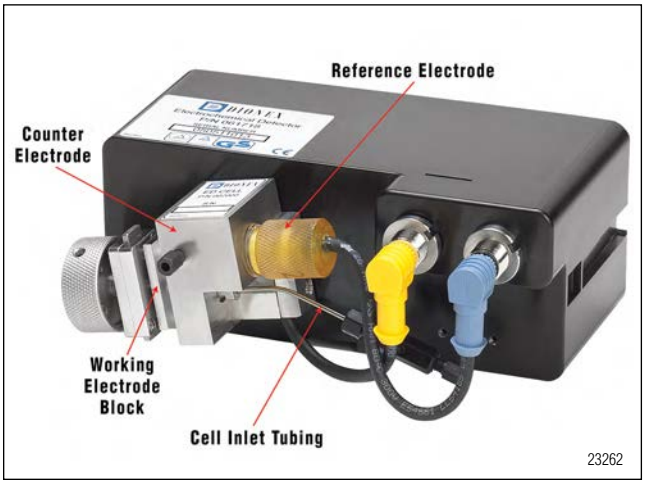


Figure 1. Amperometry cell.

ELECTROCHEMICAL DETECTOR AND AMPEROMETRY CELL

Install the electrochemical detector ED and amperometry cell (Figure 1)[20] and calibrate the combination pH-Ag/AgCl reference electrode. Install the ED module in the middle DC chamber, above Injection Valve 1. Remove the storage cap from the reference electrode but leave the storage cap O-ring in place on top of the reference electrode. The storage cap O-ring will be used again when the reference electrode is removed someday and sealed into the storage cap. It does not interfere with the installation of the reference electrode. Rinse the

KCl storage solution off the reference electrode, pat dry, and place the reference electrode in pH 7 buffer. Open Chromeleon and connect to the cyanide timebase. Click on the Chromeleon Panel icon, expand the cyanide timebase panel, and select the EC Detector tab. Connect the blue lead of the reference electrode to the ED black port. Check the cell on/off button to ensure that the cell is turned off. The pH electrode remains active regardless of the cell power. Click on the "Calibration" button which opens the ED Wellness Panel. Follow the calibration instructions in the "instructions" button or in the ICS-3000 Operator's Manual. Wait for the pH reading to stabilize, then press the "pH Offset Cal" button and wait while it calculates the pH offset. After the reference electrode is finished reading, remove, rinse, and pat it dry. Place the reference electrode in pH 10 buffer and wait until the reading is stable. Enter the "10.00" in the pH slope buffer value, press the "pH slope Cal." button, and wait while it calculates the slope intercept. When the slope intercept is calculated, save, upload the new calibration values, and close the ED Wellness Panel.

Assemble the electrochemical cell. Check that the reference electrode O-ring on the bottom of the reference electrode is in place (install one if it is missing or damaged). Gently screw the reference electrode into the electrochemical cell body. Tighten to a snug fit (finger-tight, do not use tools). Install the Certified Disposable Silver Electrode in the electrochemical cell, according to the Disposable Electrode Ag Installation Guide[21] received with the electrodes. Install the electrochemical cell into the ED. Connect the "yellow" lead on the cell to the "yellow" port on the ED and connect the "blue" lead to the "black" port on the ED. Connect the red PEEK tubing exiting the columns to the cell inlet and direct the cell outlet tubing to waste.

RESULTS AND DISCUSSION

The Cyanide Waveform

The cyanide waveform is a three-potential waveform using E_1, E_2, and E_3. These voltages are applied at the designated times during a 1-sec waveform. E_1, detection and integration potential, is -0.10 V vs Ag/AgCl and maintained from 0.00 to 0.90 sec. E_2 is -1.0 V vs Ag/AgCl at 0.91 sec, and E_3 is -0.30 V vs Ag/AgCl from 0.93 to 1.00 sec. E_2 and E_3 clean and restore the working electrode (Table 1). The waveform is applied continuously when the amperometric cell is turned on.

Chromatography

In the publication that reported the above waveform, the recommended eluent concentration was 62.5 mM sodium hydroxide.[11] In this experiment, the eluent concentration used was 63 mM sodium hydroxide, however, a 200 mM sodium hydroxide solution was prepared because it is easier to prepare consistently and requires less frequent preparation. The pump was programmed to proportion this prepared 200 mM sodium hydroxide solution to create the desired eluent concentration. Cyanide elutes at 5.8 min. Figure 2 shows both 5 and 10 µg/L cyanide standards in 100 mM sodium hydroxide.

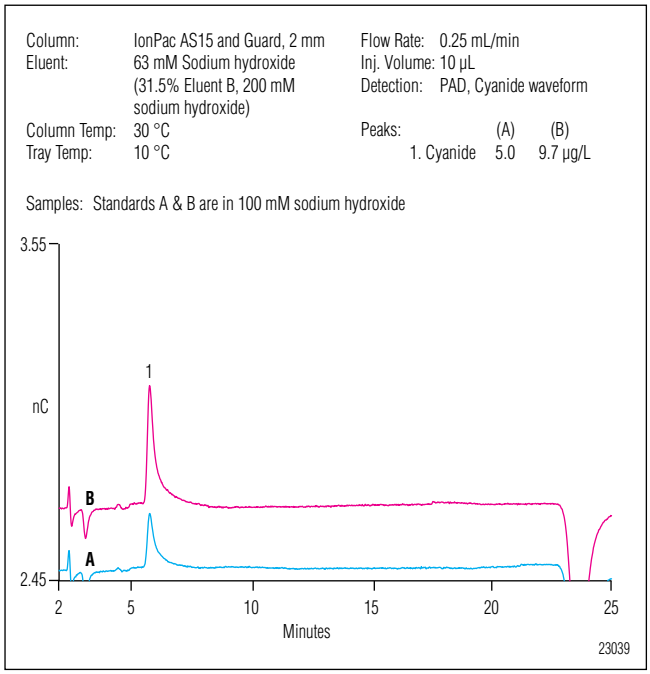


Figure 2. Cyanide standards in 100 mM sodium hydroxide.

INTERFERENCES

In the EPA methods, sulfide and sulfide-generating compounds are cited most often as potential interferences. Sulfide complexes with free cyanide to form thiocyanate. Other interferences cited are nitrate, nitrite, and chlorine. Copper and other transition metals complex with cyanide, preventing the measurement of free cyanide. Copper (II) rapidly oxidizes cyanide to cyanogen gas and copper (I) cyanide precipitates. Acidification will volatilize cyanide to the poisonous hydro-

Table 3. Effect of Bromide, Iodide, Sulfide, Sulfite, Thiocyanate, and Thiosulfate on Cyanide Recovery

Anion[a]	Recovery of 10 µg/L Cyanide
None	102.9 ± 2.3%
Bromide	99.9 ± 2.6%
Iodide	99.9 ± 2.9%
Sulfide	95.9 ± 1.7%
Sulfite	97.7 ± 2.5%
Thiocyanate	110.1 ± 2.6%
Thiosulfate	99.9 ± 2.7%

n=10 for each experiment.

[a] The concentration of each anion was 20 µg/L.

gen cyanide gas, thus preventing it from being measured as free cyanide. In this application, electroactive anions (iodide, thiosulfate, bromide, thiocyanate, and sulfide) are potential inferences, that is, anions detected using a silver working electrode and this waveform.

Possible Anionic Interferences

We determined the interference effects of the non-oxidized and partially oxidized sulfur-containing anions (sulfide, thiosulfate, thiocyanate, and sulfite), bromide, and iodide by analyzing solutions of 10 µg/L cyanide and 20 µg/L of each potential interfering anion. Table 3 shows that the free cyanide concentrations were not significantly affected by any of the anions. The free cyanide concentration did show a small decrease with sulfide and a small increase with thiocyanate. Sulfide and thiocyanate are not expected at high concentrations in drinking water. Because sulfide is not desirable in drinking water due to its disagreeable odor and taste, it is typically removed from municipal water systems by oxidation during the sanitation process. Noise levels always increase during the thiosulfate experiments because thiosulfate is a reducing agent and interacts with the working electrode. These experiments confirmed that the cyanide waveform detects thiosulfate, sulfide, and bromide under these conditions. Although sulfite, thiocyanate, and iodide are also potential interfering anions to cyanide, they were not detected by this waveform (sulfite and thiocyanate) or elute under these conditions (iodide). For applications requiring resolution of cyanide from sulfide, the IonPac AS7[22] column set should be selected.[9,10]

Metal Interferences

We determined the effects of dissolved iron, copper, and nickel on free cyanide determinations. We treated a 10 µg/L cyanide standard with each of the dissolved metal solutions and compared these three solutions to an untreated standard. We selected the iron concentration (600 µg/L) based on the expected levels in drinking water.[23] We arbitrarily set the copper and nickel concentrations for this experiment to 300 µg/L, 50% of the iron levels. We also treated the metal solutions with OnGuard II H cartridges to remove the metals and then added these solutions to cyanide standards.

The results show that copper and nickel reduce free cyanide concentrations. In the iron solution, the free cyanide concentration loss was comparable to the control, 7–10% over 3 days (Figure 3). After 92 h, only 28% of free cyanide remained in the copper solution (Figure 4). In the nickel solution, the free cyanide decreased to 75% within 20 h and then stabilized for the remainder of the 3-day experiment (Figure 5).

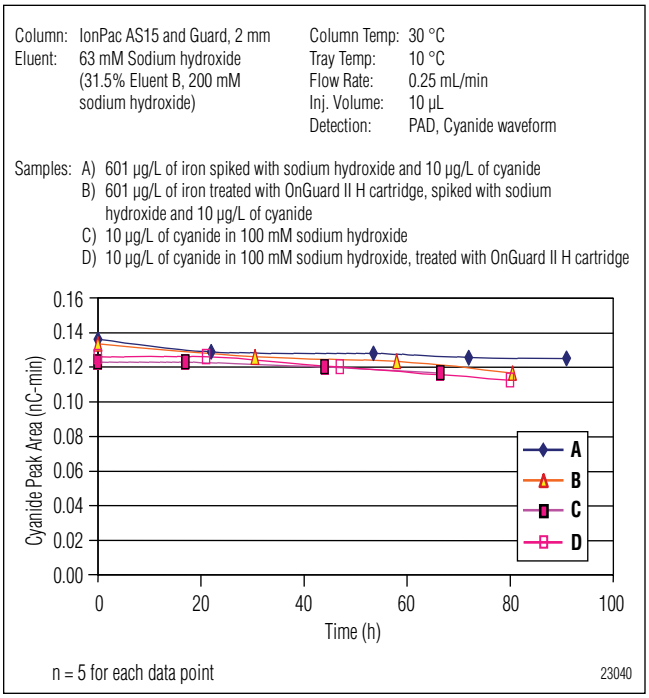


Figure 3. Effect of dissolved iron on free cyanide (10 µg/L).

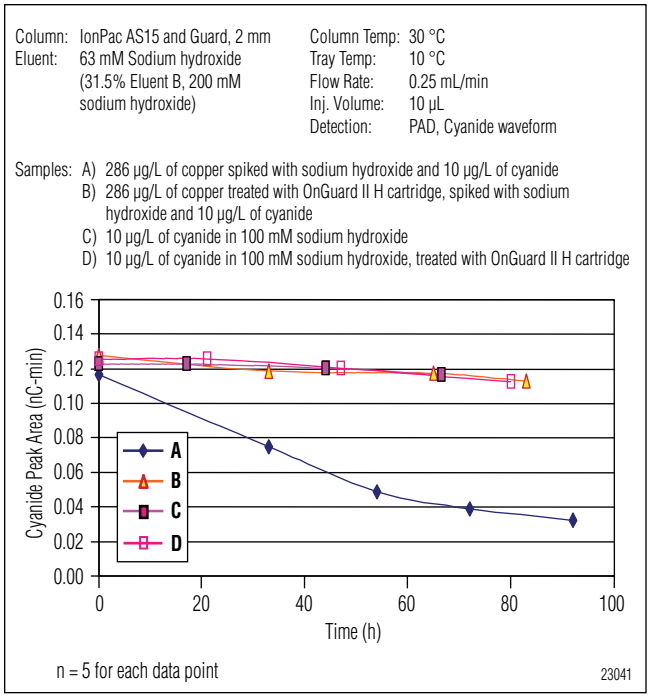


Figure 4. Effect of dissolved copper on free cyanide (10 µg/L).

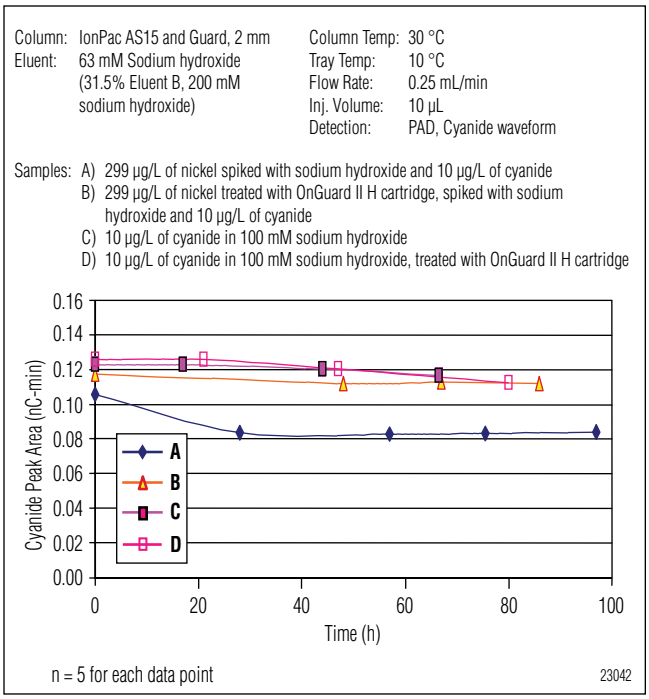


Figure 5. Effect of dissolved nickel on free cyanide (10 µg/L).

The cyanide control sample was not affected (102% ± 1% recovery) by the OnGuard II H treatment. Most importantly, the free cyanide concentration is as stable as the untreated standard when the metal-containing solutions are treated with the OnGuard II H cartridges (7–10% loss over three days) and, therefore, the cartridges effectively remove the dissolved metals. The results also confirm that the free cyanide concentration declines over three days and that the samples should be analyzed as soon as possible.

METHOD QUALIFICATION

The cyanide method was qualified prior to determining cyanide in real drinking water samples by determining the linearity over a 50-fold concentration range, typical noise, the method detection limit (MDL), reproducibility, and ruggedness. The linearity of cyanide response was determined by measuring cyanide in six replicates each of six standards (2.0, 3.0, 5.0, 10.0, 50.0, and 100 µg/L). The calibration results showed good linearity over this concentration range ($r^2 > 0.999$).

For each of the five disposable electrodes, the noise was determined over two 60-min runs, when no sample was injected, by measuring the noise in 1-min intervals from 5 to 60 min. The noise value determined by this experiment was 7.0 ± 1.8 pC (n = 10). The method detection limit (MDL) was defined as the peak in a standard with a peak height that is three times the noise level. For this application, the MDL was 1.0 µg/L. The signal to noise ratio of the 2.0 µg/L cyanide standard was 16.3 ± 4.8 (n = 10).

The reproducibility and ruggedness of the cyanide method was determined over 140 injections, ~62 h. During this study, cyanide in 10 µg/L cyanide standards was measured and the same standards spiked in copper, iron, and nickel metal solutions pretreated with OnGuard II H. Deionized water injections were inserted between the sample groups. The results (Figures 6 and 7) showed that retention time and peak areas were stable over 62 h of the experiment. The retention time and peak area reproducibilities were 5.78 ± 0.027 min and 0.1232 ± 0.0016 nC-min, respectively.

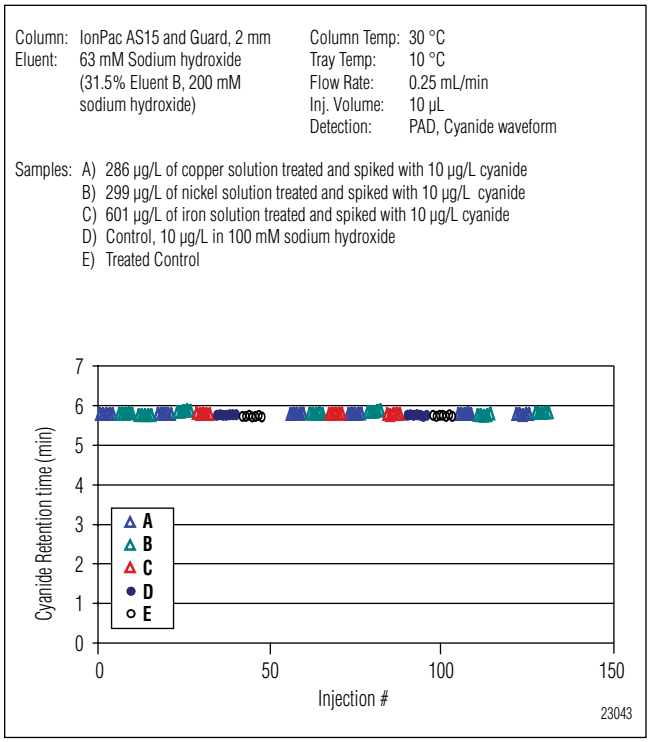


Figure 6. Retention time stability of 10 µg/L cyanide.

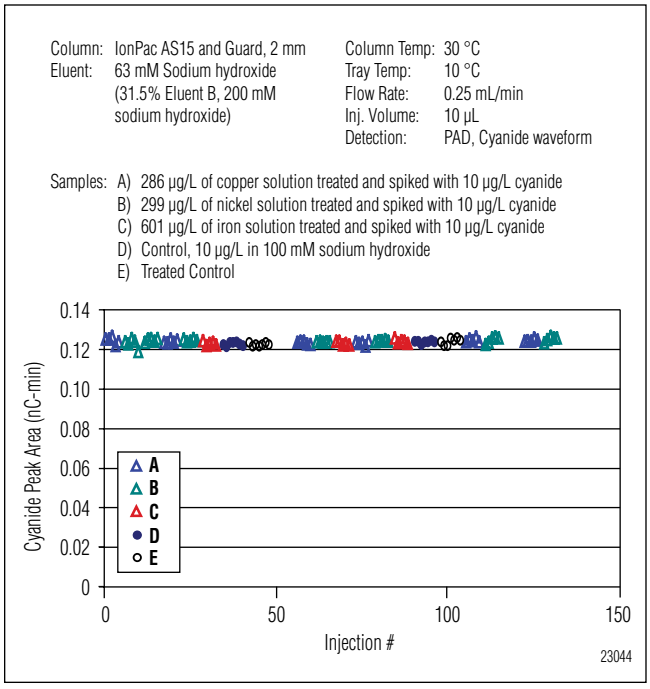


Figure 7. Peak area stability of 10 µg/L cyanide.

DETERMINATION OF CYANIDE IN DRINKING WATER AND SURFACE WATER

The characteristics of most drinking water change with the season. Free cyanide concentrations and spike recoveries of 5 and 10 µg/L cyanide in the City of Sunnyvale water were determined throughout the course of the application experiments and some changes were observed.

Sunnyvale drinking water sampled during the summer showed good spike recovery of cyanide for 5 µg/L and 10 µg/L of spiked cyanide (91.5 % ± 1.0% [n=10] and 98.2 % ± 1.7% [n=10], respectively). Cyanide also showed good recovery when 10 µg/L of sulfide was added (91.9% ± 1.7%). Spiking cyanide into 100 mM sodium hydroxide yielded similar recoveries. No free cyanide was measured in the unspiked samples of City of Sunnyvale drinking water (Figure 8).

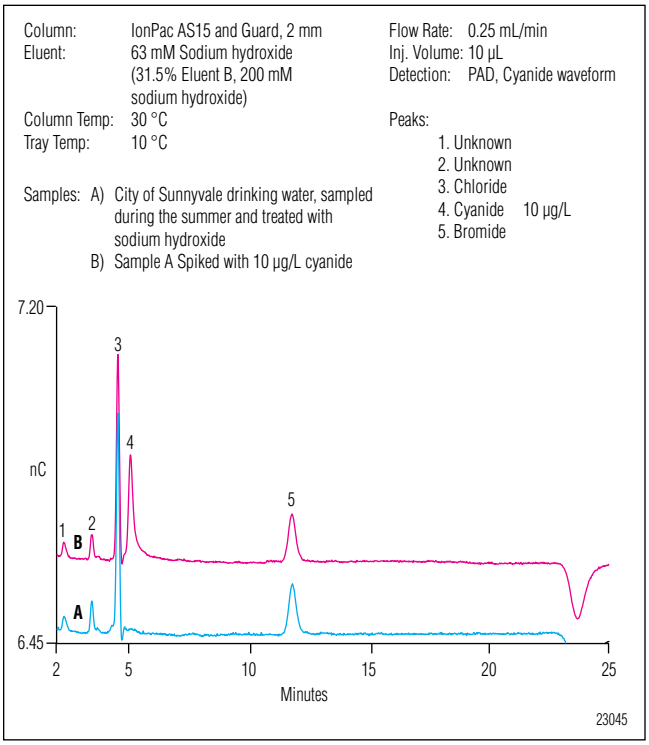


Figure 8. City of Sunnyvale drinking water with and without spiked cyanide.

Table 4. Recovery of Cyanide from Untreated Drinking Water and Surface Water Samples[a]

Concentration of Cyanide Spike (µg/L)	City of Sunnyvale Drinking Water[a]	City of San Jose Drinking Water	Alamitos Creek in Almaden
5	9.6 ± 3.0%	74.3 ± 11.8%	102.0 ± 1.3%
10	55.5 ± 2.8%	99.6 ± 0.5%	97.3 ± 2.4%

n=5 for each sample.
*Sampled during the fall months.

Table 5. Recovery of Cyanide in Treated Water Samples[a] (OnGuard II H Cartridges)

Concentration of Cyanide Spike (µg/L)	City of Sunnyvale Drinking Water	City of San Jose Drinking Water	Twain Harte Valley Drinking Water	Twain Harte Valley Flume
5	80.6 ± 5.5%	87.3 ± 6.4%	95.9 ± 2.5%	81.1 ± 3.2%
10	99.5 ± 2.8%	99.4 ± 2.5%	96.8 ± 3.1%	93.1 ± 1.5%

n=5 for each sample.
*Sampled during the fall months.

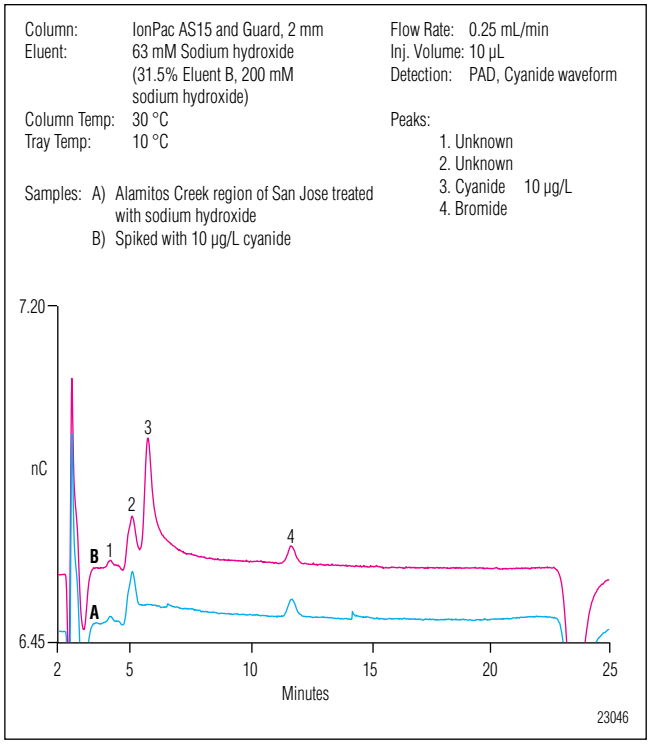


Figure 9. Alamitos Creek surface water sample with and without cyanide.

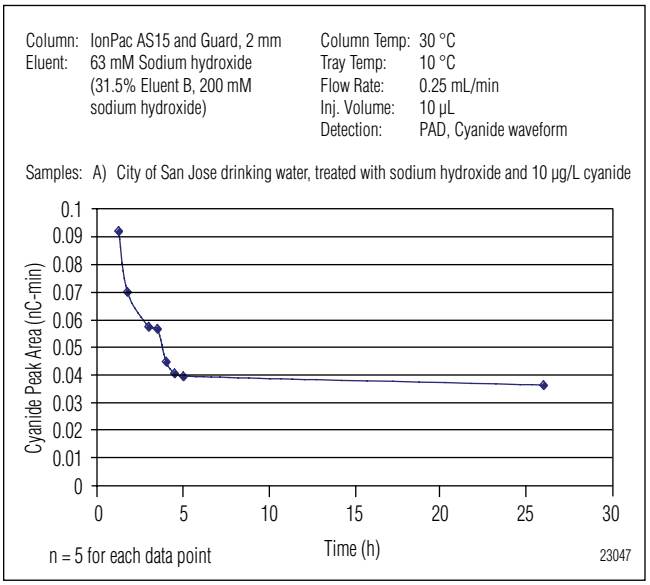


Figure 10. Degradation of cyanide spiked into the city of San Jose drinking water.

The method described in this document was used to measure free cyanide and the recovery of cyanide from two drinking water samples sampled in the fall (City of San Jose and City of Sunnyvale) and one surface-water sample (Alamitos Creek in the old Almaden mining region of San Jose) that were collected in the fall. The results showed no initial concentrations of free cyanide and variable recovery of cyanide spikes (Table 4). Only the Alamitos Creek surface water sample (Figure 9) exhibited acceptable recovery. The City of Sunnyvale drinking water had poor recovery at both spike levels (9.6% ± 3.0% and 55.5% ± 2.8%, for 5 µg/L and 10 µg/L cyanide, respectively). The City of San Jose drinking water samples had mixed results (74.3% ± 11.8% recovery of 5 µg/L and 99.6% ± 0.5% for 10 µg/L cyanide). These cyanide recovery results for the City of Sunnyvale sample were contradictory to the initial results (Figure 8). It is possible that City of Sunnyvale drinking water changed since the initial sampling. The cyanide recovery from the City of San Jose drinking water over time showed a trend similar to those observed with metal interferences (Figure 10). Therefore, the samples were treated with OnGuard II H cartridges and the recovery experiments repeated. Drinking and surface water samples were also analyzed from Twain Harte Valley, an old gold mining region. The results (Table 5) show good recovery for all samples (Figure 11–13) and good stability (> 84% of the initial peak response) for 31 h (not shown). No free cyanide was measured in any of the drinking or surface water samples.

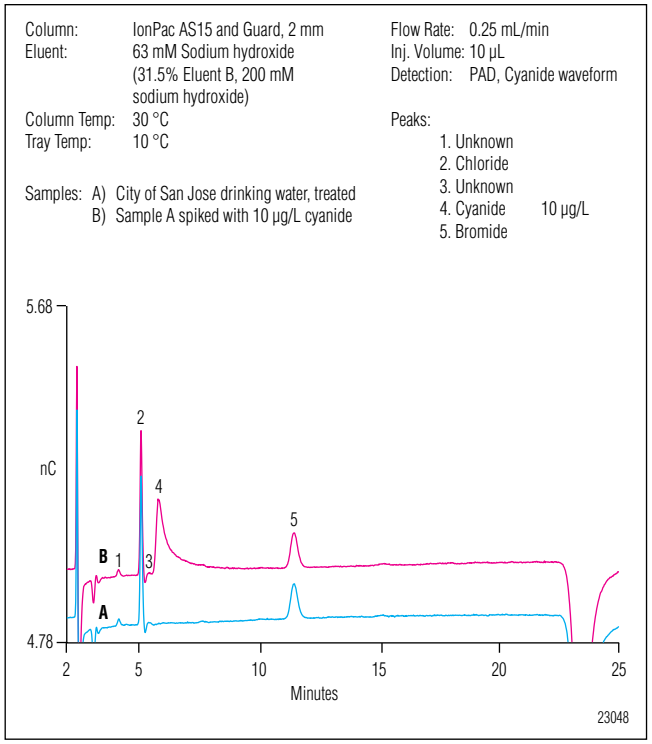


Figure 11. Treated city of San Jose drinking water with and without 10 µg/L of cyanide.

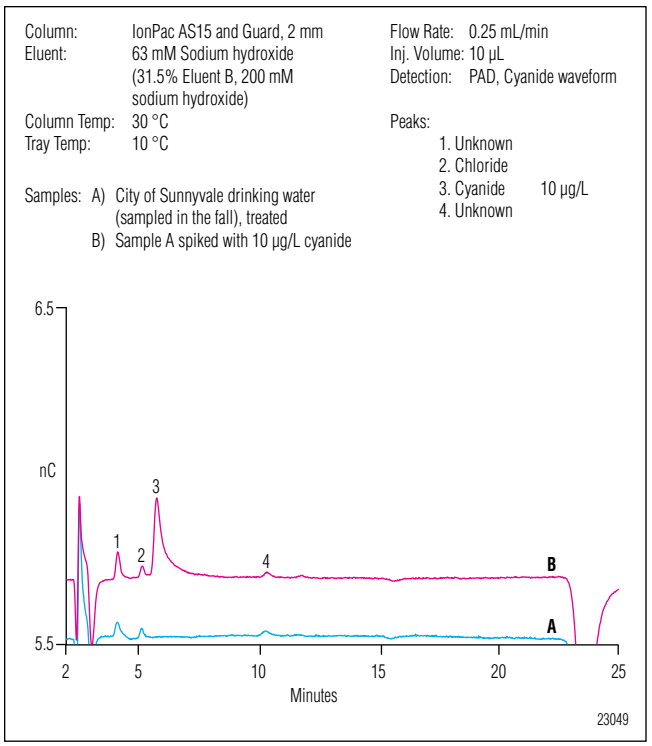


Figure 12. Treated city of Sunnyvale drinking water with and without 10 µg/L of cyanide.

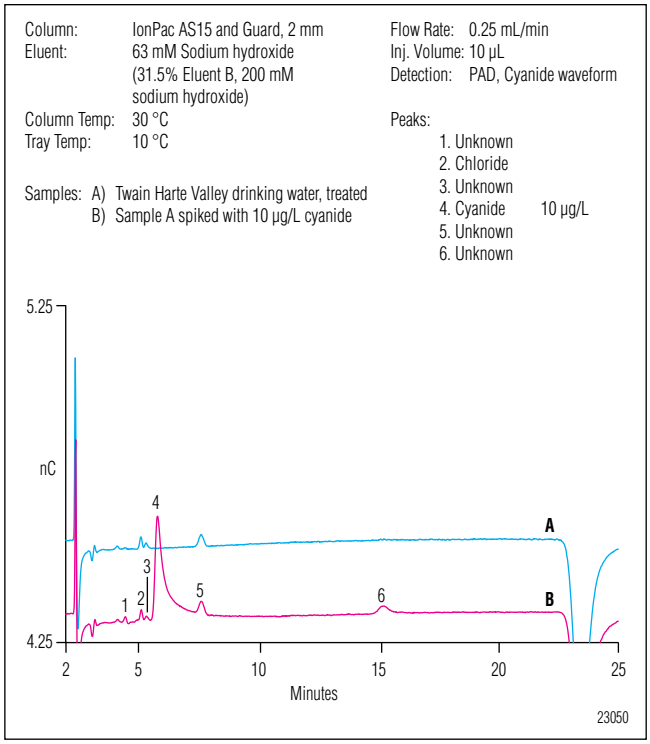


Figure 13. Treated city of Twain Harte Valley drinking water with and without 10 µg/L of cyanide.

DISPOSABLE SILVER WORKING ELECTRODES

In a published application using a waveform to determine iodide, the disposable silver working electrodes exhibited comparable or better reproducibility, linearity, and sensitivity than the conventional working silver electrodes.[24] The authors reported that they also saved time by discarding the disposable silver working electrodes at 80% of the peak response rather than re-polishing the conventional working electrode. In this study, the lifetimes of five disposable silver working electrodes were evaluated during the interference experiments, method qualification, and the testing of the municipal drinking water samples. Each electrode was installed, tested, and removed after two weeks of continuous use.

The average peak areas of 10 µg/L cyanide in 100 mM sodium hydroxide were compared over the five electrodes. The average peak area was 0.1206 ± 0.0038 nC-min, less than 1% variation. All five of the disposable silver working electrodes exceeded the 14-day lifetime specification (>80% of the peak response). Three of the five disposable silver working electrodes were only removed after three weeks so that another electrode could be tested. The other two electrodes showed >10 pC of noise during the last few days of operation.

CONCLUSION

Free cyanide can be determined in drinking water by IC-PAD. This method exhibits good sensitivity (MDL of 1 µg/L) and recovery, and exhibits linearity from 2 to 100 µg/L. This method can tolerate basic pH solutions, therefore it is believed that this method can determine cyanide in samples prepared for total cyanide determinations without dilution or neutralization of pH 13 distillation samples.

Transition metals can interfere with free cyanide determinations in drinking water. Dissolved transition metals are often present in drinking water and, therefore, it is a prudent to eliminate this possible interference by treating the water samples with OnGuard II H cartridges prior to analysis. Cyanide determinations in drinking water should always include spike recovery to ensure accurate determinations.

PRECAUTIONS

Warning: Cyanide is a poison. Never add cyanide to any solutions that have not been stabilized with base to a pH >9. Read and follow all safety precautions, handling, and waste disposal information prior to handling or using cyanide. Cyanide solutions cannot be poured into the water system without treatment. Consult your local safety representative for waste handling.

The Eluent Generator is not recommended as the eluent source for this application because of undesirably high noise levels.

Drinking and surface water samples should be stabilized immediately with sodium hydroxide. Cyanide solutions in 100 mM sodium hydroxide are stable for about one week. In drinking water samples without metal or other cation interferences, cyanide is stable for about three days.

Sodium hydroxide will etch glass and will foul the silver electrode, therefore, use only plastic pipettes, vials, and bottles for this application.

REFERENCES

1. Simeonova, P. Prof., Fishbein, L. Dr. Concise International Chemical Assessment Document 61, Hydrogen Cyanide and Cyanides: Human Health Aspects, Executive Summary section. Jointly by United Nations Environment Programme, International Labour Organization, and the World Health Organization in Inter-Organization Programme for the Sound Management of Chemicals. Geneva, **2004**, 10–11.
2. Maximum Contaminant Levels for Inorganic Contaminants. *Fed. Regist.* Code of Federal Regulations, Section 162, Title 40, Revised July 1, **2002**, 428–429.
3. Simeonova, Prof. P., Fishbein, Dr. L. Concise International Chemical Assessment Document 61, Hydrogen Cyanide and Cyanides: Human Health Aspects, Environmental Levels and Human Exposure, section 5. Jointly by United Nations Environment Programme, International Labour Organization, and the World Health Organization in Inter-Organization Programme for the Sound Management of Chemicals, Geneva, **2004**, 1.
4. *U.S. EPA Method 335.2*, U.S. Environmental Protection Agency: Cincinnati, OH, **1980**.
5. *U.S. EPA Method 335.1*, U.S. Environmental Protection Agency: Cincinnati, OH, **1974**.
6. *U.S. EPA Method 335.3*, U.S. Environmental Protection Agency: Cincinnati, OH, **1978**.
7. *Standard Methods SM-4500-CN-F*. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C., **1998**.
8. *Determination of Cyanide in Strongly Alkaline Solutions.* Application Update 107, LPN 0754. Dionex Corporation, Sunnyvale, CA, 2003.
9. Heckenberg, A., Cheng, J., Jandik, P., Cavalli, S., Abballe, F. Determination of Sulfide and Cyanide Using Integrated Pulsed Amperometric Detection on a Disposable Silver Electrode. *LC-GC*, The Application Notebook, September **2004**, 32.
10. Giuriati, C., Cavalli, S., Gorni, A., Badocco, D., Pastore, P. Ion Chromatographic Determination of Sulfide and Cyanide in Real Matrices by Using Pulsed Amperometric Detection on a Silver Electrode. *J. Chromatogr. A* **2004**, *1023*, 105–112.

11. Cheng, J., Jandik P., Avdalovic, N. Pulsed Amperometric Detection of Sulfide, Cyanide, Iodide, Thiosulfate, Bromide and Thiocyanate with Microfabricated Disposable Silver Working Electrodes in Ion Chromatography. *Anal. Chim. Acta*. **2005**, *536*, 267–274.
12. Operator's Manual for ICS-3000 Ion Chromatog raphy System, Sections 9.25.1, Disconnecting the Amperometric Cell and 9.25.5, Calibrating the Reference Electrode. LPN 065031, 242, 252-253, Dionex Corporation, Sunnyvale, CA, 2005.
13. Product Manual for Gold and Silver Disposable Electrodes. LPN 065040, Dionex Corporation, Sunnyvale, CA, 2005.
14. Product Manual for AminoPac PA10, *AAA-Direct*. Section 5 Eluent Preparation, p. 14, LPN 031481, Dionex Corporation, Sunnyvale, CA, 2005.
15. Product Manual for OnGuard II Cartridges. LPN 031688, Dionex Corporation, Sunnyvale, CA, 2004.
16. Operator's Manual for ICS-3000 Ion Chromatography System. LPN 065031, Dionex Corporation, Sunnyvale, CA, 2005.
17. Installation Manual for ICS-3000 Ion Chromatography System. LPN 065032, Dionex Corporation, Sunnyvale, CA, 2005.
18. Product Manual for IonPac AG15 Guard Column, IonPac AS15 Analytical Columns. LPN 031362, Dionex Corporation, Sunnyvale, CA, 2002.
19. AS Autosampler Operator's Manual, LPN 065051, Dionex Corporation, Sunnyvale, CA, 2005.
20. Operator's Manual for ICS-3000 Ion Chromatography System, Figure 2-22 Amperometric Cell, Section 2.16.1 Amperometric Cell. LPN 065031, p. 54, Dionex Corporation, Sunnyvale, CA, 2005.
21. Disposable Electrode Ag Installation Guide. LPN 065086, Dionex Corporation, Sunnyvale, CA, 2005.
22. Product Manual for IonPac AS7 Analytical Column and IonPac AG7 Guard Column. LPN 031299, Dionex Corporation, Sunnyvale, CA, 2005.
23. Montgomery, J. M. Water Treatment Principles and Design. J. Wiley, New York, **1985**, 11–13.
24. Liang, L., Cai, Y., Mou, S., Cheng, J. Comparisons of Disposable and Conventional Silver Working Electrodes for the Determination of Iodide using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. *J. Chromatogr., A* **2005**, *1085*, 37–41.

SUPPLIERS

Fisher Scientific International Inc., Liberty Lane, Hampton, NH 03842 USA 1-800-766-7000. www.fisherscientific.com

Sigma-Aldrich Corp., St. Louis, MO, USA. 1-800-325-3010. www.sigmaaldrich.com

U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 179, USA. 1-800-227-8772. www.usp.org

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA 19380 USA 1-800-932-5000. www.vwrsp.com

Determination of Sub-µg/L Bromate in Municipal and Natural Mineral Waters Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection

INTRODUCTION

Ozone is a powerful drinking water disinfectant that is effective in treating chlorine resistant organisms, such as *Cryptosporidia*.[1] For bottled water, ozonation is generally preferred over other available disinfection treatment methods because it does not leave a taste or residual disinfectant, due to the short lifetime of ozone.[2,3] Ozone also improves the quality of finished drinking water, by reducing filtered water turbidity and decreasing the formation of many halogenated disinfection by-products. However, ozonation of drinking water containing bromide can result in the formation of the disinfection by-product bromate, a potential human carcinogen even at low µg/L concentrations.[4] The U.S. EPA and European Commission have established a regulatory maximum contaminant level (MCL) of 10 µg/L bromate in drinking waters.[5,6] In the U.S., bottled water is considered a food product and is therefore regulated by the U.S. Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act.[7] In 2001, the U.S. FDA also established an MCL of 10 µg/L bromate in bottled drinking water.[7] More recently, the European Commission set a lower MCL of 3 µg/L bromate for natural mineral waters and spring waters treated by ozonation.[8] However, these limits were based on the feasibility of detection and removal, even though studies suggest concentrations lower than 1 µg/L pose increased lifetime cancer risks.[4,9]

Published EPA methods for determining low concentrations of bromate in drinking waters using direct injection have focused primarily on using columns designed specifically for carbonate eluents combined with suppressed conductivity detection or postcolumn reaction followed by UV/Vis detection.[10-12] Dionex Application Note 167 demonstrated that the use of a high-capacity hydroxide-selective IonPac® AS19 column, an electrolytically generated hydroxide eluent, a large loop injection, and suppressed conductivity detection can significantly reduce the bromate detection limit from 1.4 µg/L, reported in EPA Method 300.1, to 0.34 µg/L.[13] In addition, the use of a hydroxide eluent produced a bromate MDL of <0.2 µg/L for absorbance detection after postcolumn addition using EPA Methods 317.0 and 326.0.[14,15]

However, determining low concentrations of bromate in high ionic strength matrices using suppressed conductivity detection is subject to potential interferences and loss of sensitivity. Although postcolumn reaction methods do not generally suffer from interferences by common anions, column overloading with high ionic strength samples can still cause peak broadening and an associated loss of response. In particular, natural mineral waters typically contain elevated levels of common anions that can significantly exceed the concentrations present in most municipal drinking water samples, presenting an additional challenge for the currently available methods to determine <1 µg/L bromate. Natural mineral waters previously have been analyzed for trace concentrations of bromate using the the IonPac AS19 column with a hydroxide eluent or the IonPac AS23 column and a carbonate/bicarbonate eluent using a large loop injection and suppressed conductivity detection.[16]

In this application note, we demonstrate the use of a two-dimensional (2-D) ion chromatography (IC) system for the determination of trace concentrations of bromate in municipal and natural mineral waters with high ionic strength matrices. The first dimension uses a high capacity

4-mm IonPac AS19 column to resolve the bromate from the matrix ions. The matrix ions are diverted to waste while a 2 mL plug (cut volume) containing the bromate is transferred to the second dimension for analysis. Bromate is well resolved in the second dimension using a 2-mm IonPac AS24 column. This method is fully automated using an ICS-3000 Reagent-Free™ IC (RFIC™) system. In addition, this 2-D IC method achieves bromate detection limits equivalent to or better than postcolumn addition methods. The 2-D IC method avoids the cost and disposal of the chemicals required for postcolumn configurations and simplifies the experimental setup. Additionally, it avoids potential column overload during analysis of high ionic strength matrices.

EQUIPMENT

Dionex ICS-3000 Reagent-Free Ion Chromatography system consisting of:
- DP Dual Pump module
- EG Eluent Generator module with a dual setup
- DC Detector/Chromatography module
- (single or dual temperature zone configuration)

AS Autosampler with a 5 mL syringe (P/N 053915), 8.2 mL sampling needle assembly (P/N 061267)

Two EluGen® EGC II KOH cartridges (P/N 058900)

Two Continuously-Regenerated Anion Trap Columns, CR-ATC (P/N 060477)

Four 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.8 Chromatography Management Software

REAGENTS AND STANDARDS

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

Bromate standard (1000 mg/L, Ultra Scientific, VWR P/N ULICC-010)

Sodium bromate ($NaBrO_3$) (EM Science SX0385-1)

Sodium chloride (NaCl) (J.T. Baker; VWR P/N JT3625-1)

Sodium nitrate ($NaNO_3$) (Fisher Scientific S343-500)

Sodium bicarbonate ($NaHCO_3$) (EM Science SX0320-1)

Sodium sulfate (Na_2SO_4) (Aldrich 29,931-3)

Sodium phosphate, dibasic, anhydrous (Na_2HPO_4) (JT Baker 4062-1)

CONDITIONS

First Dimension

Columns:	IonPac AG19 guard, 4 × 50 mm (P/N 062887) IonPac AS19 analytical, 4 × 250 mm (P/N 062885)
Eluent:	10 mM potassium hydroxide 0–12 min,[a] step to 65 mM at 12 min, 65 mM 12–35 min[b]
Eluent Source:	EGC II KOH with CR-ATC
Flow Rate:	1 mL/min
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Injection Vol:	1000 µL
Detection:	Suppressed conductivity, ASRS® ULTRA II (4 mm), AutoSuppression® external water mode (flow rate: 3–5 mL/min) Current setting: 161 mA
System Backpressure:	~2300 psi
Expected Background Conductance:	<0.5 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	35 min

[a]The step change described here should occur after the valve on system #2 has switched from the load to inject position.
[b]The method equilibrates for 5 min at 10 mM KOH prior to injection.

Second Dimension

Columns:	IonPac AG24 guard, 2 × 50 mm (P/N 064151) IonPac AS24 analytical, 2 × 250 mm (P/N 064153)
Eluent:	10 mM potassium hydroxide 0–24 min, step to 65 mM at 24 min, 65 mM 24–35 min[b]
Eluent Source:	EGC II KOH with CR-ATC
Flow Rate:	0.25 mL/min
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Cut Volume:	2 mL (on the concentrator column)

Concentrator:	TAC-ULP1, 5 × 23 mm (P/N 061400)
Detection:	Suppressed conductivity, ASRS ULTRA II (2 mm), AutoSuppression external water mode (flow rate: 1–3 mL/min) Current setting: 41 mA
System Backpressure:	~2400 psi
Expected Background Conductance:	<0.8 µS
Noise:	~2–3 nS/min peak-to-peak
Run Time:	35 min

[b]*The method equilibrates for 5 min at 10 mM KOH prior to injection.*

PREPARATION OF SOLUTIONS AND STANDARDS

Stock Bromate Standard Solution

Dissolve 0.1180 g sodium bromate in 100 mL of deionized water for a 1000 mg/L standard solution. This standard is stable for at least six months when stored at 4 °C.

Bromate Primary Dilution Standard

To prepare a 10 mg/L bromate solution, add 1 mL of the 1000 mg/L stock standard to a 100 mL volumetric flask. Bring to volume with deionized water. When stored at 4 °C, the resulting solution is stable for at least one month.

Bromate Secondary Dilution Standard

To prepare a 1 mg/L bromate solution, add 10 mL of the primary dilution standard to a 100 mL volumetric flask. Bring to volume with deionized water. When stored at 4 °C, the resulting solution is stable for at least one month.

Bromate Calibration Standards

To prepare bromate calibration standards at concentrations of 0.15, 0.25, 0.50, 1, 3, 5, 10, and 15 µg/L, add the appropriate volumes of the bromate secondary dilution standard to separate 100 mL volumetric flasks. Bring to volume with deionized water.

Common Anion Stock Solutions

Prepare 1000 mg/L each of chloride, nitrate as N, bicarbonate, sulfate, and phosphate as P. Dissolve 0.1649 g sodium chloride in deionized water and dilute to 100 mL. Dissolve 0.6068 g sodium nitrate in deionized water and dilute to 100 mL. Dissolve 0.1377 g sodium bicarbonate in deionized water and dilute to 100 mL. Dissolve 0.1479 g sodium sulfate in deionized water and dilute to 100 mL. Dissolve 0.4583 g anhydrous sodium phosphate, dibasic in deionized water and dilute to 100 mL.

Laboratory Synthetic Sample Matrix (LSSM)

The LSSM contains 100 mg/L each of chloride, bicarbonate, and sulfate and 10 mg/L each of nitrate-N and phosphate-P. Prepare this solution by adding 10 mL each of chloride, bicarbonate, and sulfate from their respective 1000 mg/L stock solutions to a 100 mL volumetric flask. Add 1 mL each of nitrate-N and phosphate-P from their respective 1000 mg/L stock solutions to the volumetric flask containing chloride, bicarbonate, and sulfate. To fortify this solution with 0.5 µg/L or 5 µg/L bromate add 0.05 mL or 0.5 mL, respectively of the 1 mg/L bromate secondary dilution standard to the volumetric flask and bring to volume with deionized water.

SYSTEM PREPARATION AND SETUP

Install an EGC II KOH cartridge for each system channel. Install backpressure tubing temporarily in place of the columns on both system channels to produce a total backpressure of 2000–2500 psi at a flow rate of 1 mL/min. Condition the cartridges by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After completing the conditioning process, disconnect the backpressure tubing. Install a CR-ATC between the EGC II KOH cartridge and the EGC degas. Hydrate the CR-ATC prior to use by following the instructions outlined in the EluGen Cartridge Quickstart Guide (Document No. 065037-02). Figure 1 shows a detailed schematic diagram of the system setup.

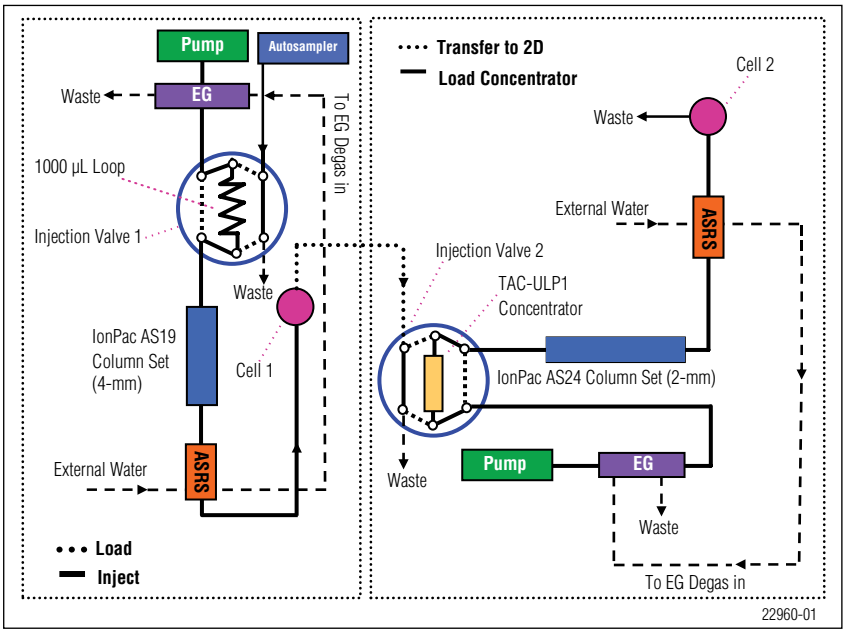


Figure 1. Schematic diagram of an ICS-3000 two-dimensional ion-chromatography system for the determination of trace concentrations of bromate.

Install and configure the AS Autosampler. The most accurate and precise sample injections with the AS Autosampler are made with a calibrated sample loop, flushed with about four to five times the loop volume. Because this application requires large sample injection volumes, a sample syringe of at least 5 mL (P/N 053915) should be installed. To accommodate the larger volume, an 8.2 mL sampling needle assembly (P/N 061267) is also required for operation. The largest injection possible with a 5 mL syringe installed on the AS Autosampler is 4000 µL. To inject 1000 µL, select the normal mode from the front panel of the autosampler. The normal mode will allow the autosampler to flush the sample loop prior to injection. Enter the correct Sample Loop Size and Sample Syringe Volume in the AS Plumbing Configuration screen. Instruct the AS to inject 1000 µL with the Chromeleon software.

Prepare a 1000 µL sample loop by measuring approximately 86.4 in. of 0.030 in. i.d. tubing. To verify the volume of the loop, first weigh the empty tubing. Fill the tube with deionized water then reweigh the filled tube and calculate the volume. The total sample volume should be 1000 µL ± 5%. Install the sample loop on Injection Valve 1 of the DC-3000.

Because the two dimensions are working as one system, a second timebase does not need to be created. However, to allow independent control of the DC-3000 injection valves in the timebase, the DC settings in the Chromeleon system configuration must be changed. To modify this configuration, go to the DC High Pressure Valves tab in the system configuration, double-click InjectValve_2, and change controlled by AS to DC.

Install a 4 × 50 mm IonPac AG19 and a 4 × 250 mm IonPac AS19 column on system #1 in the lower compartment of the DC. Install a 2 × 50 mm IonPac AG24 and a 2 × 250 mm IonPac AS24 column on system #2. Connect a piece of 0.01 in. i.d. PEEK™ tubing from the Cell Out on system #1 to the Sample Inlet Port on Injection Valve #2. The length of this tubing should be kept to a minimum. Install a 5 × 23 mm TAC-ULP1 concentrator in place of the sample loop on system #2. The direction of sample loading on the TAC-ULP1 should be in the opposite direction of the analytical flow.

Make sure the pressure for both systems is from 2200–2500 psi using the operating conditions described earlier to allow the degas assembly to effectively remove electrolysis gases from the eluent. If necessary, install additional backpressure tubing between the degas assembly and the injection valve to achieve the recommended pressure setting. Monitor the pressure periodically as it can gradually rise over time. To reduce pressure, trim the backpressure tubing.

Hydrate the ASRS ULTRA II suppressors prior to installation using a disposable plastic syringe. Push 3 mL of degassed deionized water through the Eluent Out port and 5 mL of degassed deionized water through the Regen In port. Allow the suppressors to stand for 20 min to fully hydrate the suppressor screens and membranes. Before installing the suppressors, rinse the analytical column with 65 mM KOH while diverting to waste. Install the ASRS ULTRA II for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the CR-ATC and connect the Regen In of the

suppressor to the external water source. The Regen Out of the CR-ATC is connected to the Regen In of the EG degasser.

Equilibrate the AS19 and AS24 columns with 65 mM KOH at their respective flow rates, shown in the Conditions section, for approximately 60 min. Analyze a matrix blank by injecting 1000 µL deionized water using 8–10 min as the default cut time. An equilibrated system has background conductances of < 0.5 µS and < 0.8 µS for the AS19 and AS24 columns, respectively. Determine the final cut time (preconcentration time) for the second dimension, as described in the next section, before injecting a bromate standard.

Determining the Cut Time for the Second Dimension

Because there may be slight variations in system plumbing, column capacity, and tubing lengths, individual laboratories should first determine the optimum cut time (from the first dimension) before determining bromate in the second dimension. To determine the cut time for analysis in the second dimension, we recommend performing duplicate 1000 µL injections of 15 µg/L bromate prepared in deionized water and 15 µg/L bromate prepared in a LSSM containing 100 mg/L each of chloride, sulfate and bicarbonate and 10 mg/L each of nitrate-N and phosphate-P.

For this application, it is important that valve #2 on the second dimension remains in the inject position during this time to avoid any baseline disturbances that may occur in the first dimension. This can be accomplished by placing a semicolon (";") before the DC inject commands in the Chromeleon program.

Determine the start time for placing valve #2 in the load position by subtracting 1 min from the retention time (RT) of bromate in the LSSM sample (in this experiment, bromate RT = 9 min – 1 min = 8 min). Determine the time for switching valve #2 to the inject position by adding 0.2 min to the time when the bromate peak in deionized water returns to the baseline (in this experiment, bromate returned to baseline = 9.8 min + 0.2 min = 10.0 min). The chromatograms used for the present experiment are shown in Figures 2A and 2B.

Note: it is important to verify the retention time of bromate on the AS19 column weekly to ensure good trapping efficiency on the TAC-ULP1 concentrator.

After the cut time has been established, enable valve #2 by removing the semicolons prior to the DC inject commands in the Chromeleon program. Perform duplicate injections of a 5 µg/L bromate standard to verify that nearly identical bromate retention times are achieved on the AS24 column in the second dimension. Figure 3 shows an example chromatogram of 5 µg/L bromate separated on the AS24 column in the second dimension.

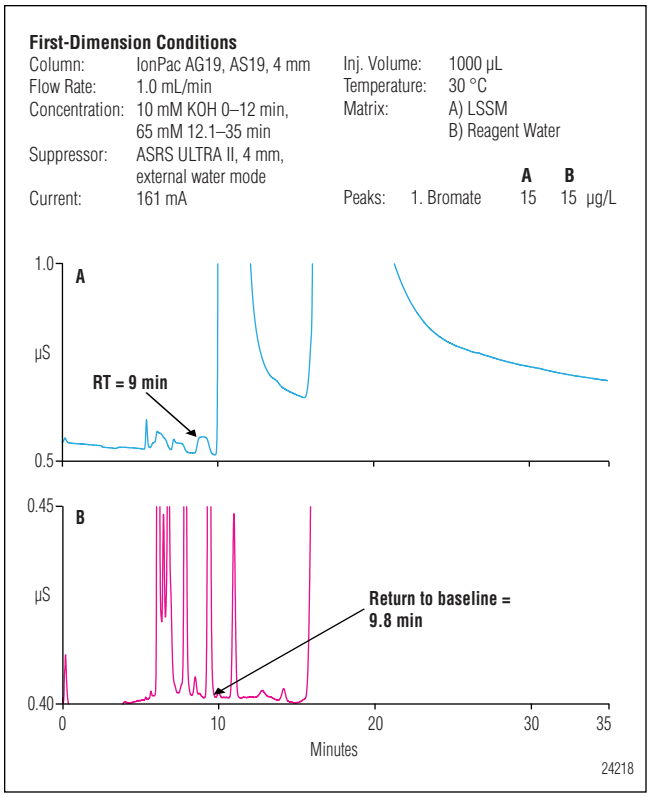


Figure 2. Determination of the cut window in the first dimension.

RESULTS AND DISCUSSION

The bromate in the second dimension was calibrated by injecting a 1000 µL water blank and a duplicate injection of eight calibration standards in the first dimension to cover the desired concentration range. The peak area response generated by the calibration standards was tabulated against the bromate concentration using a quadratic regression curve. Table 1 summarizes the calibration data obtained from injecting standards in the range of 0.15-15 µg/L bromate. The accuracy of the calibration curve was verified by injecting a 5 µg/L bromate standard prepared from a second source, producing a calculated recovery of 97.1%.

Table 1. Calibration Data and Method Detection Limits for Bromate

Analyte	Range (µg/L)	Linearity[a] (r^2)	MDL Standard (µg/L)	SD (µg/L)	Calculated MDL (µg/L)
Bromate	0.15–15	0.9995	0.20	0.012	0.036

[a] Quadratic fit

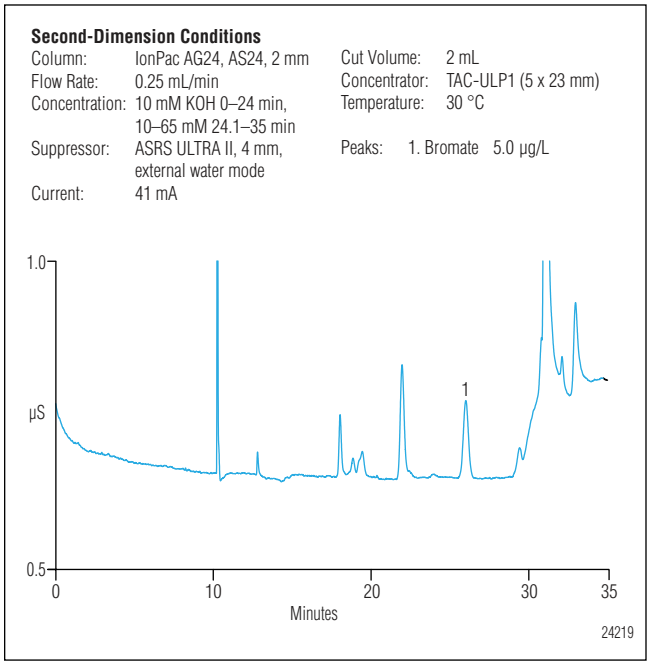


Figure 3. Chromatogram of a 5 µg/L bromate standard with the IonPac AS24 column in the second dimension.

During the initial development of the 2-D method for determining trace concentrations of bromate, the lowest concentration minimum reporting level (LCMRL) was determined. The U.S. EPA has developed a statistical approach for determining a single-laboratory LCMRL using linear regression and prediction intervals and has found this to be a more systematic procedure for determining the minimum reporting level (MRL).[17] The MRL is the lowest analyte concentration that demonstrates known quantitative quality, whereas the LCMRL is the lowest true concentration at which the future recovery is predicted to fall with a 99% confidence between 50% and 150% recovery. The LCMRL can be used to determine the MRL for a particular analyte by either using a multiplying factor or by combining results from a multi-laboratory study. Although the EPA encourages all laboratories to determine the LCMRL to aid in evaluating the performance of spiked recoveries at or below the MRL, it does not mandate LCMRL determinations.

In this study, the LCMRL was determined to be 0.15 µg/L bromate by preparing and analyzing seven individual replicate injections of 0.15, 0.20, 0.30, 0.40, and 0.50 µg/L bromate. The data from these replicate injections were then inserted into the statistical program provided on the EPA website (http://www.epa.gov/OGWDW/methods/sourcalt.html) to determine the LCMRL. The target MRL for this application was established at 0.50 µg/L bromate, which is just over three times the calculated LCMRL.

EPA Method 314.2, also using 2-D IC, does not require the determination of the detection limit for validation of the method. However, some laboratories may require this determination due to the various regulatory bodies associated with compliance monitoring. The limit of detection (LOD) of the 2-D method was determined for bromate by performing seven replicates of reagent water fortified with 0.20 µg/L bromate and using the following equation:

$$LOD = St_{(n-1,\ 1-\alpha = 0.99)}$$

where:

$t_{(n-1,\ 1-\alpha = 0.99)}$ = student's t-value for a 99% confidence level with n - 1 (t = 3.14 for seven replicate injections)

n=number of replicates

S=standard deviation of replicate analyses

A calculated LOD of 0.036 µg/L bromate was determined as shown in Table 1. The bromate LOD using 2-D IC is significantly lower than the bromate detection limits of 0.12 µg/L and 0.17 µg/L reported in EPA Methods 317.0 and 326.0, using postcolumn addition and absorbance detection.[11,12]

The performance of the 2-D bromate method was evaluated through a single-operator precision and accuracy study using fortified municipal and natural mineral water samples. The recovery of bromate was evaluated by analyzing eight different matrices, including reagent water, LSSM, two municipal drinking waters from different sources, and four natural mineral waters from different countries (France, Japan, Switzerland, United Kingdom). Each sample was fortified with 0.5 and 5 µg/L bromate. To ensure the accuracy of the calibration curve, quality control standards prepared at 0.5, 5, and 15 µg/L bromate were analyzed at the beginning, middle, and end of each sample analysis batch.

Table 2 summarizes the performance of the method for determining trace concentrations of bromate in municipal drinking waters using 2-D IC. As shown, trace concentrations of bromate were detected in both municipal drinking water samples, well below the current regulatory limit of 10 µg/L. The most likely source of bromate in these samples is the hypochlorite solution used for disinfection treatment, as ozonation is not used at either of the tested drinking water treatment facilities.

For the municipal drinking waters fortified with 0.5 µg/L bromate, the calculated recoveries were between 98 and 99%. For the same samples fortified with 5 µg/L bromate, the recovery was approximately 105%. Figure 4 shows chromatograms of drinking water B, unfortified and fortified with 0.5 µg/L bromate, using the combined IonPac AS19/AS24 columns with 2-D IC. As shown, bromate is well-resolved from any potential inference on the IonPac AS24 column, and therefore produces an excellent recovery of 98.7%.

Table 2. Bromate Recoveries from Fortified Reagent Water, LSSM, and Municipal Drinking Water Matrices

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Average Recovery (%)	Peak Area Precision (RSD)
Reagent Water	—	0.5	7	101.5	1.98
		5.0	7	105.6	0.66
LSSM[a]	—	0.5	7	96.1	5.75
		5.0	7	106.7	1.66
Drinking Water A	0.45	0.5	7	98.2	6.06
		5.0	7	104.5	1.71
Drinking Water B	1.19	0.5	7	98.7	2.51
		5.0	7	105.6	1.91

[a] LSSM = Laboratory Synthetic Sample Matrix containing 100 mg/L each of chloride, sulfate, and bicarbonate and 10 mg/L each of nitrate-N and phosphate-P

Some drinking water samples may contain elevated concentrations of chloride, sulfate, and bicarbonate that can increase peak broadening of bromate and therefore lower recovery. To determine whether the 2-D IC method can analyze these types of samples, a LSSM was prepared and fortified with 0.5 and 5 µg/L bromate. The excellent recoveries shown in Table 2 for this sample and system configuration indicate that bromate was not influenced by the increased concentrations of common anions.

In general, the ionic strength of natural mineral waters significantly exceeds the concentrations found in typical municipal drinking water samples. Determining low concentrations of bromate in these matrices using currently available methods is a challenging analytical problem because of column overloading. In some cases, sample dilution is required, increasing the MRL in proportion to the dilution factor. Alternatively, samples can be treated with OnGuard® cartridges to remove most of the chloride, carbonate, and sulfate in the sample. However, this requires additional time and increases the cost of each analysis. One of the primary advantages of 2-D IC is that most samples can be injected directly without any sample pretreatment, thereby simplifying analysis.

In this study, four different natural mineral water samples were analyzed for bromate. Table 3 summarizes the ionic properties of three of the investigated samples according to the manufacturers' specifications. As shown, the ionic strength of the mineral waters analyzed in this study varied significantly. None of the bottled minerals waters indicated that ozonation was used as a disinfection treatment method; therefore the detection of bromate was not anticipated.

Table 3. Concentrations (mg/L) of Cations and Anions of the Investigated Mineral Water Samples

Mineral Water	Na^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_3^-	HCO_3^-	SO_4^{2-}
A	5.5	0.7	9.5	50.8	—[a]	5.9	<3	190	5.8
B	9.0	0.6	3.4	147.3	0.12	21.5	18	390	33
C	4.2	—[a]	117	510	1.8	3.0	<0.1	278	1445

[a] Not specified

Table 4. Bromate Recoveries from Fortified Natural Mineral Water Samples

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Average Recovery (%)	Peak Area Precision (RSD)
Mineral Water A	<MDL[b]	0.5	7	95.2	3.37
		5.0	7	103.9	1.22
Mineral Water B	<MDL	0.5	7	95.2	5.85
		5.0	7	105.5	0.62
Mineral Water C[a]	<MDL	0.5	7	95.6	7.23
		5.0	7	103.8	1.22
Mineral Water D	<MDL	0.5	7	96.5	4.00
		5.0	7	103.8	1.35

[a] Cut time changed from 8–10 min to 7–10 min due to the increased amount of sulfate (1445 mg/L) that shifted retention times on the first dimension column

[b] <MDL = less than the method detection limit

The samples were fortified with 0.5 and 5 µg/L bromate to evaluate the accuracy of the 2-D method for determining bromate in natural mineral waters (Table 4). As shown, recoveries were in the range of 95-105% with peak area precisions for seven replicate injections in the range of 0.6-7.2%. Previously, a 250 µL direct injection of mineral water C was analyzed with the IonPac AS19 column followed by suppressed conductivity detection.[16] Using the method parameters described in Application Note 184 required a 1:5 sample dilution to reduce the 1445 mg/L sulfate in the sample to avoid column overloading. This would result in an increase in the MRL by a factor of five. However, because the 2-D method removes most of the interfering matrix ions in the first dimension, the sample can be injected directly without any sample preparation steps, thereby maintaining the method's MRL of 0.5 µg/L bromate. However, the increased sulfate concentration did shift the retention time of bromate in the first dimension, resulting in a lower bromate recovery than expected. To improve the bromate recovery, the cut window was increased from 8-10 min to 7-10 min to account for the shift in retention time on the AS19 column. This minimal

change in cut time resulted in a significant improvement in recovery from <50% to ~96%. Figure 5 shows example chromatograms of unfortified and fortified mineral water C. As shown in these chromatograms, a significant amount of chloride was transferred to the AS24 column due to the shift in retention times on the AS19 column. The IonPac AS24 is a high-capacity anion-exchange column that provides an excellent bromate/chloride resolution as demonstrated in this example and thereby allowed good quantification of bromate from the sample.

CONCLUSION

This application note describes a 2-D IC system for the determination of ≥0.5 µg/L bromate in municipal and natural mineral waters. The method provides an improvement to existing EPA methods for bromate by providing lower detection limits and improved recoveries of bromate in high ionic strength matrices. In addition, samples can be injected directly without requiring the use of OnGuard cartridges, sample dilution, or sample degassing for carbonate removal prior to analysis. The elimination of time-consuming off-line sample preparation improves consistency between different analysts and laboratories. The method also allows the determination of trace concentrations of bromate in a wide range of sample matrices.

REFERENCES

1. Bonacquisti, T. A Drinking Water Utility's Perspective on Bromide, Bromate, and Ozonation. *Toxicology* **2006**, *221*, 145–148.
2. U.S. Environmental Protection Agency, *Water Health Series: Bottled Water Basics,* Sept 2005.
3. U.S. Environmental Protection Agency, *Occurrence Assessment for the Final Stage 2 Disinfectants and Disinfection Byproducts Rule*, Document No. 815-R-05-011, December 2005.
4. World Health Organization, *Bromate in Drinking Water*–Background document for the development of WHO Guidelines for Drinking Water Quality, 2005.
5. U.S. EPA. National Primary Drinking Water Regulations. Disinfectants and Disinfection By-Products. *Fed. Reg.* **1998**, *63* (241), 69389-69476.
6. Fawell, J.; Walker, M. Approaches to Determining Regulatory Values for Carcinogens with Particular Reference to Bromate. *Toxicology* **2006,** *221,* 149–153.

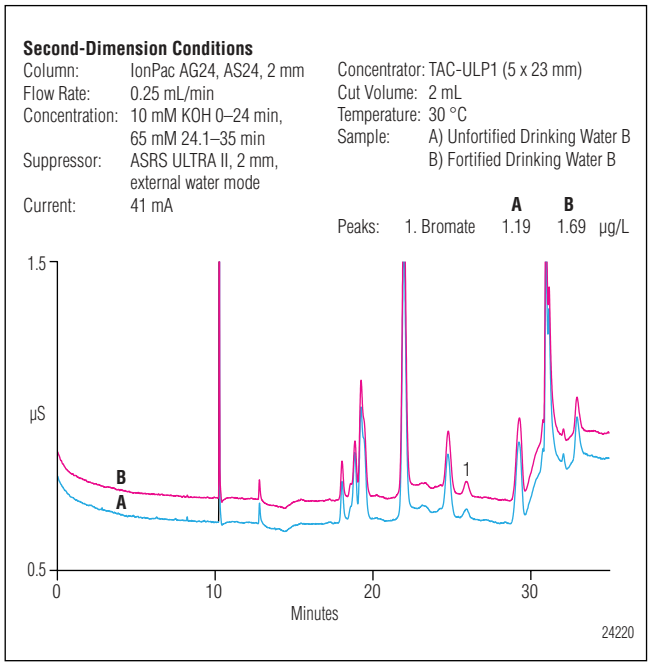


Figure 4. Chromatogram of (A) drinking water B and (B) drinking water B fortified with 0.5 µg/L bromate.

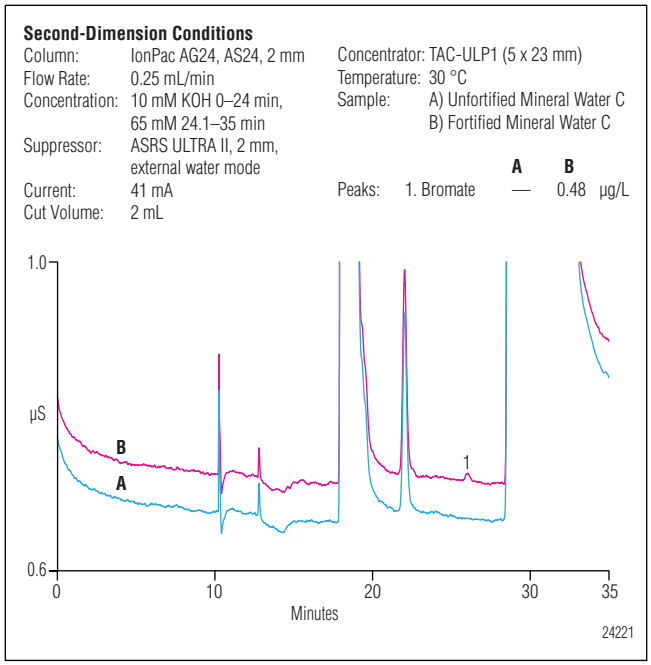


Figure 5. Chromatogram of (A) mineral water C and (B) mineral water C fortified with 0.5 µg/L bromate.

7. Food and Drug Administration. Beverages: Bottled Water. *Fed. Reg.* **2001,** *66* (60), 16858–16868.
8. European Parliament and Council Directive No. 2003/40/EC, *Establishing the List, Concentration Limits and Labeling Requirements for the Constituents of Natural Mineral Waters and the Conditions for Using Ozone-Enriched Air for the Treatment of Natural Mineral Waters and Spring Waters,* 2003.
9. Toxicological Review of Bromate, U.S. Environmental Protection Agency, Washington, D.C., 2001.
10. U.S. EPA Method 300.1, The Determination of Inorganic Anions in Drinking Water by Ion Chromatography, U.S. Environmental Protection Agency, Cincinnati, OH, 1997.
11. U.S. EPA Method 317.0, rev 2.0, Determination of Inorganic Oxyhalide Disinfection By-Products in Drinking Water Using Ion Chromatography with the Addition of a Postcolumn Reagent for Trace Bromate Analysis, U.S. Environmental Protection Agency, Cincinnati, OH, 2001.
12. U.S. EPA Method 326.0, Determination of Inorganic Oxyhalide Disinfection By-Products in Drinking Water Using Ion Chromatography Incorporating the Addition of a Suppressor Acidified Postcolumn Reagent for Trace Bromate Analysis, U.S. Environmental Protection Agency, Cincinnati, OH, 2002.
13. Dionex Corporation. *Determination of Trace Concentrations of Oxyhalides in Municipal and Bottled Waters Using a Hydroxide-Selective Column with a Reagent-Free Ion Chromatography System;* Application Note 167, LPN 1662: Sunnyvale, CA, 2004.
14. Dionex Corporation. *Determination of Trace Concentrations of Disinfection By-Product Anions and Bromide in Drinking Using Reagent-Free Ion Chromatography Followed by Postcolumn Addition of o-Dianisidine for Trace Bromate Analysis;* Application Note 168, LPN 1706: Sunnyvale, CA, 2005.
15. Dionex Corporation. *Determination of Trace Concentrations of Disinfection By-Product Anions and Bromide in Drinking Using a Reagent-Free Ion Chromatography System Followed by Postcolumn Addition of an Acidified On-Line Generated Reagent for Trace Bromate Analysis;* Application Note 171, LPN 1767: Sunnyvale, CA, 2006.
16. Dionex Corporation. *Determination of Trace Concentrations of Chlorite, Bromate, and Chlorate in Bottled Natural Mineral Waters;* Application Note 184, LPN 1890; Sunnyvale, CA, 2007.
17. U.S. Environmental Protection Agency, Statistical Approach for the Determination of the Single-Laboratory Lowest Concentration Minimum Report Level (LCMRL) and Validation of Laboratory Performance At or Below the Minimum Reporting Level, EPA Document No. 815-R-05-006, November 2004, available at http://www.epa.gov/OGWDW/methods/pdfs/method_lcmrl.pdf.

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections

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 have traditionally been separated using HPLC, but method detection limits (MDLs) of HPLC techniques employing direct injection of samples are too high for the detection of the low concentrations in real samples that are near the regulated limit. Therefore, the analytes in these samples require preconcentration before analysis.

The U.S. EPA prescribes liquid-liquid extraction[1] and liquid-solid extraction[2] (also called solid-phase extraction, SPE) methods for preconcentrating PAHs in drinking water samples. However, preparing an individual samples is time consuming for each of the two extraction methods, and a new SPE cartridge must be used for each sample when using the SPE method. The expense of using multiple SPE cartridges and the associated manual labor can be eliminated with online SPE combined with the subsequent HPLC analysis. This technique delivers a simple, rapid, and accurate means for determining PAHs at low concentrations in water samples. For example, Zhou et al[3] prepared a copper (II) isonicotinate $[Cu(4\text{-}C_5H_4N\text{-}COO)_2(H_2O)_4]$ coordination polymer as adsorbent for online SPE coupled with HPLC and UV detection for determining eight trace PAHs in environmental waters.

The Dionex UltiMate® 3000 ×2 Dual HPLC system has already been used to execute the online SPE method coupled with HPLC to determine phenols in drinking and bottled waters,[4] and PAHs in edible oils.[5] The Acclaim® Polar Advantage II (PA2) is a polar-embedded column designed for enhanced hydrolytic stability within a wide range of pH values (pH 1.5–10). It is compatible with 100% aqueous mobile phases, overcoming the limitations of conventional C8 and C18 reversed-phase columns. Thus, the Acclaim PA2 is a good choice as an SPE column for concentrating polar and non-polar components in large volume water samples (e.g., tap water, pH ~8) without adding any organic solvents.

This application note details an online SPE method followed by HPLC with fluorescence and UV detections on the UltiMate 3000 ×2 Dual HPLC system for determining the 16 PAHs specified in the US EPA Priority Pollutants List (structures shown in Figure 1) at the concentrations required by world regulatory agencies. PAHs from water samples are trapped on the Acclaim PA2 column, and then separated on a Supelcosil™ LC-PAH column. This automated method is a cost-effective and accurate way to determine PAHs in drinking water samples.

EQUIPMENT

UltiMate 3000 ×2 Dual system consisting of:

- DPG-3600A pump with SRD-3600 Solvent Rack with degasser
- WPS-3000TSL autosampler (with 2.5 mL injection loop (P/N 6820.2416) installed)
- TCC-3100 thermostatted column compartment with one 2P-6P valve
- VWD-3400RS Variable Wavelength Detector
- RF2000 fluorescence detector

Chromeleon® 6.80 (SP4) Chromatography Workstation

Device configurations for the online SPE with analytical HPLC are as shown in Figure 2.

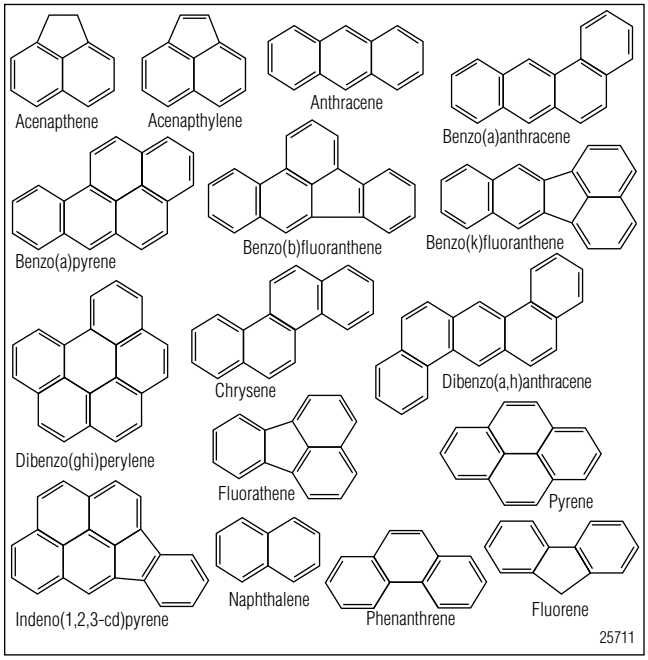


Figure 1. Structures of the 16 PAHs specified in U.S. EPA Method 550.1.

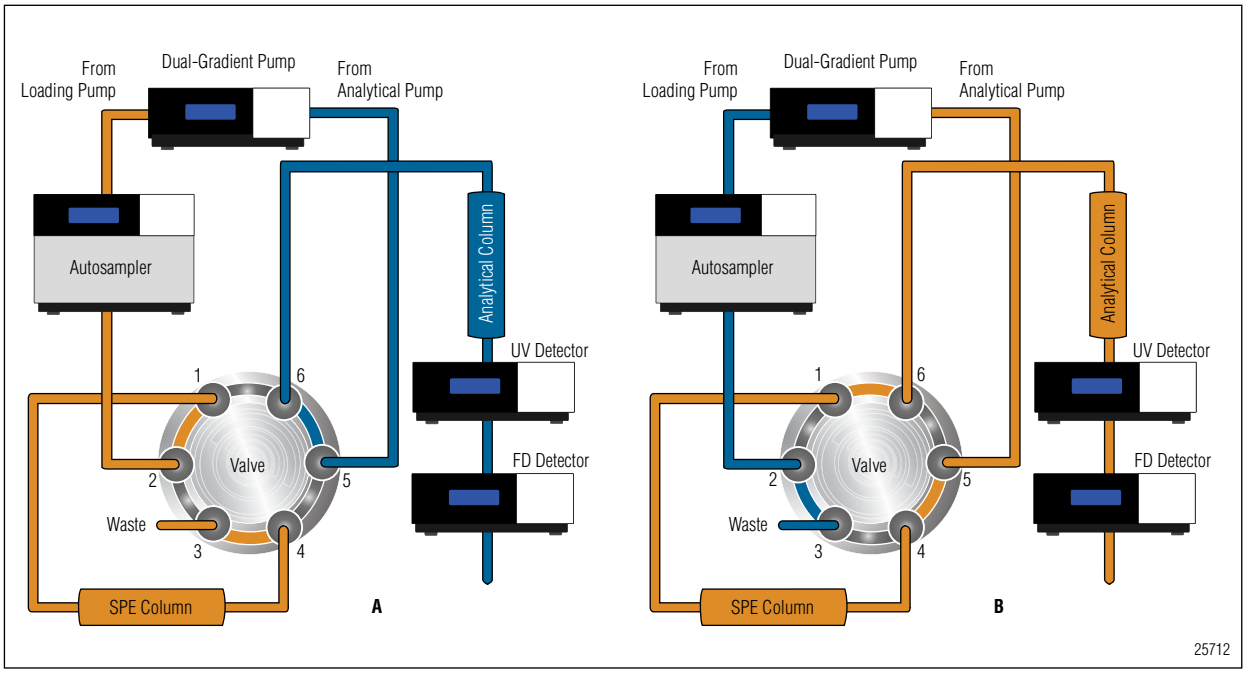


Figure 2. Flow scheme for on-line sample preparation and analysis. (A) The valve is positioned for injection of the sample on the online SPE column, and for equilibration of the online SPE column. (B) The online SPE column is switched into the analytical flow path, eluting the PAHs onto the analytical column for gradient separation followed by UV and fluorescence detections.

REAGENTS AND STANDARDS

Deionized water from a Milli-Q® Gradient A10

Acetonitrile (CH3CN), HPLC grade (Fisher Scientific)

Methanol (CH3OH), HPLC grade (Fisher Scientific)

Mix of PAHs standard, EPA Sample for Method 610, 550 and 550.1, (AccuStandard®) the concentration of each component is 2000 µg/mL for Acenaphthylene, 1000 µg/mL for Acenaphthene and Naphthalene, 200 µg/mL for Fluorene, Fluoranthene, Benzo(b) fluoranthene, Dibenzo(a,h)anthracene, and Benzo(g,h,i)perylene, 100 µg/mL for Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(k) fluoranthene, Chrysene, Indeno(1,2,3-cd)pyrene, Phenanthrene, and Pyrene

CONDITIONS

Analytical Column: Supelcosil LC-PAH columns, 4.6 × 250 mm (Supelco Cat. # 58229)

Online SPE Column: Acclaim PA2, 3 µm, 4.6 × 50 mm (P/N 063189)

Mobile Phases: For both loading and analysis pumps

A. Water

B. Acetonitrile

Injection Volume: 2.0 mL on the SPE column

Column Temperature: 20 °C

Detection: UV (on 254 nm) and Fluorescence (at various excitation and emission wavelengths), in series.

Table 1 shows the gradient for on-line SPE using the loading pump, Table 2 the gradient for separation using the analysis pump, and Table 3 the valve switching program.

The PAHs have good fluorescent responses except for acenaphthylene. Because their fluorescent responses occur at different excitation and emission wavelengths, it is necessary to change these wavelengths based on individual PAH retention times. Table 4 shows the program for wavelength changes. UV detection is used to determine four compounds including acenaphthylene, naphthalene, acenaphthene, and fluorene according to EPA method 550.1 requirements.

Table 1. Gradient Program for On-line SPE

Time (min)	Flow rate (mL/min)	Solvent A (H_2O) (% vol.)	Solvent B (CH_3CN) (% vol.)	Curve
0	1.0	95	5	
8	1.0	95	5	5
8.5	0.5	0	100	5
54	0.5	0	100	5
54.5	1.0	95	5	5
65	1.0	95	5	5

Table 2. Gradient Program for Separation

Time (min)	Flow rate (mL/min)	Solvent A (H_2O) (% vol.)	Solvent B (CH_3CN (% vol.)	Curve
0	1.0	60	40	
10	1.0	60	40	5
30	1.0	0	100	6
54	1.0	0	100	5
54.5	1.0	60	40	5
65	1.0	60	40	5

Table 3. Valve Switching Program

Time (min)	Position
0	1 – 2
8	6 – 1
54	1 – 2

Table 4. Wavelength Changes for RF2000 Fluorescence Detector

Time (min)	Ex / Em Wavelength (nm)	Gain
0	256 / 390	1
31.5	275 / 420	4
34	270 / 385	1
37	290 / 430	1
51	305 / 480	4
65	256 / 390	1

PREPARATION OF STANDARDS AND SAMPLES

Preparation of Stock and Working Standards

To prepare a mixed stock standard solution, add 5 µL of the mix of PAHs standard, using a 10 µL syringe, to a 10 mL vial, and then add 9995 µL CH3CN-Methanol-H_2O (2:2:1, v/v), using a 5 mL pipette. The mixed stock standard solution is used to prepare working standards for calibration as described in Table 5.

SYSTEM SETUP

Description of the On-Line SPE-HPLC Method

The flow scheme, shown in Figure 2, couples the SPE directly with the analytical HPLC run, using a second gradient pump and one two-position, six-port (2P-6P) column-switching valve. Figure 2, diagram A shows the valve positions at the time of the injection. The filtered sample is injected directly onto the system, and delivered to the SPE column for enrichment. The analytical column is equilibrated with the second pump at the same time. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to flush out the bound analytes with CH_3CN/water (Figure 2, diagram B), and then the analytes are separated on the analytical column and detected by UV and fluorescence in series.

Selection of the Online SPE and Analytical Columns

The Acclaim PA2 is good choice for concentration of PAHs in water samples as explained in the introduction. The experiments showed that a Donor-Acceptor Complex Chromatography (DACC) column (Varian, ChromSpher Pi) also can be used as the SPE column in this method with similar performance under the same conditions.

The Supelcosil LC-PAH column was used for separation in this experiment, but another analytical PAH column such as the Phenomenex® Envirosep PP can be used for this application. If another PAH column is used, the separation conditions will need to be adjusted to account for the different column chemistry. Experiments showed that a longer analytical column is better for the online SPE application. This is because possible peak deterioration caused by the sample transfer from the online SPE column to the analytical column decreases with increasing SPE column length.

Table 5. Preparation of the Working Standards

Vial # (10 mL)		Vial 1	Vial 2	Vial 3	Vial 4	Vial 5
Volume of mix of stock standard solution (µL)		20	50	100	200	500
Volume of deionized water (µL)		9980	9950	9900	9800	9500
Concentration of PAHs (µg/L)	Naphthalene	1.0	2.5	5.0	10	25
	Acenaphthylene	2.0	5.0	10	20	50
	Acenaphthene	1.0	2.5	5.0	10	25
	Fluorene	0.20	0.50	1.0	2.0	5.0
	Phenanthrene	0.10	0.25	0.50	1.0	2.5
	Anthracene	0.10	0.25	0.50	1.0	2.5
	Fluoranthene	0.20	0.50	1.00	2.0	5.0
	Pyrene	0.10	0.25	0.50	1.0	2.5
	Benzo(a)anthracene	0.10	0.25	0.50	1.0	2.5
	Chrysene	0.10	0.25	0.50	1.0	2.5
	Benzo(b)fluoranthene	0.20	0.50	1.00	2.0	5.0
	Benzo(k)fluoranthene	0.10	0.25	0.50	1.0	2.5
	Benzo(a)pyrene	0.10	0.25	0.50	1.0	2.5
	Dibenzo(a,h)anthracene	0.20	0.50	1.0	2.0	5.0
	Benzo(g,h,i)perylene	0.20	0.50	1.0	2.0	5.0
	Indeno(1,2,3-cd)pyrene	0.10	0.25	0.50	1.0	2.5

Conversion of WPS-3000TSL Autosampler for Online SPE

The WPS-3000TSL autosampler has 15 positions for 10 mL vials that can accommodate the 2 mL injection volume.

Because a 2 mL sample needs to be injected, the semipreparative version of WPS-3000 autosampler is required (S/N 5822.0028 with temperature control, or 5822.0018 without temperature control). It may be more convenient to install a 2500 µL semipreparative sample loop (P/N 6820.2416) to the current analytical WPS-3000 autosampler for this application. The following parts, which belong to the WPS-3000SL Semipreparative Upgrade Kit (P/N 6822.2450), need to be installed in place of the parts used in common analytical version: a buffer loop (P/N 6820.2421), a needle for semipreparative sample loop (P/N 6820.2419), and a 2500 µL syringe (P/N 6820.0006). There is no need to install the other parts of the Upgrade Kit.

SAMPLE PREPARATION

Tap water was collected at the Dionex Shanghai Applications Lab located in the Pudong District, Shanghai, China. Spiked tap water samples were prepared by adding 400 µL of the mixed stock standard solution to a 50 mL conical flask (with plug), then adding 39.6 mL of tap water filtered through a 0.45 µm membrane (Millex-HN).

RESULTS AND DISCUSSION

Reproducibility, Detection Limits, and Linearity

Method reproducibility was estimated by making eight consecutive replicate injections of tap water spiked with the PAHs standard mix (Figure 3). Table 6 summarizes the retention time and peak area precision data. Calibration linearity for the determination of PAHs was investigated by making four replicate injections of a mixed standard of PAHs prepared at four different concentrations. The external standard method was used to calculate the calibration curve and for 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, demonstrating that they can match the MDLs obtained in EPA 550.1.

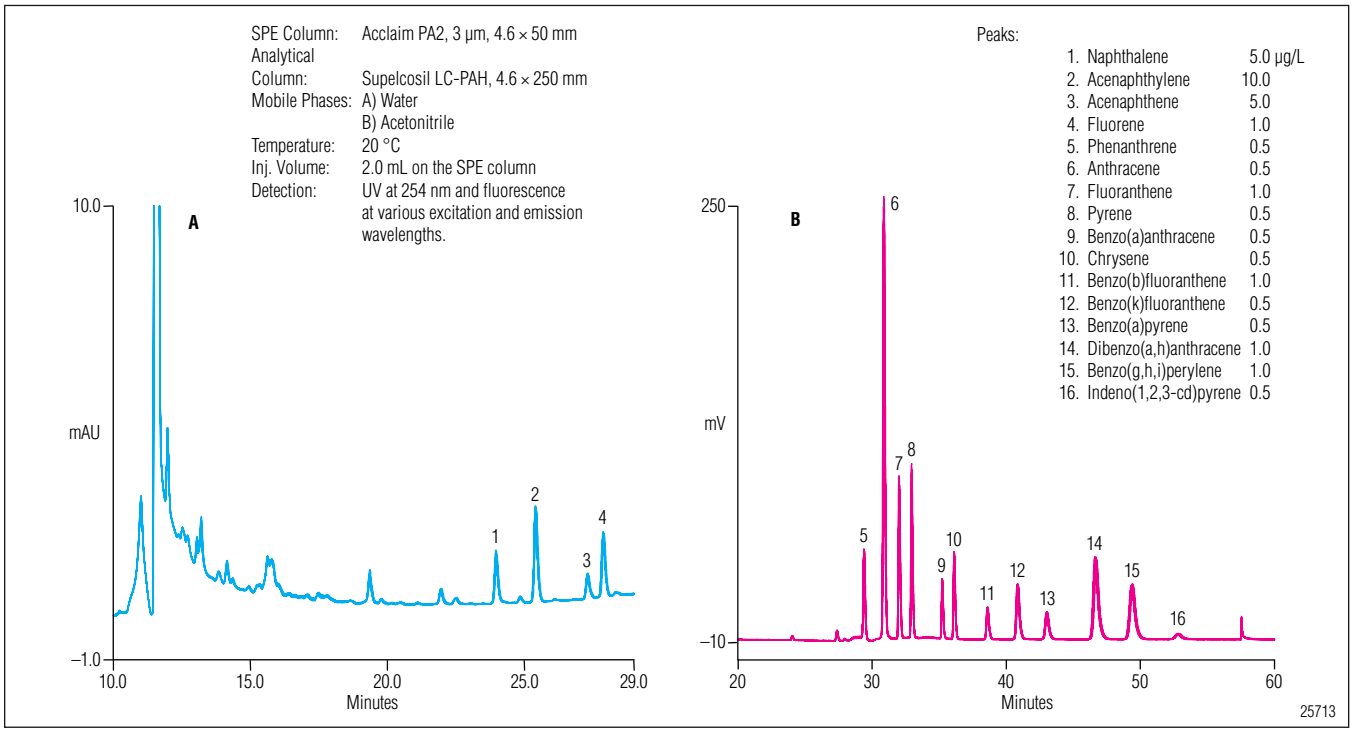


Figure 3. Overlay of chromatograms of eight consecutive injections of a tap water sample spiked with the PAHs standard mix, obtained by A) UV at 254 nm and B) Fluorescence at different wavelengths.

Table 6. Reproducibility of Retention Times and Peak Areas[a]		
PAH	**RT RSD**	**Area RSD**
Naphthalene	0.057	10.808
Acenaphthylene	0.049	4.093
Acenaphthene	0.051	6.211
Fluorene	0.049	3.535
Phenanthrene	0.048	7.861
Anthracene	0.046	1.792
Fluoranthene	0.040	2.754
Pyrene	0.034	3.591
Benzo(a)anthracene	0.033	1.635
Chrysene	0.039	2.015
Benzo(b)fluoranthene	0.052	1.013
Benzo(k)fluoranthene	0.067	2.018
Benzo(a)pyrene	0.074	1.593
Dibenzo(a,h)anthracene	0.106	2.266
Benzo(g,h,i)perylene	0.101	2.057
Indeno(1,2,3-cd)pyrene	0.132	5.777

[a]Eight consecutive injections of a tap water sample spiked with a mixed PAH standard.

Tap Water Sample Analysis

Figure 4 shows chromatograms of tap water and the tap water spiked with PAHs. The results are summarized in Table 8. Only Naphthalene (peak 1) was found in the tap water sample, and its concentration (0.46 µg/L) is below the calculated detection limit (1.17 µg/L for naphthalene). Recoveries of all PAHs in the spiked sample were acceptable.

Table 7. Calibration Data for the 16 PAHs					
PAH	**Equation**	**r (%)**	**Detection**	**MDL (µg/L)**	**MDL (µg/L), EPA method 550.1 required**
Naphthalene	A = 0.0500c - 0.0097	99.50	UV	1.17	2.20
Acenaphthylene	A = 0.0399c - 0.0092	99.94	UV	1.08	1.41
Acenaphthene	A = 0.0229c - 0.0041	99.86	UV	0.84	2.04
Fluorene	A = 0.2644c - 0.0103	99.94	UV	0.11	0.126
Phenanthrene	A = 17.17c - 1.035	99.86	FL	0.15	0.15
Anthracene	A = 71.17c - 4.346	99.69	FL	0.08	0.14
Fluoranthene	A = 20.72c – 0.959	99.43	FL	0.09	0.009
Pyrene	A = 44.77c - 10.50	99.45	FL	0.26	0.126
Benzo(a)anthracene	A = 11.34c - 0.7228	99.43	FL	0.08	0.004
Chrysene	A = 27.27c - 3.799	99.52	FL	0.15	0.160
Benzo(b)fluoranthene	A = 6.276c + 0.192	99.64	FL	0.017	0.006
Benzo(k)fluoranthene	A = 42.93c - 3.965	99.91	FL	0.01	0.003
Benzo(a)pyrene	A = 16.05c - 0.1821	99.82	FL	0.022	0.016
Dibenzo(a,h)anthracene	A = 23.29c – 0.480	99.84	FL	0.025	0.035
Benzo(g,h,i)perylene	A = 22.12c – 0.455	99.89	FL	0.070	0.020
Indeno(1,2,3-cd)pyrene	A = 6.184c - 0.1596	99.92	FL	0.059	0.036

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 eight injections of tap water sample spiked with mixed PAHs standard is multiplied by 3.50 (at n = 8) to yield the MDL.

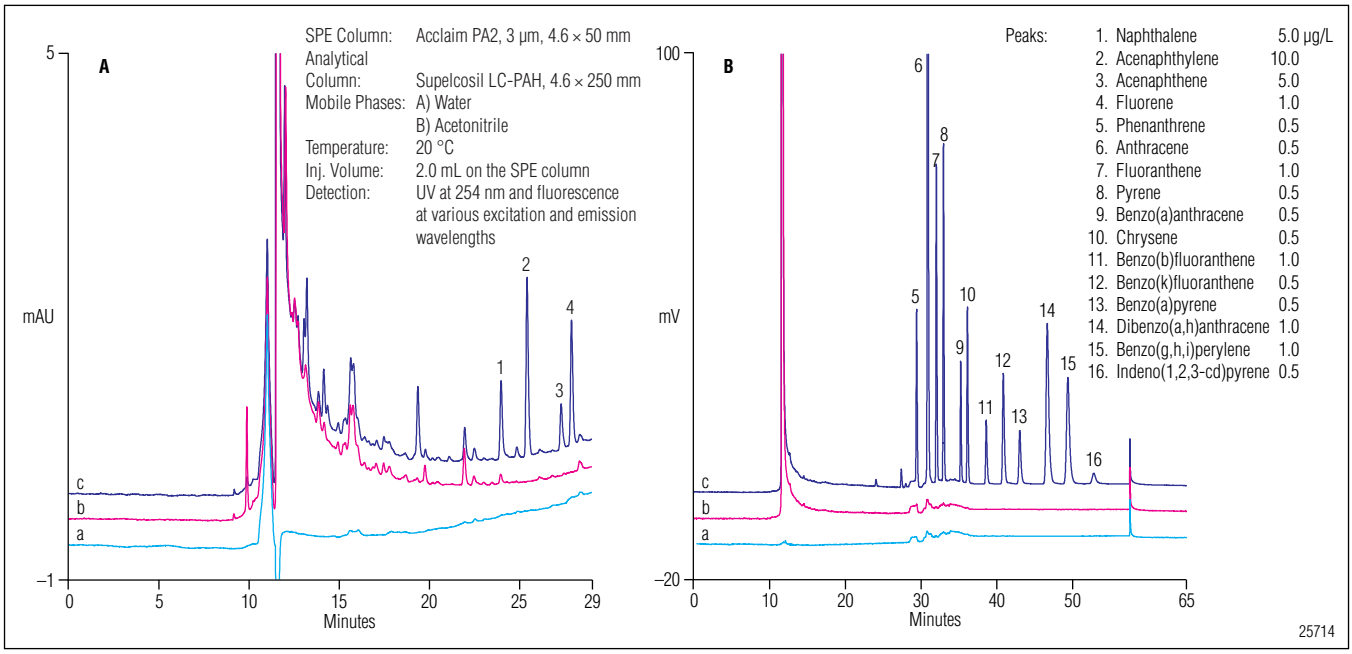


Figure 4 Chromatograms obtained by A) UV at 254 nm and B) FL at different wavelengths. Chromatograms of (a) blank, (b) tap water, and (c) tap water spiked with a PAH standard mixture.

CONCLUSION

This application note demonstrates that PAHs can be successfully determined in drinking water at concentrations that meet the detection limits specified in EPA Method 550.1[2] using an online SPE method with an UltiMate 3000 ×2 dual HPLC system. This method saves analyst time and the expense of consumables compared to offline SPE.

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. Fresh acetonitrile must be used.

A command for washing the 2.5 mL sample loop was added to the program to reduce carryover when the SPE column is on the analytical flow path, as follows:

20.000 WashSampleLoop Volume=2500.000
22.000 Wash
25.000 InjectValveToInject

It is advisable to add an on-line filter (2 µm) between the injector and switching valve to protect the SPE and analytical columns when running a large number of samples.

Table 8. Analytical Results for Tap Water Samples

PAH	Tap water		
	Detected (µg/L)	Added (µg/L)	Recovery (%)
Naphthalene	< MDL	5.0	72
Acenaphthylene	ND	10	85
Acenaphthene	ND	5.0	80
Fluorene	ND	1.0	90
Phenanthrene	ND	0.50	92
Anthracene	ND	0.50	106
Fluoranthene	ND	1.0	102
Pyrene	ND	0.50	99
Benzo(a)anthracene	ND	0.50	84
Chrysene	ND	0.50	76
Benzo(b)fluoranthene	ND	1.0	98
Benzo(k)fluoranthene	ND	0.50	104
Benzo(a)pyrene	ND	0.50	104
Dibenzo(a,h)anthracene	ND	1.0	90
Benzo(g,h,i)perylene	ND	1.0	76
Indeno(1,2,3-cd)pyrene	ND	0.50	96

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

REFERENCES

1. Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Liquid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection, Method 550, U.S. EPA.
2. Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection, Method 550.1, U.S. EPA.
3. Zhou, Y.Y.; Yan, X.P.; Kim, K.N.; Wang, S.W.; Liu, M.G. Exploration of Coordination Polymer as Sorbent for Flow Injection Solid-Phase Extraction On-Line Coupled with High-Performance Liquid Chromatography for Determination of Polycyclic Aromatic Hydrocarbons in Environmental Materials. *J. Chromatogr. A* **2006,** 1116, 172–178.
4. Dionex Corporation. *Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection,* Application Note 191, LPN 1949-02. Sunnyvale, CA, 2008.
5. Dionex Corporation. *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection,* Application Note 196, LPN 1998. Sunnyvale, CA, 2008.

Determination of Haloacetic Acids in Water Using IC-ESI-MS/MS

INTRODUCTION

This method allows separation and detection of sub-µg/L levels of nine haloacetic acids (HAAs) in high-ionic strength matrices. Using this method, the analytes are separated from chloride, sulfate, nitrate, bromide and bicarbonate, and detected using a triple quadrupole mass spectrometer with an electrospray interface. Quantification is achieved using internal standards.

Haloacetic acids occur in drinking water during the disinfection process, as a result of the reaction between chlorine and natural organic materials, such as humic and fulvic acids.[1,2] The iodoacids (e.g. iodoacetic acid) are much less stable and are not included in this analysis. When bromide is present in the water, bromoacetic acids and mixed chloro- and bromoacetic acids can also be generated. Haloacetic acids have been linked to possible health threats to human health. Monitoring for monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA) and dibromoacetic acid (DBAA) has been in effect since they were first regulated under the Stage I Disinfection Byproducts (DBP) Rule, Dec. 16, 1998, with a Minimum Contamination Level (MCL) set at 60 µg/L. Stage II DBP Rule, Jan. 4, 2006, maintained the MCL, but also instituted minimum reporting limits (MRL) requirements of 2 µg/L for MCAA and 1 µg/L for the other HAAs. The remaining four HAAs that may be present in drinking water are: chlorobromoacetic acid (CBAA), chlorodibromoacetic acid (CDBAA), dichlorobromoacetic acid (DCBAA), and tribromoacetic acid (TBAA).

The determination of the chloro-, bromo-, and mixed haloacetic acids in waters destined for human consumption, including drinking water and swimming pool water, has been reported using a variety of analytical techniques.[3] USEPA Methods 552.2 and 552.3 use acidic methanol derivatization followed by gas chromatography with electron capture detection.[4] This method is both labor-intensive and time-consuming. Bruzzoniti[5] recently published a table summarizing the existing IC columns and methods used for HAAs analysis, including this method, which uses the IonPac® AS24 column. Asami[6] used offline sample pretreatment with external standard calibration and MS/MS detection for calibration of haloacetic acids and oxyhalides, using perchlorate as an internal standard. Only the method using the IonPac AS24 method addresses the issue of high-ionic strength matrices. All the IC methods take advantage of the low pKa values of HAAs (~ 0.7–2.8) by using anion-exchange separation mode. Hydroxide-based eluents are used in conjunction with chemical suppression, so the background signal entering the mass spectrometer is as low as that of water. When mass spectrometric detection is used, the matrix ions are typically diverted to waste during the analytical run to avoid contamination of the detector. USEPA Method, 332.0[7] uses the same configuration as discussed in this paper for the determination of perchlorate in drinking water; namely, ion chromatography with matrix diversion and detection using suppressed conductivity followed by electrospray mass spectrometry. The selectivity of the analytical column in a method using matrix diversion must be designed such that the matrix ions are sufficiently resolved from target analytes.

Four internal standards are used in our method for the nine target analytes. These were chosen because they elute throughout the chromatographic run, thus allowing easy tracking of close-eluting analytes. Stuber and Reemtsma[8] discuss the challenges of quantification using LC-ESI-MS in the presence of significant matrix effects, and provide some guidance for using internal standards. Most of the currently published work describing various analytical approaches for determination of HAAs, however, does not adequately address the challenges of very high-ionic strength matrices, or the need for internal standards to obtain accurate and precise quantification.

Figure 1 shows the chromatogram of the nine standards, the two matrix diversion windows, and the general form of the KOH gradient. The three periods noted in the figure correspond to three periods for data collection in the Analyst method program. Figure 2 shows the general instrumentation schematic.

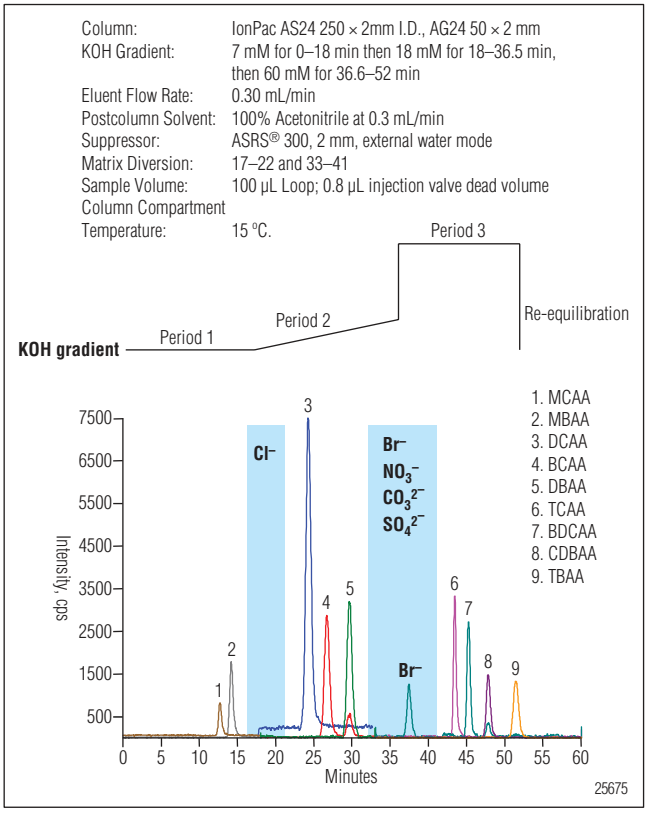


Figure 1. Chromatogram produced by chromatography conditons and mass spectrometer conditions provided in Table 1. The shaded areas show the time windows for matrix diversion to waste and the matrix ions that elute in those windows. The time windows for data collection in Periods 1, 2, and 3 are indicated.

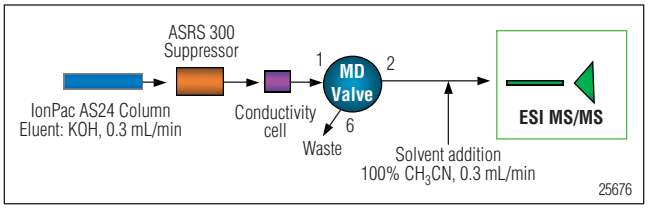


Figure 2. ICS-3000 system schematic, showing matrix diversion and mass spectrometric detection.

RECOMMENDED EQUIPMENT

ICS3000 Chromatography System

DP Dual Pump module (or SP and an AXP)

DP1 Analytical Pump

DP2 pump is used to deliver post-suppressor acetonitrile

DC Dual-Zone Chromatography Module

CD Conductivity Detector

AS Autosampler with sample tray cooling

EG Eluent Generator

Mixing Tee (Upchurch, part number U-466)

Mass Spectrometer

ABI-Sciex (Toronto, Canada) API2000™ Triple Quadrupole Mass Spectrometer with electrospray interface capable of negative ion detection, or equivalent

Nitrogen and Zero Air supplies as specified by MS manufacturer

25-pin relay cable connecting mass spectrometer to DP pump module; pins 19 (red) and 7 (black) in the DP connector

Software

Dionex DCMSLink™ 2.0 software or higher

ABI Sciex Analyst software (version 1.4.2 or higher)
XCalibur 2.0 or higher

Standards

Deionized water: 18 MΩ or better

Acetonitrile (HPLC grade)

Analyte standard mix (1000 µg/mL) of the nine nativeHAAs; monochloroacetic acid (MCAA),monobromoacetic acid (MBAA), dichloroaceticacid (DCAA), trichloroacetic acid (TCAA),dibromoacetic acid (DBAA), chlorobromoacetic acid(CBAA), chlorodibromoacetic acid (CDBAA),dichlorobromoacetic acid (DCBAA), and tribromoacetic acid (TBAA) purchased from Restek (P/N31896).

The internal standards from Dionex are: monochloroacetic acid-2-13C (1000 µg/mL, P/N 069406), monobromoacetic acid-1-13C (1000 µg/mL, P/N 069407), dichloroacetic acid-2-13C (1000 µg/mL, P/N 069408), and trichloroacetic acid-2-13C (1000 µg/mL, P/N 069409).

Standards are dissolved in methyl tert-butyl ether (MtBE). A working standard mixture of the four internal standards was prepared in deionized water. All standard solutions were kept refrigerated at 4 °C when not in use. Standards in the 2-5 µg/L range are stable for 14 days when stored at 4 °C with PTFE/silicone septa. Because the standards are purchased in MtBE, which has limited solubility in water (~ 5%), not more than ~ 0.5% of MtBE is added when making the mixtures, relative to the total water volume.

Sample Preparation

Samples were collected in amber glass bottles with PTFE-lined screw caps. Crystalline or granular ammonium chloride is added to the sample containers to produce a final concentration of 100 mg/L of ammonium chloride. The preservation requirements are exactly the same as those described in EPA Method 552.3.

CONDITIONS

Chromatography Conditions

Column: IonPac AS24 250 × 2 mm I.D., IonPac AG24 50 × 2 mm

KOH Gradient:

Time	*KOH (mM)*
–7.0	7
0.0	7
18.0	7
36.5	18
36.6	60
52.0	60

Eluent Flow Rate: 0.30 mL/min

Postcolumn Solvent: 100% Acetonitrile at 0.3 mL/min

Suppressor: ASRS 300 (2 mm) External water mode

Anion Trap: CR-ATC (2-mm)

Matrix Diversion: 17–22 and 33–41

Sample Volume: 100 µL sample loop

Column Compartment Temperature: 15 ºC

Autosampler Temperature: 8 °C

Detector Compartment Temperature: 30 °C

Mass Spectrometric Conditions: Tables 1–5

Table 1. API2000 Conditions

Analyte	KOH Gradient	Transition	Source-Dependent Parameters	Declustering Potential (V)	Focusing Potential (v)	Collision Energy (eV)	Entrance Potential (V)	Collsion Cell Entrance Potential (V)	Collision Cell Exit Potential (V)	Dwell Time (mSec)
MCAA MCAA-21-13C	7 mM 0 – 18 min	92.9/34.9 93/34.9	Curtain 20 CAD 2 Ionspray -4500 Temp 475 °C GS1/GS2 90/90	-20	-300	-12	-10	-12	-6	600 each
MBAA MBAA-1-13C		137/78.8 138/78.8		-11	-350	-12	-7	-10	-14	600 each
Dalapon	18 mM 18 – 36.5 min.	141/97	Curtain 25 CAD 4 Ionspray -4500 Temp 475 °C GS1/GS2 90/90	-13	-350	-11	-8	-13	-6	500
DCAA DCAA-2-13C		127/82.9 128/84		-11	-320	-12	-6.5	-12	-6	500 each
BCAA		170.8/78.8		-16	-300	-28	-6	-14	-8	500
DBAA		214.7/170.7		-11	-350	-12	-4.5	-15	-10	500
TCAA TCAA-2-13C	60 mM 36.6 – 52 min	161/116.9 162/118	Curtain 25 CAD 4 Ionspray -4500 Temp 475 °C GS1/GS2 90/90	-6	-290	-7	-7	-13.7	-13.7	400 each
BDCAA		207/81 or 79/79		-12	-300	-6	-4	-15	-14	400
CDBAA		207/78.8		-11	-300	-20	-4	-15	-6	400
TBAA		250.75/78.8		15	-350	-28	-5	-12	-12	400

Table 2. API3200 Conditions									
Analyte	**KOH Gradient**	**Transition**	**Source-Dependent Parameters**	**Declustering Potential (V)**	**Collision Energy (eV)**	**Entrance Potential (V)**	**Collsion Cell Entrance Potential (V)**	**Collision Cell Exit Potential (V)**	**Dwell Time (mSec)**
MCAA MCAA-2-13C	7 mM 0 – 18 min	92.9/34.9	Curtain 30 CAD 2 Ionspray -4500 Temp 500 °C GS1/GS2 70/70	-15	-3	-5	-17	-5	600 each
MBAA MBAA-1-13C		137/78.8		-14	-7	-8	-20	-1.5	600 each
Dalapon	18 mM 18 – 36.5 min.	141/97	Curtain 30 CAD 3 Ionspray -4500 Temp 500 °C GS1/GS2 70/70	-17	-5	-5	-11	-1	500
DCAA DCAA-2-13C		127/82.9		-15	-3	-5	-17	-1	500 each
BCAA		170.8/78.8		-26	-4	-8	-32	-1.5	500
DBAA		214.7/170.7		-22	-3.5	-22.8	-18	-1.5	500
TCAA TCAA-2-13C	60 mM 36.6 – 52 min	161/116.9	Curtain 30 CAD 3 Ionspray -4500 Temp 250 °C GS1/GS2 70/70	-12	-3	-6	-19	-1	400 each
BDCAA		207/81 or 79/79		-85	-4	-15.1	-10	-1.5	400
CDBAA		207/78.8		-12	-3	-16.8	-6	-14	400
TBAA		250.75/78.8		-13	-2.5	-13	-32	-1.5	400

Table 3. API4000 Conditions								
Analyte	**KOH Gradient**	**Transition**	**Source-Dependent Parameters**	**Declustering Potential (V)**	**Collision Energy (eV)**	**Entrance Potential (V)**	**Collision Cell Exit Potential (V)**	**Dwell Time (mSec)**
MCAA MCAA-2-13C	7 mM 0 – 18 min	92.9/34.9	Curtain 20 CAD 2 Ionspray -4000 Temp 500 °C GS1/GS2 50/50	-25	-15	-2	-3	600 each
MBAA MBAA-1-13C		137/78.8		-25	-15	-2	-3	600 each
Dalapon	18 mM 18 – 36.5 min.	141/97	Curtain 20 CAD 8 Ionspray -4300 Temp 500 °C GS1/GS2 50/50	-33	-15	-4	-13	500
DCAA DCAA-2-13C		127/82.9		-43	-31	-5	-2	500 each
BCAA		170.8/78.8		-31	-17	-5	-9	500
DBAA		214.7/170.7		-21	-11	-4	-5	500
TCAA TCAA-2-13C	60 mM 36.6 – 52 min	161/116.9	Curtain 20 CAD 10 Ionspray -4200 Temp 250 °C GS1/GS2 50/50	-35	-22	-4.5	-12	400 each
BDCAA		206.8/81		-35	-22	-4.5	-12	400
CDBAA		206.8/81		-35	-22	-4.5	-12	400
TBAA		250.9/78.8		-30	-34	-4	-12	400

Table 4. Thermo Quantum Access Conditions								
Analyte	**Q1/Q3**	**CE**	**Tube Lens**	**Cap Temp (°C)**	**Sheath Gas/Aux Gas**	**Ion Sweep**	**Skimmer Offset (V)**	**Scan Time/ Section (s)**
MCAA MCAA-2-13C	93/35.6 94/35.6	10	26	270	40/15	0.1	0	1.25
MBAA MBAA-1-13C	137/79.1 138/79.1	12	33	270	40/15	0.1	0	1.25
DCAA DCAA-2-13C	127/83.2 128/84	11	26	270	40/15	0.1	0	1.25
DBAA	214.8/79.2	24	33	270	40/15	0.1	0	1.25
BCAA	171/79.2	35	44	270	40/15	0.1	0	1.25
TCAA TCAA-2-13C	161.1/117.1 162/118	10	69	270	40/15	0.1	0	1.6
BDCAA	79/79	15	30	270	40/15	0.1	0	1.6
CDBAA	206.7/79.1	15	30	270	40/15	0.1	0	2.5
TBAA	250.7/79.1	25	26	270	40/15	0.1	0	2.5

Table 5. Waters Quattro Premier Parameters					
Analyte	**Transition**	**Dwell (sec)**	**Cone, V**	**Extractor/RF Lens/Source Block Temp (V/V/oC)**	**Collision Energy, V**
MCAA MCAA-2-13C	92/35 93/93	1.0 0.5	15	-3/-0.5/120	8
MBAA MBAA-1-13C	136.9/78.9	0.5	15	-3/-0.5/120	10
DAL	140.9/97	0.5	18	-3/-0.5/120	8
DCAA DCAA-2-13C	126.9/83	0.5	17	-3/-0.5/120	10
BCAA	172.9/128.9	0.5	17	-3/-0.5/120	10
DBAA	216.8/172.84	0.5	18	-3/-0.5/120	12
TCAA TCAA-2-13C	160.9/116.9 162.9/118.9	0.5 0.5	16	-3/-0.5/120	8
BDCAA	162.9/80.9	1.0	25	-3/-0.5/120	10
CDBAA	206.8/78.9	1.0	28	-3/-0.5/120	10
TBAA	250.8/78.9	1.0	28	-3/-0.5/120	12

Other Conditions:
Desolvation Gas: 350 °C @ 940 L/hr
Capillary: -2.8V
Collision Pressure: 5.5×10^{-3} (0.15 flow @ 7 psig)
Cone Flow: 100 L/hr,
ACN Flow Rate: 0.2 mL/min

RESULTS AND DISCUSSION OF THE METHOD

Separation

One of the most important features of this method is the ability to quantify the HAAs in the presence of matrices of high-ionic strength. Initially, the separation was achieved using the IonPac AS20 (250 × 2 mm I.D., 78 µEq/column). Reduced peak height, lower peak efficiencies, and shifting retention times were observed when the matrix composition exceeded 100 mg/L chloride and sulfate. The IonPac AS24 column (250 × 2 mm I.D.) 140 µEq/column provides approximately twice the anion-exchange capacity as the AS20.9 This improved capacity is required for this application, where the concentration of common matrix ions can be as high as 250 mg/L chloride, 250 mg/L sulfate, 150 mg/L bicarbonate, and 30 mg/L nitrate. The high capacity of the column insures that the ion-exchange sites are not consumed with matrix ions during the separation.

Figure 1 shows separation of nine HAA standards and the time windows for the common matrix ions. The common ions shown are separated from the HAAs, and this separation allows time for the diversion of these ions to waste using a three-way valve before they can enter the mass spectrometer.

Solvent Addition

Addition of 0.3 mL/min of 100% acetonitrile after the IC suppressor and before the ESI inlet improves sensitivity from twofold to 10-fold depending on the analyte and condition of the mass spectrometer. The flow of solvent to the mass spectrometer continues during matrix diversion for stability of the electrospray.

Temperature

The retention times for the HAAs increase as column temperature increases. In addition, some of the HAAs–most notably the brominated species–are less stable at higher temperatures and high pH. The autosampler temperature was set to 8 °C and the column compartment temperature to 15 °C to maximumize analyte and internal standard stability and retention time reproducibility. In addition, stable retention times are critical to maintain the times for the matrix diversion windows.

The ESI source temperature was optimized for maximum sensitivity of all analytes. With this method, trisubstituted HAAs are more susceptible to source temperature changes, and the best sensitivity was achieved at the minimum temperature needed for desolvation in the electrospray interface.

Matrix Diversion

The IonPac AS24 column manual contains a procedure for setting the correct matrix diversion window times and method parameters.

Internal Standards and Calibration

As is common, the ratios of peak areas for analytes and internal standards versus analyte concentration are used to produce the calibration plots. Internal standards were chosen that elute in each of the three sections of the gradient method due to changes in the background and eluent composition over the course of the run. Several choices for Multiple Reaction Monitoring (MRM) transitions were available due to the presence of Cl and Br isotopes. MCAA-2-13C (*m/z* 94 > 35), MBAA-1-13C (*m/z* 138 > 79), DCAA-2-13C 1 (*m/z* 128 > 84), and TCAA-2-13C 13C (*m/z* 162 > 118) were chosen because they exhibited low background and good sensitivity. Other choices may be appropriate depending on sample matrix.

Referring to Figure 1, Period 1 uses 7 mM KOH eluent and the analytes are MCAA and MBAA. Chloride elutes at the end of this region, so a matrix diversion window separates this first section of the gradient from the second section. The brominated acetic acids–especially MBAA–are known to be susceptible to decomposition at elevated temperature and pH, so stable-labeled MBAA-1-13C is used for accurate tracking of the MBAA analyte. MCAA-2-13C is also used as an internal standard in the first section of the chromatogram for the quantification of MCAA. The stable-labeled internal standard for Period 2 of the gradient is DCAA-2-13C. In this section, the KOH concentration ramps to 18 mM and the analytes are the dihaloacetic acids, including DCAA, BCAA, and DBAA. Period 2 ends with the diversion of sulfate, nitrate, bromide, and bicarbonate to waste. The concentration of KOH eluent is increased to 60 mM in Period 3 of the gradient and the trihaloacetic acids TCAA, BDCAA, DBCAA and TBAA elute. The internal standard for this section is TCAA-2-13C.

The system was calibrated using a mixture of nine haloacetic acids at levels of 0.25, 1.0, 2.5, 5.0, 10.0, and 20.0 µg/L, with the four isotopically labeled internal standards at 3.0 µg/L added to each sample and standard. A relative response ratio was generated to produce the calibration plots. A linear fit was used with 1/× weighting. Correlation coefficients in deionized water were 0.998 or better

Precursor and Product Ions

Precursor ions are generally the result of deprotonation (M-H)- of the organic acid. Because the target species all have halide substituents, there are multiple choices for possible transitions. The specific transitions are:

MCAA (92.9 > 34.9), MBAA (137 > 78.8),
DCAA (127 > 82.9), BCAA (170.8 > 78.7),
DBAA (214.7 > 170.7), TCAA (161 > 116.9),
BDCAA (207 > 81 or 79 > 79), CDBAA (207 >78.8),
TBAA (250.7 > 78.8).

The trivalent compounds BDCAA and CDBAA are difficult to optimize, and BDCAA often fragments to *m/z* 79 in Q1, so the best sensitivity can usually be found at 79 > 79, although other transitions can be used if they provide adequate sensitivity. The MS/MS voltages are relatively low, suggesting the general fragility of these analytes. Tables 1–5 provide working conditions for five different mass spectrometers tested with this method.

Analytical Results

Table 6 shows linearity in deionized water and a matrix composed of 250 mg/L chloride, 250 mg/L sulfate, 30 mg/L nitrate, and 150 mg/L bicarbonate. The fitting method was linear with 1/× weighting using Analyst software. At the maximum matrix concentrations (250 mg/L chloride, 250 mg/L sulfate, 150 mg/L bicarbonate and 30 mg/L nitrate) linear range is 0.5-10 µg/L with $r^2 = 0.997$ or better. Minimum detection limits (MDLs) were calculated using the Student's t-test calculation with seven injections. The MDL values were 0.1–1.0 µg/L for the nine HAAs in the high-level matrix. DCAA showed the highest sensitivity, and the trivalent mixed acids BDCAA and CDBAA showed the least sensitivity.

Calibration check standards (CCS) were placed in each sequence at approximately every 10 sample injections at levels of 0.5 and 5.0 µg/L, and at the end of every sequence. The recovery of each CCS was 95–105% in every instance. In addition, the sample was spiked with 2.5 µg/L of the native calibration mixture to calculate percent recovery.

Figure 3 shows the extracted ion currents for Periods 1 and 2, and Figure 4 shows the extracted ion current for Period 3 from a water sample with high-ionic strength. This sample is from within the pressure zone of a southwest public water utility whose source is primarily surface water. The chloride concentration of the sample was 170 mg/L and the sulfate concentration was 215 mg/L. Concentrations were determined by ion chromatography, and the sample was not diluted before analysis. The monosubstituted and disubstituted halogenated analytes found in Period 1 and 2 are: MCAA (1.2 µg/L), MBAA (0.8 µg/L), DCAA (6.1 µg/L), BCAA (5.8 µg/L), and DBAA (2.9 µg/L). Figure 4 includes the trisubstituted HAAs for the sample. The analytes found in this sample are: TCAA (1.6 µg/L), BDCAA (4.3 µg/L), CDBAA (3.8 µg/L), and TBAA (0.7 µg/L) (See Table 7). These chromatograms were processed using Gaussian smoothing for 10 cycles. Some analytes can be found at several MRM transitions. Analytes that are seen on two MRM transitions used in the method are indicated with arrows. These results were compared to amounts quantified using Method 552.2 and amounts range from 65–130% of that method. (See Table 8).

Figure 4B shows the brominated species in the sample which experienced some degradation in Q1. An unidentified brominated compound elutes just prior to TCAA in the sample; this explains the sharp front on the TCAA peak. The 251 > 79 transistion is the most sensitive for quantification of CDBAA, although, with optimized tuning, monitoring mass 79 (79 > 79) and transistion *m/z* 207 > 81 was useful. The m/z 207 ion is the nominal mass for BDCAA and the decarboxylated CDBAA. As the number of bromide substitutions increases, the parent ion becomes less stable. The MRM for TBAA is *m/z* 251 > 79 where the *m/z* 251 ion is the result of decarboxylation of the parent ion. With the isotopes and the presence of the multiple halogens, there are several possibilities for MRM transitions.

Table 6. Linearity and MDL in Deionized Water and Matrix

Analyte	ISTD 5 µg/L	R^2 (Calibration Range 0.250-20 µg/L) DIW/Matrix	MDL µg/L/%RSD (n=7, 1 µg/L)	DI water MDL µg/L/%RSD (n=7, 1 µg/L) In Matrix
MCAA	MCAA-1-13C	0.9997/0.9989	0.51/3.5	0.44/14.7
MBAA	MBAA-1-13C	0.9999/0.9990	0.08/3.6	0.13/4.2
DCAA	DCAA-2-13C	0.9999/0.9991	0.39/2.0	0.10/3.3
BCAA	DCAA-2-13C	0.9999/0.9992	0.20/0.8	0.10/0.8
DBAA	DCAA-2-13C	0.9999/0.9993	0.16/5.5	0.33/10.8
TCAA	TCAA-2-13C	0.9999/0.9993	0.24/0.5	0.09/0.3
BDCAA	TCAA-2-13C	0.9991/0.9991	0.26/5.0	0.64/18.9
CDBAA	TCAA-2-13C	0.9992/0.9994	0.38/5.5	0.52/16.4
TBAA	TCAA-2-13C	0.9994/0.9998	0.26/9.2	0.36/9.9

Table 7. Summary of IC-ESI-MS/MS Analytical Results for Real Samples										
Sample	**Cl^--SO_4^{2-} (mg/L)**	**MCAA IC/MSMS (µg/L) %Spike Rec**	**MBAA IC/MSMS (µg/L) %Spike Rec**	**DCAA IC/MSMS (µg/L) %Spike Rec**	**BCAA IC/MSMS (µg/L) %Spike Rec**	**DBAA IC/MSMS (µg/L) %Spike Rec**	**TCAA IC/MSMS (µg/L) %Spike Rec**	**BDCAA* IC/MSMS (µg/L) %Spike Rec**	**CDBAA IC/MSMS (µg/L) %Spike Rec**	**TBAA IC/MSMS (µg/L) %Spike Rec**
Treated Reservoir Water	163 243	1.11 93%	1.08 103%	15.1 72%	8.5 76%	3.72 84%	5.85 80%	7.13 104%	4.75 92%	1.07 106%
Sample M	93 237	2.31 118%	1.16 106%	15.0 56%	9.4 65%	4.40 80%	6.2 70%	7.49 99%	5.12 72%	1.19 125%
Sample O	170 215	1.21 116%	0.82 105%	6.11 96%	5.83 94%	2.93 98%	1.59 91%	4.27 92%	3.85 100%	0.76 95%

Table 8. Summary of Method 552.2 Results for Real Samples										
Sample	**Cl^--SO_4^{2-} (mg/L)**	**MCAA (µg/L) 552.2 %Rec**	**MBAA (µg/L) 552.2 %Rec**	**DCAA (µg/L) 552.2 %Rec**	**BCAA (µg/L) 552.2 %Rec**	**DBAA (µg/L) 552.2 %Rec**	**TCAA (µg/L) 552.2 %Rec**	**BDCAA (µg/L) 552.2 %Rec**	**CDBAA (µg/L) 552.2 %Rec**	**TBAA (µg/L) 552.2 %Rec**
Treated Reservoir Water	163 243	1.31 85%	0.95 113%	17.33 87%	10.53 81%	4.74 78%	7.81 75%	7.75 104%	6.39 74%	Na
Sample M	93 237	2.12 109%	0.89 130%	16.33 92%	9.86 95%	4.44 100%	7.09 87%	7.03 106%	6.03 85%	Na
Sample O	170 215	1.33 91%	0.64 128%	6.23 98%	6.54 89%	3.43 85%	2.24 71%	4.32 99%	5.95 65%	Na

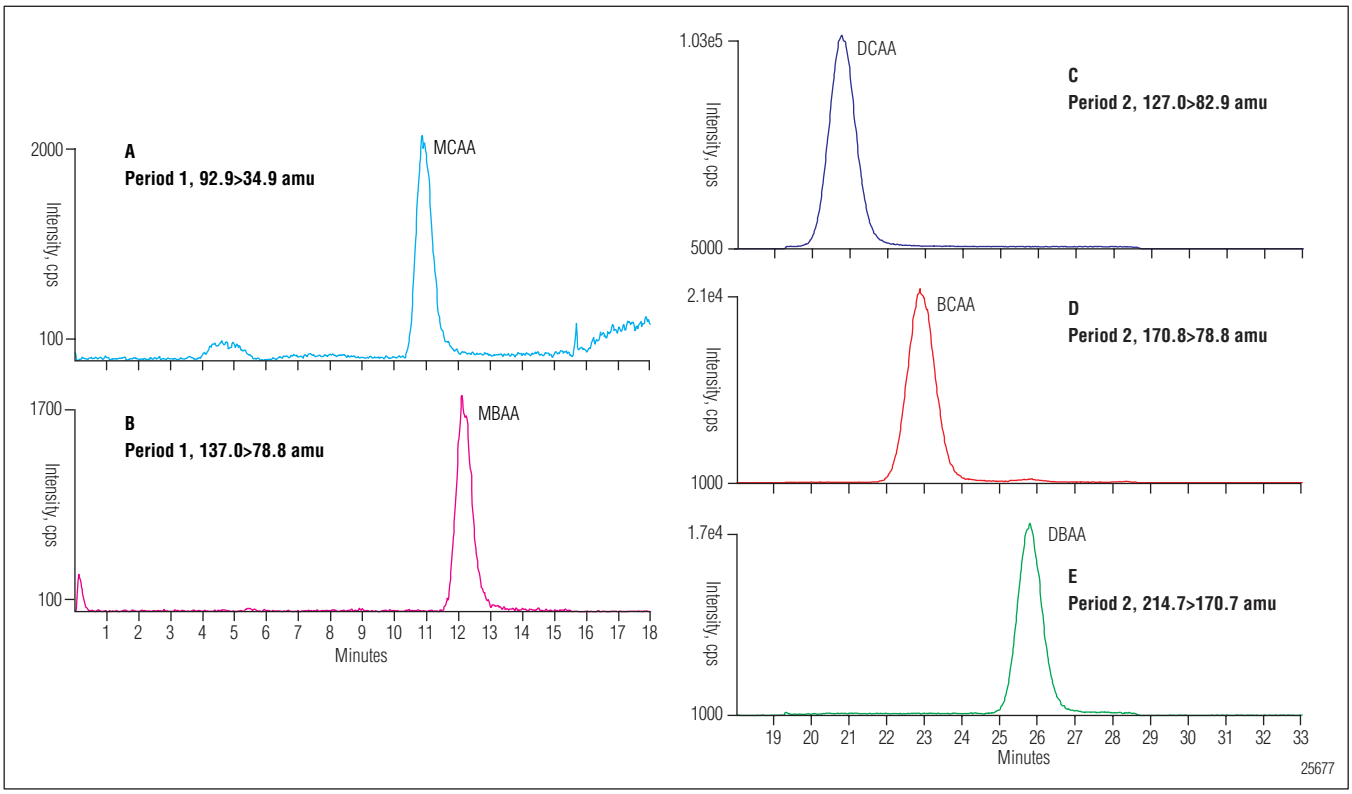


Figure 3. Extracted ion currents for Periods 1 and 2 for the treated water reservoir sample. A) monochloroacetic acid, 1.2 µg/L found; B) monobromoacetic acid, 0.82 µg/L found; C) dichloroacetic acid, 6.1 µg/L found; D) bromochloroacetic acid, 5.8 µg/L found; E) dibromo-acetic acid, 2.9 µg/L found. MRMs are as indicated. For conditions, see Figure 1.

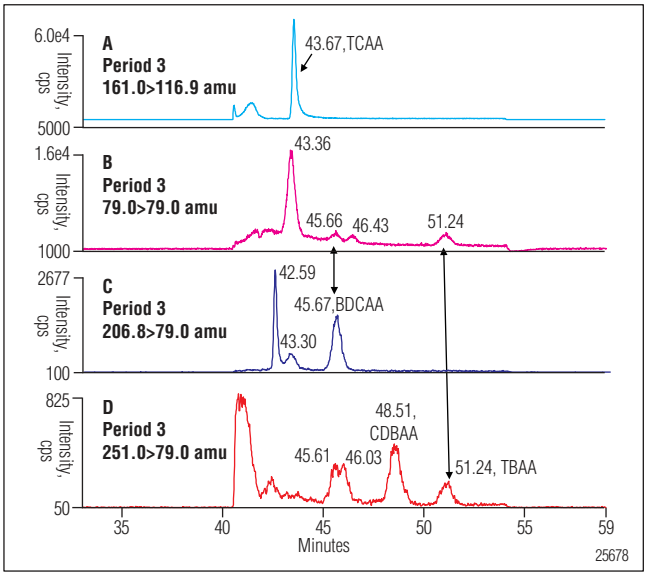


Figure 4. The extracted ion chromatograms for the indicated transistions of a water sample with high-ionic strength. A) 1.6 µg/L trichloroacetic acid found; B) bromide fragments; C) 4.3 µg/L bromodichloroacetic acid found; D) 3.8 µg/L chlorodibromoacetic acid and 0.7 µg/L tribromoacetic acid found

Conclusion

This application note describes a method for the determination of haloacetic acids without sample preparation. Using IC-MS/MS with the RFIC™ system and matrix diversion, this method provides sub-µg/L level detection of nine haloacetic acid compounds with direct injection of a sample with high-ionic strength. The parameters used in this method were used in the development of EPA method 557 for determination of haloacetic acids, bromide, and Dalapon, a general-use pesticide.

REFERENCES

1. H. Weinberg. Disinfection byproducts in drinking water: the analytical challenge. *Anal. Chem. News Features* 71: 801A-808A **(1999).**
2. D.A. Reckhow, P.L. S. Rees and D. Bryan. Watershed sources of disinfection byproduct precursors. *Water Science and Technology:* Water Supply 4: 61-69 **(2004).**
3. B. Paull and L. Barron. Using ion chromatography to monitor haloacetic acids in drinking water: a review of cuffent technologies. *J. Chromatogr. A* 1046: 1-9 **(2004).**
4. http://www.epa.gov/safewater/methods/pdfs/met552_3.pdf
5. M. C. Bruzzoniti, R. M De Carlo, K. Horvath, D. Perrachon, A, Prelle, R. Tofalvi, C. Sarzanini and P. Hajos, High performance ion chromatography of haloacetic acids on macrocyclic cryptand anion exchanger. *J. Chromatogr. A* 1187: 188-196 **(2008).**
6. M. Asami, K. Kosaka, Y. Matsuoka and M. Kamosita. An analytical method for haloacetic acids and oxo halides using ion chromatography coupled with tandem mass spectrometry and detection of perchlorate in environmental and drinking waters. *J. Environ. Chem*. 17:363-375 **(2007).**
7. http://www.epa.gov/microbes/m_332_0.pdf.
8. M. Stuber and T. Reemtsma, Evaluation of three calibration methods to compensate matrix effects in environmental analysis with LC-ESI-MS. *Anal. Bioanal. Chem*. 378: 910-916 **(2004).**
9. IonPac AS24 Product Manual, Dionex Corp, June 2007.

Determination of Total Cyanide in Municipal Wastewater and Drinking Water Using Ion-Exclusion Chromatography with Pulsed Amperometric Detection (ICE-PAD)

INTRODUCTION

Cyanide is a well known acute toxin that prevents cellular respiration by irreversibly binding with the iron in cytochrome C oxidase.[1,2] In addition, thiocyanate, which is metabolized from cyanide, interferes with iodine uptake by the thyroid gland, causing goiters and other long-term iodine deficiency diseases.[1] Cyanide is regulated as an environmental contaminant by the US EPA for drinking water, surface water, and wastewater due to these health concerns.[3–5]

Total cyanide is defined by the US EPA as free cyanide ion and complex cyanides that are converted to hydrocyanic acid (HCN) during strong acid digestion.[6] More recently, total cyanide also includes ferrocyanide and ferricyanide due to free cyanide formed by exposure to light.[7] For drinking and surface waters, the US EPA has established a maximum contamination level (MCL) of 200 µg/L free cyanide determined by a total cyanide assay.[3] To determine total cyanide, the sample is digested with sulfuric acid to convert the cyanide to hydrogen cyanide gas, aspirated into a strong caustic solution, then assayed.

In wastewater, the EPA specifies cyanide discharge limits by industry and size of the facility (<38,000 or >38,000 liters per day).[8] The typical sources of cyanide contamination are industrial waste from plating and mining industries, burning coal and plastics, and effluent from publicly owned treatment works (POTW).[1,2] The EPA specifies 5.2 µg/L total cyanide continuous discharge limits for POTW and 22 µg/L maximum discharges into fresh water.[4,5] For salt water bodies, the continuous and maximum discharges are 1 µg/L total cyanide. The EPA defines these continuous (4 d) and maximum (1 h average) limits to ensure that aquatic life is unharmed.

Ninety percent of the cyanide in POTW influent and flow-through are attributed to the metal finishing and organic chemical industries.[2] However, many POTWs report that cyanide concentrations in wastewater effluents are higher than those from the influent levels.[9] Cyanide concentrations as high as 60 µg/L have been reported at discharge sites.[10] This cyanide generation is associated with chlorination and chloramination processes used for waste disinfection.[9,10] Nitrate formed from chlorination of ammonium creates unstable intermediates that degrade to cyanide during the harsh acid and temperature conditions typically used for acid-distillation in total cyanide determinations.

In EPA methods 335.2, 335.3, and 335.4, samples are individually acid- or UV-digested to convert all cyanide compounds to hydrogen cyanide gas which is distilled into sodium hydroxide (pH 13). Total cyanide is then determined spectrophotometrically or by titration.[6,11,12] These methods are complicated, often requiring multiple distillation apparatuses, and they are subject to interference from high-pH solutions, oxidizers, and sulfur-containing compounds.[13] Chromatography methods, such as ion-exchange (IE) and ion-exclusion (ICE) can eliminate some of these interferences by separation. With IE chromatography, cyanide is not fully

resolved from chloride and sulfide concentrations at mg/L levels. ICE is preferred because strong acid anions such as chloride and sulfate are excluded from the column, and cyanide is resolved from sulfide. Electrochemical detection by direct current (DC) amperometric, or pulsed amperometric detection (PAD), is sensitive, selective, and suitable for direct determinations of cyanide.[14,15] PAD is preferred over DC amperometry because in PAD the working electrode is cycled through three or four voltage potentials every second, resulting in an electrode surface which is continually cleaned, whereas in DC amperometry, the working electrode can foul over time, leading to a loss in peak response.[13] In the previous PAD methods used to detect cyanide, the silver working electrode also detected chloride and was incompatible with samples containing mg/L concentrations of sulfide.[15] Using PAD with a Pt working electrode, chloride is not detected, and the Pt working electrode is stable with mg/L sulfide concentrations. None of the previous ICE-PAD methods were used to determine cyanide.[15–17] With this method, the authors combine the advantages of ICE with the sensitivity, selectivity, and stability of PAD using a Pt working electrode to directly detect cyanide without interferences from chloride and sulfide.

In this Application Note, the authors describe a method with PAD using a Pt disposable working electrode and a waveform optimized for determination of total cyanide in drinking and wastewater. Prior to analyses, the samples are acid distilled, trapped in 1 M NaOH, and diluted to 250 mM NaOH using the EPA-approved MICRO DIST™ sample preparation system. This ICE-PAD method has the advantages of eluting cyanide before sulfide ($R_s > 3$) and excluding chloride and sulfate, which typically interfere in ion exchange methods. This ICE-PAD method provides a fast, reliable, sensitive, and selective method to directly determine µg/L to sub- µg/L concentrations of total cyanide in wastewater. The authors also demonstrate linearity, detection limits, accuracy, and precision for determination of total cyanide in drinking water and wastewater samples using the MICRO DIST system and ICE-PAD.

EXPERIMENTAL

Equipment

Dionex ICS-3000 Ion Chromatography system consisting of:

Single Gradient Pump (SP) module with degas option

Detector and Chromatography Module (DC) with single or dual heating zone, and 6-port injection valve

Electrochemical Detector ED (P/N 061718)

AS Autosampler with Sample Tray Temperature Controlling option, and 10 mL sample tray

An electrochemical cell containing a combination pH–Ag/AgCl reference electrode (cell and reference electrode, P/N 061756) and a disposable (Pt) working electrode (P/N 064440 package of six)

Chromeleon® 6.8 Chromatography Workstation

Vial Kit, 10 mL polystyrene with caps and septa (P/N 055058)

Knitted reaction coil, 375 µL, (P/N 043700) with two PEEK™ unions (¼-28 thread female to 10-32 thread female, P/N 042806)

MICRO DIST System for sample distillation (Lachat Instruments/Hach Company, P/N MDD001) with user filled tube kit (Hach Company, P/N A17117 package of 100), heating block, protective gloves, test tube racks, and a small mechanical press.

Filter unit for vacuum filtration, 0.2 µm nylon (Nalgene® Media-Plus with 90 mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter.

Vacuum pump

Syringe filter (Pall Life Sciences, GHP Acrodisc® 25 mm with 0.45 µm GHP membrane, P/N 4560T) or filter unit for sample filtration, 0.45 µm nylon (Nalgene Media-Plus with 50 mm filter, Nalge Nunc International, P/N 153-0045) or equivalent nylon filter

PEEK Tubing:

Red (0.127 mm or 0.005 in i.d., P/N 052310 for 5 ft) tubing used for liquid line connections from injection valve to the guard and analytical columns, and cell.

Yellow (0.76 mm or 0.003 in i.d., P/N 052301 for 5 ft) tubing used for system backpressure loop.

50 µL PEEK sample loop (P/N 042950)

REAGENTS AND STANDARDS

Reagents

Deionized water, Type 1 reagent grade, 18.2 MΩ-cm resistivity, freshly degassed by ultrasonic agitation and applied vacuum.

Use only ACS reagent grade chemicals for all reagents and standards.

Magnesium chloride, hexahydrate (VWR, P/N JT2444-1)

Methanesulfonic acid (Aldrich, P/N 64280; Dionex, P/N 033478)

pH 7 (yellow) buffer solution (VWR International, P/N BDH5046)

pH 4 (red) buffer solution (VWR International, BDH5018)

Sodium cyanide, anhydrous (Aldrich, P/N 20,522-2)

Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

Sulfuric acid (VWR, P/N JT9681-33)

For Interference Experiments

Ammonium chloride (Aldrich, P/N 213330, FW 53.49)

Sodium cyanate (Aldrich, P/N 185086, FW 65.01)

Sodium sulfide, nonahydrate, > 99.99% (Aldrich, P/N 431648, FW 240.18)

Sodium thiocyanate, (Aldrich, P/N 251410, FW 81.07)

Sodium nitrate (Aldrich, P/N SS506, FW 84.99)

Sodium sulfate (Aldrich, P/N 239313, FW 142.04)

Samples

Certified Wastewater Standard for cyanide, 40 µg/L total cyanide (20 µg/L free cyanide from potassium cyanide and 20 µg/L complexed cyanide from potassium ferricyanide in 0.5% potassium hydroxide) (High-Purity Standards, P/N CWW-CN-D).

Municipal wastewater effluent samples were collected at the same time and location. Sodium hydroxide was added to one of the samples immediately after collection.

A municipal drinking water sample stabilized with 2 g of 50% sodium hydroxide per 250 mL of sample.

CONDITIONS

Column:	IonPac® ICE-AG1 Guard, 4 × 50 mm (P/N 067842) IonPac ICE-AS1 Analytical, 4 × 250 mm (P/N 064198)
Flow Rate:	0.2 mL/min
Eluent:	50 mM Methanesulfonic acid
Column Temperature:	30 °C
Tray Temperature:	10 °C
Inj. Volume:	50 µL
Detection:	Pulsed Amperometric Detection (PAD)
Waveform:	See Table 1.
Reference Electrode:	pH-Ag/AgCl electrode (P/N 061879) in AgCl mode
Working Electrode:	Disposable Platinum
Typical Background:	70–120 nC
Typical System Backpressure:	2200 psi
Noise:	20–30 pC
Typical pH:	1.2–1.3
Run Time:	30 min

Table 1: Cyanide Detection Waveform Optimized for Acid Eluents[17]

Time (sec)	Potential vs. Ag/AgCl (V)	Gain Region[a]	Integration	Ramp[a]
0.00	+ 0.30	Off	Off	Ramp
0.31	+ 0.30	On	Off	Ramp
0.32	+ 1.15	On	Off	Ramp
0.64	+ 1.15	On	On (Start)	Ramp
0.66	+ 1.15	On	Off (End)	Ramp
0.67	– 0.30	On	Off	Ramp
1.06	–0.30	Off	Off	Ramp
1.07	+ 0.30	Off	Off	Ramp

[a]The gain and ramp are instrument settings for the ICS-3000 IC electrochemical detector.

PREPARATION OF SOLUTIONS AND REAGENTS

When preparing eluents, it is essential to use high quality, Type 1 water (18.2 MΩ-cm resistivity or better) that contains as little dissolved gas as possible. Dissolved gases can cause higher noise levels. Degas the deionized water before eluent preparation using a Nalgene filter flask (P/N 164-0020) with 0.2 µm nylon filter with applied vacuum. Prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Preparation of Eluent

To prepare 2 L of 50 mM methanesulfonic acid (MSA) eluent, pipette 4.5 mL (9.6 g) MSA (FW 96.10) into a 2 L glass eluent bottle containing 1993 g of Type 1 degassed, deionized water. Immediately cap the bottle, connect it to the Eluent A line, and place the eluent under ~4–5 psi of helium or other inert gas. Thoroughly mix the eluent solution and prime the pump with the new eluent.

Preparation of Standards

Warning: Cyanide is a poison by inhalation, contact, and ingestion. Solutions containing cyanide can generate hydrogen cyanide gas at neutral or acidic pH, and must be stabilized with base. Read and follow the material safety data sheet (MSDS) instructions for personnel handling, exposure, and disposal information. Consult local safety personnel for regulations concerning the proper disposal of cyanide. Add 100 mL of 50% NaOH into the system waste container. Hydrogen cyanide gas is created during the acid digestion of cyanide-containing samples. Conduct the acid digestion sample preparation in a well-ventilated hood.

Use high quality, 50% (w/w) sodium hydroxide solution for diluent preparation. Sodium hydroxide pellets are coated with sodium carbonate and cannot be used for this application.

Preparation of 100 mM Sodium Hydroxide Diluent

To prepare 250 mL of 100 mM sodium hydroxide (NaOH) diluent, pipette 1.3 mL (2.0 g) of 50% NaOH into a 250 mL HDPE bottle containing 248.7 g degassed Type 1 deionized water. Swirl the bottle gently to thoroughly mix the solution. Use this solution as the diluent for all cyanide standards. Prepare a fresh solution daily or as needed.

Cyanide Standards

To prepare a 1000 mg/L stock solution, weigh 0.189 g of reagent grade sodium cyanide into a 100 mL polyethylene bottle and dissolve thoroughly in 100 g of 100 mM NaOH diluent. Prepare an intermediate 1.0 mg/L cyanide standard by pipetting 50 µL of the 1000 mg/L stock solution into a 50 mL polyethylene bottle and diluting with 100 mM NaOH to a final weight of 50.00 g.

To prepare 1.0, 2.0, 5.0, 10, and 25 µg/L cyanide working standards from the 1.0 mg/L intermediate standard, pipette 20, 40, 100, 200, and 500 µL respectively, of the intermediate standard into 20 mL polyethylene bottles. Dilute these working standards with 100 mM NaOH to 20.00 g total weight. The stock solution and the intermediate standard are stable for more than a month when refrigerated. The working standards should be prepared daily.

Standards for Interference Experiments

As a test for positive interferences of cyanide methods, the ASTM D19.06 Cyanide Task Group devised an ASTM Challenge Matrix stock solution,[18] containing 17.8 mM ammonium chloride (FW 53.49), 17.8 mM potassium nitrate (FW 101.10), 49.4 mM sodium sulfate (FW 142.04), 5.95 mM potassium cyanate (FW 81.12), 2.6 mM potassium thiocyanate (FW 97.18), and 12 mM NaOH (1 mL of 12 M NaOH in 1 L). The Challenge Matrix working solution is a 10-fold dilution of the stock solution.

Individual interference stock solutions (Table 2) were prepared at 10 times the concentration of the ASTM Challenge Matrix Stock to facilitate preparation of individual interference solutions. Sulfide causes a negative interference with cyanide determinations in some methods, and was therefore added to the interference testing solution. Sulfide was prepared at the same molar concentration (17.8 mM) as nitrate, ammonium, and chloride. To prepare individual stock solutions (ammonium, chloride, cyanate, nitrate, sulfate, sulfide, thiocyanate), add the amount of reagent grade compound (Table 2) to a 100 mL polyethylene bottle and dilute with 100 g of deionized water.

Table 2. Amount of Compound Used to Prepare 100 mL of Individual Stock Solutions			
Ion	**Compound**	**Mass (g)**	**Concentration mg/L (mM)**
Ammonium	Ammonium chloride (NH_4Cl, FW 53.49)	0.954	3220 (178)
Chloride			6320 (178)
Cyanate	Sodium cyanate (NaOCN, FW 65.01)	0.387	2500 (59.5)
Nitrate	Sodium nitrate ($NaNO_3$, FW 84.99)	1.51	11,000 (178)
Sulfate	Sodium sulfate (Na_2SO_4, FW 142.04)	7.03	4750 (494)
Sulfide	Sodium sulfide, nonahydrate ($Na_2S \bullet 9H_2O$, FW 240.18)	4.28	1900 (178)
Thiocyanate	Sodium thiocyanate (NaSCN, FW 81.07)	0.209	1500 (26)

To prepare separate or combined interference standards, dilute the stock solutions 100-fold with 100 mM NaOH by pipetting 200 µL of the stock solutions into 19.8 g of 100 mM NaOH. Prepare the combined 5 µg/L cyanide/19 mg/L sulfide interference standard by pipetting 100 µL of the 1 mg/L cyanide intermediate standard and 200 µL of the 1900 mg/L sulfide stock solution into 19.7 g of 100 mM NaOH.

Sodium sulfide solutions degrade quickly upon exposure to air. Prepare sulfide solutions from a new bottle of sodium sulfide nonahydrate solid and store at 4 °C, as degradation accelerates as temperature increases. The 1900 mg/L sulfide stock solution must be prepared every 2 weeks when stored at 4 °C. Sulfide solutions at concentrations <1 mg/L should be prepared every two days. With the 1900 mg/L sulfide stock solution, long-term stability can only be achieved by freezing at –10 °C.

Sample Preparation

Free cyanide is reactive and unstable, and therefore water samples should be stabilized at the time of collection. Oxidizing agents decompose free cyanide and any free cyanide present at neutral pH will volatilize to hydrogen cyanide. Sodium hydroxide solution (2 g of 50% (w/w)) was added to ~250 g of municipal drinking water samples for preservation. The cyanide certified wastewater (CWW) sample was prepared according to the instructions then diluted 10-fold by combining 10 mL of the prepared CWW sample with 90 mL 100 mM NaOH diluent. The municipal wastewater effluent samples were filtered with 0.45 µm syringe filters prior to sample digestion to remove particulate matter and bacteria. Control samples of 100 mM NaOH blank and 5 µg/L cyanide standard samples were prepared in the same manner. To filter samples >50 mL, the authors used the 150 mL Nalgene filter apparatus (0.45 µm, nylon).

Separate 1 µg/L cyanide spike recovery samples were prepared from the municipal drinking water samples by pipetting 20 µL of 1.0 mg/L cyanide standard into separate 20 mL polyethylene bottles containing 20 g of base-treated water sample. The 5 µg/L cyanide spiked samples of municipal wastewater effluent and the 10-fold dilution of the cyanide CWW samples were prepared similarly with 100 µL of 1.0 mg/L cyanide standard added into 20 g of sample.

Acid Digestion

The MICRO DIST sample preparation system uses a three-part tube (Figure 1) and a digestion block designed to hold 21 assembled tubes. The tube includes a polypropylene sample tube, hydrophobic membrane, and a polypropylene collector tube that contains the trapping solution and functions as a measuring tube. The membrane separates the sample tube from the collector tube and allows only the gaseous sample to pass into the trapping solution. During the initial experiments, both the prefilled (assembled with the trapping solution) and user-filled (unassembled without the trapping solution) tubes were tested. The user-filled collector tubes were used for the final development of this Application Note. During digestion at 120 °C, hydrogen cyanide gas is generated in the sample tube from the reaction of cyanide in the sample with 7.11 M sulfuric acid and 0.75 M magnesium chloride solution. Hydrogen cyanide gas passes through the sample membrane in the collector tube and is dissolved as cyanide in the 1 M NaOH trapping solution. After the 20 min digestion time, the tubes are removed from the heating block, the sample tube is discarded, and the collector tube is inverted to cool for 10 min. The condensate is collected off the walls of the collector tube by the trapping solution. To prepare the sample for dilution and analysis, the collector tube is broken at the breakaway point to yield a measuring tube (M). The distillation (D) half of the collector tube is discarded. The sample in M tube is diluted to 6 mL with deionized water for a final concentration of 250 mM NaOH.

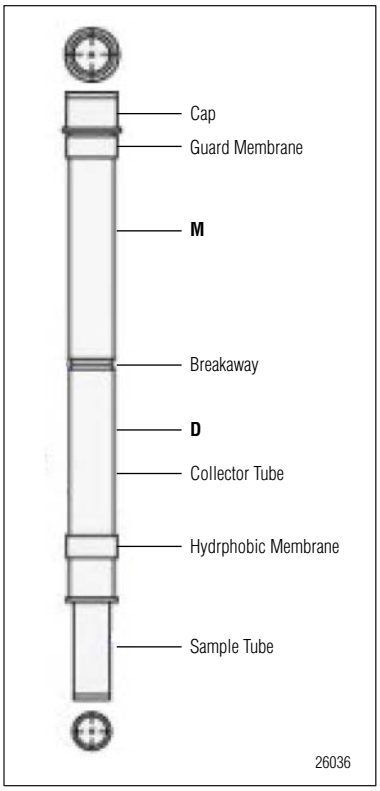


Figure 1. MICRO DIST tube assembly14

MICRO DIST Solutions

Prepare the 7.11 M sulfuric acid/0.75 M magnesium chloride digestion and 1 M NaOH trapping solutions according to the MICRO DIST Cyanide-1 Method, 10-204-00-1-X[19] instructions. Caution: Carefully prepare the sulfuric acid/ magnesium chloride solution in the exhaust hood with the hood sash positioned between you and the acid. Concentrated sulfuric acid reacts exothermically with water, and at this concentration, the solution can exceed the boiling point of water and violently boil over and splatter. To minimize isolated hot spots and violent flashbacks, add the concentrated sulfuric acid (MW 98.08, 95.7%) slowly, in 50 mL increments, pouring down the side of the flask and mixing gently between additions. Cool to room temperature and dilute to the 1 L mark.

MICRO DIST Acid Digestion

The cyanide samples, 100 mM NaOH blanks, and cyanide control standards were digested according to MICRO DIST Cyanide-1, Method 10-204-00-1-X. Each digestion experiment should include duplicate 100 mM NaOH blanks, control cyanide standards, and samples.

Use the following procedure to digest the samples:

- Place the heater block in the exhaust hood, turn it on, and set the temperature to 120 °C. Allow at least 40 min for the heating block to stabilize.
- Rinse the MICRO DIST user-filled collector tubes (Figure 1) on both sides of the D side membrane with 1 mL each of acid and base solutions prior to use to minimize contamination.
- To assemble the collector tubes, first add 1.5 mL of 1 M NaOH trapping solution to the M side of the collector tubes, then cap the collector tube (M side) with the cap and a filter membrane. The cap must be securely attached and the filter must completely cover the top of the tube. The cap and filter are responsible for containing the final solution in the collector tube.
- Label the collector tubes on each side of the breakaway point.
- Place the collector tubes in the test tube rack with the M side up.
- Label the sample tubes, weigh 6.0 g sample into each tube, and place in the test tube rack.

The next three steps must be performed quickly;

- Add 0.6 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride to one sample tube and immediately place the assembled collector tube over the sample.
- To press-fit the tubes, place the tubes in the press (D side down), support the tubes around the breakaway mark, and pull the press lever down to smoothly press-fit the collector tube into place over the sample tube.
- Using the heat-protective gloves, place the fully assembled tube in the pre-stabilized heating block (D) side down, and digest at 120 °C for 30 min. Repeat with the other samples, blanks, and controls. The manufacturer recommends adding the tubes to the heat block within one minute.
- After the 30 min digestion, quickly remove the tube from the heating block using heat-protective gloves, remove the sample tube within 4 s, and quickly invert the collector tube (D side up). Discard the sample tube and the solution from the sample tube according to safety regulations. Remove the other tubes in the same manner.
- Allow to cool for 10 min.

- To rinse the condensate off the collector tube walls, gently tip the collector tube and the trapping solution until all of the condensate is collected. Tap the collector tube to collect any droplets clinging to the membrane.
- To break off the D side of the collector tube, firmly place both hands on both sides of the breakaway point and break the tube by pushing away. Place the M side of the collector tube into test tube rack. Discard the D side of the tube.
- Dilute to the 6 mL mark with deionized water. Swirl the sample to mix.
- Transfer the samples to the AS Autosampler sample vials.

As noted in the instructions, the digestion temperature and time, the condensation time, quick removal of the sample vial after digestion, and efficient rinsing of the condensate off the collector tube walls are critical to achieving good sample recovery.

SYSTEM PREPARATION AND SETUP

The IonPac ICE-AS1 column should not exceed backpressure >1000 psi. Do not remove or install the ED module while the DC module is turned on, as power surges can cause internal damage to the ED module

Configuring Virtual Channel to Monitor pH

It is useful to monitor and record the pH during sample analyses. To continuously record the pH during sample determinations, create a Virtual Channel in the Server Configuration program according to the instructions in AN 188.[15] (The pH virtual channel becomes one of the available signal channels.) More information on Virtual Channels can be found in the Chromeleon "help" program.

Plumbing the Chromatography System

CAUTION: Cyanide is converted to hydrogen cyanide, a toxic gas, at pH < 9. Add concentrated NaOH to the waste container prior to starting the system to maintain the pH of the waste stream and to prevent evolution of gaseous hydrogen cyanide. Add 100 mL of 50% NaOH for each 5 gallons of waste. This will yield 5 gallons of NaOH at ~1–2x the MSA eluent concentration.

Use red PEEK (0.127 mm or 0.005 in i.d.) tubing for all eluent lines from after the injection valve to the cell inlet. Black PEEK (0.25 mm or 0.010 in i.d.) tubing can be used from the pump to injection valve. Install the IonPac ICE-AS1 column set according to the product manual.[20] Column pressure is typically ~850 psi, which is sufficiently below the recommended operating pressure limit of 1000 psi for this column. A 1000 psi backpressure loop can be installed between the pump and injection valve to further reduce system noise. Install the 375 µL knitted reaction coil between the IonPac ICE-AS1 column and the electrochemical cell as described in AN 188.

Assemble the Electrochemical Cell

Assemble the electrochemical cell according the instructions in AN 188. In this application, the working electrode is a disposable platinum electrode. Typically, the background will stabilize within 10 min. However, a longer equilibration may be required when initially setting up the system.

RESULTS AND DISCUSSION

Sample Preparation

Initial experiments with the MICRO DIST sample preparation system found total cyanide (1–2 µg/L) when 100 mM NaOH was used as a sample (blank) with either the prefilled collector tubes or as received user-filled tubes with lab prepared 1 M NaOH trapping solution. The source of the contamination was unknown but it is likely a non-cyanide contaminant related to the hydrophobic membrane in the collector tubes. As discussed previously, cyanide can be generated when nitrate and nitrite are present under acid-digestion conditions. This problem was eliminated for the user-filled collector tubes by pre-rinsing the sides of the tubes labled D and M with 1 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride solution and 1 M NaOH solution. These experiments illustrate the importance of control samples and standards in the acid-digestion sample preparation.

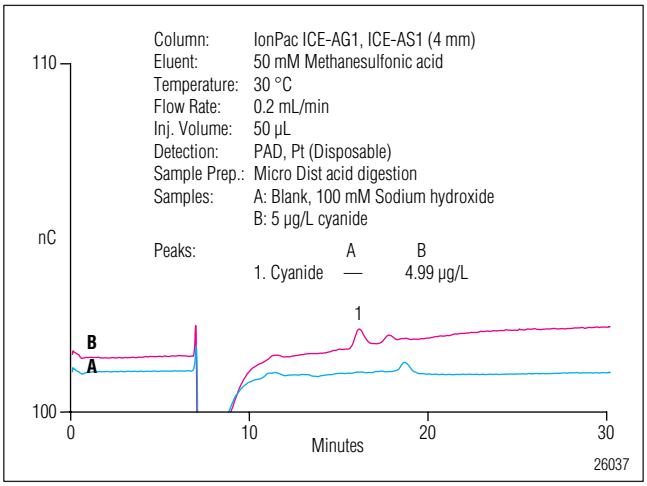


Figure 2. Comparison of A) Blank and B) 5 µg/L cyanide standard.

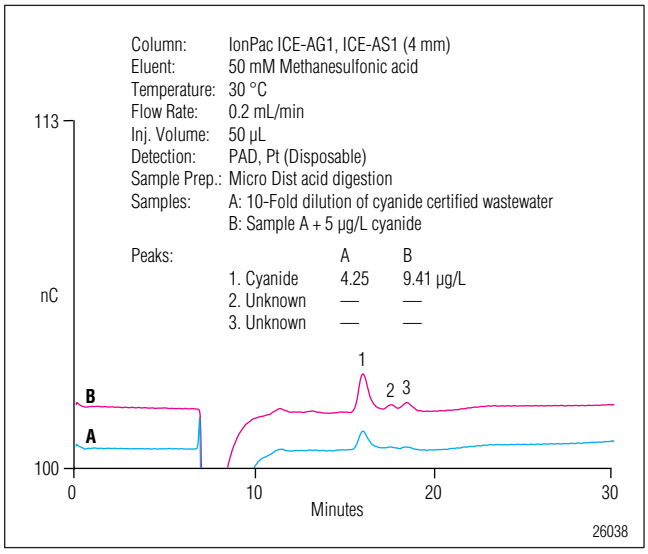


Figure 3. Comparison of A) 10-fold dilution of cyanide certified wastewater sample, and B) Sample A with 5 µg/L cyanide added.

Separation

ICE achieves better separation of cyanide from other anions in the sample compared to ion-exchange chromatography. In ICE (also known as Donnan exclusion), the fully sulfonated ion-exchange resin acts as a semipermeable membrane with separating molecular species rather than ions.[21,22] Strong acid anions, such as chloride and sulfate, are excluded by Donnan exclusion on the stationary phase and pass quickly through the column.[22] While weak acid anions, such as cyanide and sulfide, are protonated by the strong acid eluent to neutral compounds. These neutral compounds are not excluded but instead partition in the aqueous phases within and between the resin beads and separate in the order of increasing pKas.[21]

With this method, cyanide was separated by ICE using an IonPac ICE-AS1, 4 × 250 mm column using 50 mM MSA at a flow rate of 0.2 mL/min and detected by PAD using a Pt disposable working electrode with an amperometric waveform optimized for acid eluents. Figure 2 shows the separation of 5 µg/L cyanide standard prepared in 100 mM NaOH. The cyanide peak is symmetrical (A_s = 1.1 (EP)) and elutes in 16 min.

Method Qualification

The authors determined the linearity and estimated limit of detection (LOD) to qualify the method. To determine the LOD, the peak to peak noise was determined per min in three consecutive runs of deionized water for 60 min each, resulting in an average noise of 19.8 pC. The LOD of 0.27 µg/L was determined by multiplying the Student's t-test value of 3.14 for 99% confidence limits and the standard deviation (0.0085) of seven replicate injections of 0.50 µg/L cyanide standard. The linearity of cyanide detection was determined by calibrating with triplicate injections of five standards from 1.0 to 25 µg/L cyanide and comparing the peak area response to concentration (r^2 = 0.9999).

Samples

The authors applied the method to acid-digested samples of CWW, municipal drinking water, and municipal wastewater effluent. To determine total cyanide in the CWW sample, the sample was diluted 10-fold to a certified concentration of 4 µg/L total cyanide. Recovery was 4.25 ± 0.07 µg/L cyanide, 6.3% higher than the total cyanide certified value (Figure 3, chromatogram A). The cyanide peak has similar peak shape as in the prepared standard in Figure 2 with two small unknown peaks eluting at approximately 17–18 min. Determination of total cyanide was also evaluated in the municipal drinking water and wastewater effluent samples. In this study, 0.67 ± 0.02 µg/L (n = 6) total cyanide was detected in the municipal drinking water after acid digestion (Figure 4, chromatogram A). Because municipal wastewater effluent samples are known to have high levels of bacteria

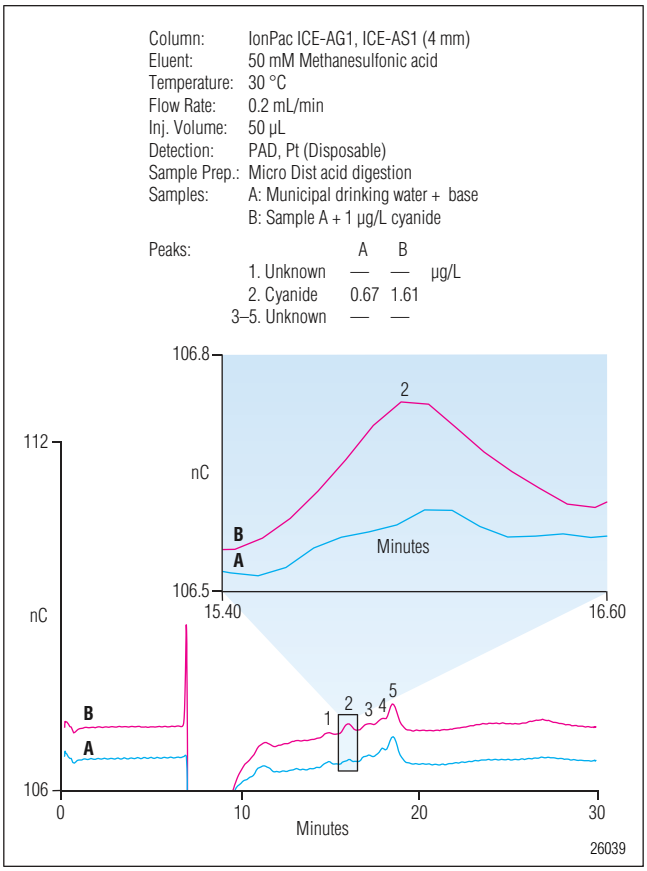


Figure 4. Comparison of A) Municipal drinking water, and B) Sample A with 1 µg/L cyanide added.

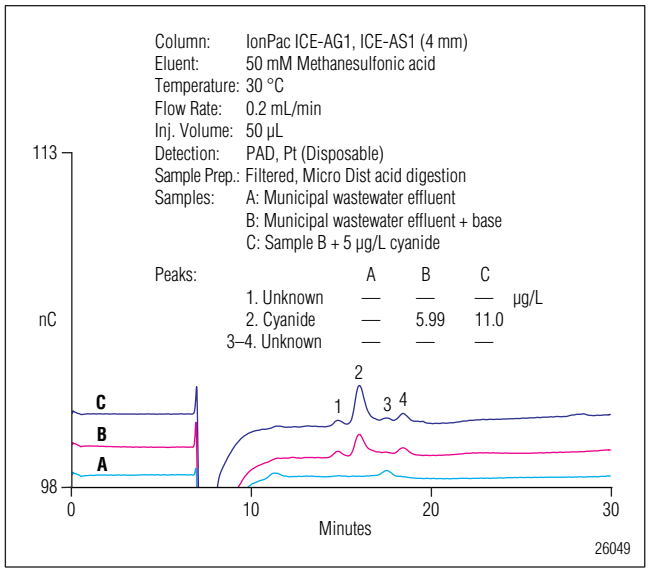


Figure 5. Comparison of A) Municipal wastewater effluent, B) second sample of A with base added, and C) Sample B with 5.0 µg/L cyanide added.

and other particulates, both samples of the municipal wastewater effluent (with and without NaOH added) were filtered prior to acid digestion. Solutions of 100 mM NaOH and 5 µg/L cyanide prepared in 100 mM NaOH were also filtered as controls. The municipal wastewater effluent samples with and without base added during collection showed 5.99 ± 0.09 µg/L cyanide and no detectable cyanide (Figure 5), respectively. These results agree with previous reports that chloramine and chlorine disinfectant treatments used in POTWs generate unstable cyanide intermediates and that NaOH may stabilize these intermediate compounds.[9,10]

To determine the method precision, six replicate injections were performed using a 5 µg/L cyanide standard, a 10-fold dilution of CWW sample, and the same sample spiked with 5 µg/L cyanide. The calculated RSDs ranged from 0.57 to 2.9%. The accuracy of the method was evaluated over three days by adding known concentrations of cyanide to the samples prior to acid digestion (Figures 3, 4, 5, chromatograms A, B, and C, respectively). Table 3 summarizes the results of this study.

Table 3. Average Cyanide Determinations Over Three Days

Sample	Amount Found (µg/L)[a]	Amount Added (µg/L)	Average Recovery[a] (%)
100 mM sodium hydroxide	<LOD	1.06	110 ± 6.4
Filtered 100 mM sodium hydroxide	<LOD	5.02	102 ± 1.0
10-fold dilution of certified cyanide wastewater sample (4.0 µg/L total cyanide)	4.25 ± 0.07	4.99	102 ± 0.9
Municipal drinking water	0.67 ± 0.02	0.99	97.4 ± 2.0
Filtered municipal wastewater effluent without base	<LOD	Not Tested	—
Filtered municipal wastewater effluent with base	5.99 ± 0.09	4.97	99.5 ± 1.0

[a]n = 6

As shown, the method demonstrated good accuracy with average recoveries of 97.4–102%.

Interferences

The ASTM Cyanide Task Group researched the effect of ions that can cause false positives for total cyanide and therefore developed a challenge matrix to evaluate results for various analytical methods.[18] The challenge matrix contains 95.4 mg/L ammonium chloride, 25 mg/L cyanate, 15 mg/L thiocyanate, 110 mg/L nitrate, and

Table 4. Effect of Potential Interferences on Total Cyanide Determinations	
Sample[a]	**Average Cyanide Found (µg/L)**
100 mM Sodium hydroxide blank	<LOD
5 µg/L cyanide	5.03
ASTM challenge matrix	32.32
Ammonium chloride, cyanate, thiocyanate, and nitrate	36.20
Ammonium chloride, cyanate, and thiocyanate	21.29
Ammonium chloride, cyanate	26.31
Ammonium chloride, thiocyanate	0.44
Ammonium chloride	<LOD
Cyanate	16.21
Thiocyanate	<LOD

n = 2
[a]Interfering ions are the same molar concentrations as in the ASTM challenge matrix: 32.2 mg/L ammonium, 63.2 mg/L chloride, 25 mg/L cyanate, 110 mg/L nitrate, 47.5 mg/L sulfate, and 15 mg/L thiocyanate.[13]
ND is not detected.

475 mg/L sulfate. To determine the potential for false positives from the challenge matrix, the authors analyzed the challenge matrix samples for total cyanide and an undigested 25 µg/L cyanate sample for free cyanide. The experiments showed that the cyanide was generated from the acid digestion of ASTM challenge matrix primarily from cyanate (Table 4). No free cyanide was found in the undigested cyanate standards. Total cyanide concentrations increased when nitrate or thiocyanate was added to cyanate-containing samples then acid-digested. These results agree with the false positives previously reported in the literature and associated with acid-digestion and oxidation of thiocyanate and cyanate by nitrate to cyanide.[9,10] In wastewater treatment plants, nitrate is formed from chlorination of ammonium which reacts with other unstable intermediates to degrade to cyanide during acid digestion.

Sulfide is a known interferent with cyanide determinations and its presence in samples can yield poor recoveries. Sulfide concentrations at mg/L concentrations can foul the silver working electrode used in electrochemical methods and cause falsely high results in flow injection methods.[13] To determine whether sulfide interfered with accurate cyanide determinations, the authors analyzed a 19 mg/L sulfide sample spiked

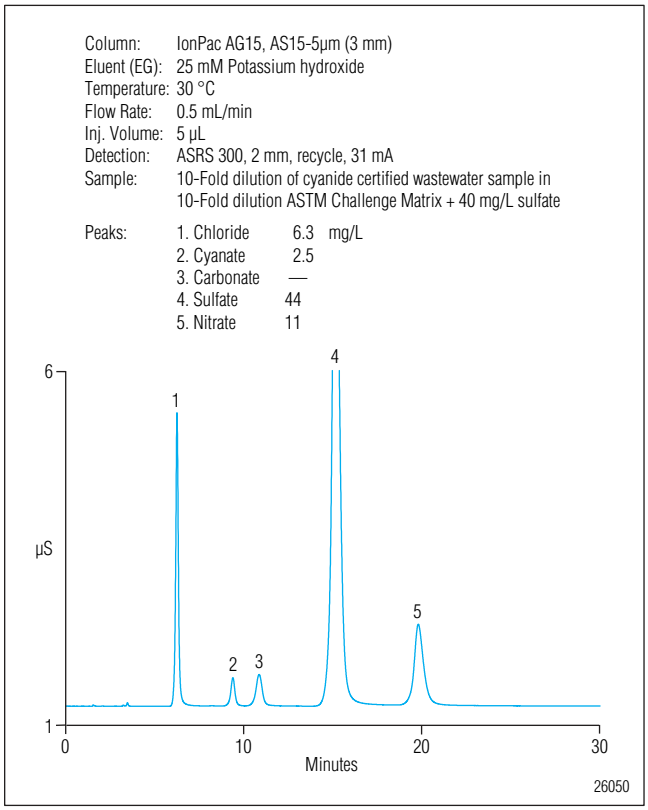


Figure 6. Determination of cyanate using a Reagent-Free IC system.

with 5.0 µg/L cyanide. Cyanide was fully resolved from sulfide, despite a cyanide-to-sulfide concentration ratio of 1:3800 (not shown), and the cyanide recovery was 99.2%. The results show that, unlike methods which use a silver working electrode or flow injection methods, sulfide does not interfere with accurate determinations of cyanide using the technique described in this application.

The effect of nitrate, nitrite, cyanate, and thiocyanate interferences and other oxidizing agents can be minimized by pre-treating the samples with sulfamic acid or sodium arsenite prior to adding base for preservation and acid digestion.[23] The presence of interfering anions can be determined by IC with suppressed conductivity detection using AN 154[24] for nitrite and nitrate, AN 138[25] for thiocyanate, and AN 200[26] for cyanate determinations. Figure 6 shows the determination of 2.5 mg/L cyanate by the conditions in AN 200 in a 10-fold dilution of the certified cyanide wastewater standard and ASTM matrix plus 40 mg/L of additional sulfate.

Table 5. Results of Robustness Experiments					
Parameter	**Value**	**Retention Time[a] (min)**	**Difference (%)**	**Peak Area[a] (nC-min)**	**Difference (%)**
Eluent Concentration (mM MSA)	47.5	15.92 ± 0.04	-0.3	0.377± 0.005	-0.8
	50	15.96 ± 0.02	—	0.380 ± 0.004	—
	52.5	15.91 ± 0.03	-0.3	0.380 ± 0.009	—
Column Temperature (°C)	28	16.01 ± 0.04	+0.3	0.385 ± 0.014	-1.3
	30	15.96 ± 0.02	—	0.380 ± 0.004	—
	32	15.89 ± 0.01	-0.4	0.373 ± 0.017	-1.8
Working Electrode	Conventional	15.96 ± 0.04	—	0.384 ± 0.006	+1.1
	Disposable Lot 080917	15.96 ± 0.02	—	0.380 ± 0.002	—
	Disposable Lot 080917	15.99 ± 0.03	+0.2	0.376 ± 0.005	-1.1
Column (Lot)	008-05-003	15.96 ± 0.02	—	0.380 ± 0.004	—
	008-05-092	16.68 ± 0.03	+4.5	0.385 ± 0.007	+1.3

[a]n = 6

Robustness

To determine the robustness of the method, the authors evaluated the effects of Pt working electrodes (conventional and disposable electrodes within the same lot), eluent concentration, column temperature, and lot-to-lot column variation on 5.0 µg/L cyanide peak responses and retention times (Table 5). The results demonstrated that slight variations in eluent concentration, column temperature, and different working electrodes had little effect on the retention times of cyanide (<0.5%). Using a column from a different lot showed the greatest effect on retention time (+4.5%). In terms of the cyanide peak area; only nominal effects (<1.5%) were observed for the variables investigated in this study.

CONCLUSION

This Application Note describes an ICE-PAD method using the EPA-approved Lachat MICRO DIST acid digestion system to determine µg/L concentrations of total cyanide in municipal drinking water and municipal wastewater effluent. The method provides low detection limits and improvement of cyanide recoveries due to exclusion of chloride and resolution from sulfide. False positives from cyanate and thiocyanate in the presence of nitrate in POTW wastewater effluent are related to the POTW chloramination processes and the acid digestion conditions during sample preparation. The effect of these false positive interferences can be minimized by identifying the presence of nitrate and nitrite, thiocyanate, and cyanate by methods described in AN 154, AN 138, and AN 200, respectively, followed by pretreatment with sulfamic acid.

SUPPLIERS

Fisher Scientific International Inc., Liberty Lane, Hampton, NH, USA 03842. 1-800-766-7000 www.fisherscientific.com

Hach Company (Lachat Instruments), PO Box 389, Loveland, CO, USA 80539. 1-800-227-4224 www.hach.com

High-Purity Standards, P.O. Box 41727, Charleston, SC, USA 29423. 1-843-767-7900 www.highpuritystandards.com

Sigma-Aldrich Corp., St. Louis, MO, USA. 1-800-325-3010 www.sigmaaldrich.com

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA, USA 19380 1-800-932-5000 www.vwrsp.com

REFERENCES

1. Simeonova, P. Prof.; Fishbein, L. Dr. Concise International Chemical Assessment Document 61, Hydrogen Cyanide and Cyanides: Human Health Aspects, Executive Summary section, jointly by United Nations Environment Programme, International Labour Organization, and the World Health Organization in Inter-Organization Programme for the Sound Management of Chemicals: Geneva, 2004, pp. 1–5, 10–11.
2. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cyanide, PB2007-100674. Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2006, pp. 153–159.
3. National Primary Drinking Water Regulation. *Code of Federal Regulations,* Part 141, Title 40, 2008; *Fed Regist.* **2008,** 22, 448.
4. Water Quality Standards. *Code of Federal Regulations,* Part 131, Title 40, 2008; *Fed Regist.* **2008,** 21, 451–463.
5. Water Quality Guidance for the Great Lakes System. *Code of Federal Regulations,* Part 132, Title 40, 2008; *Fed Regist.* **2008,** 21, 493–494.
6. EPA Method 335.2 Determination of total cyanide in water (titrametric, spectrophotometric), EPA/600/4-79-020, U.S. Environmental Protection Agency National Exposure Research Laboratory; Cincinnati, OH, 1980.
7. Final Administrative Determination on Ferric and Ferrocyanide. *Fed. Regist.* **2003,** *68* (193), 57690–57691.
8. Electroplating Point Source Category. *Code of Federal Regulations,* Part 413, Title 40, 2008; *Fed Regist.* **2008,** 28, 216–217.
9. Carr, S. A.; Baird, R. B.; Lin, B. T. Wastewater derived interferences in cyanide analysis, *Water Res.,* **1997,** 31(7), 1543–1548.
10. Watershed Investigations, Laboratory Staff Watershed Protection Group Environmental Services Department, City of San Jose. Cyanide Attenuation Study Report, 2004, 1–45.

11. U.S. EPA Method 335.3, EPA/600/4-79/020, U.S. Environmental Protection Agency National Exposure Research Laboratory; Cincinnati, OH, 1978.
12. U.S. EPA Method 335.4, EPA/600/R-93/100, U.S. Environmental Protection Agency National Exposure Research Laboratory; Cincinnati, OH, 1993.
13. Weinberg, H.S.; Cook, S. J. Segmented flow injection, UV digestion, and amperometric detection for the determination of total cyanide in wastewater treatment plant effluents, *Analy. Chem.,* 74(23), **2002**, 6055–6063.
14. Dionex Corp., *Determination of Cyanide in Strongly Alkaline Solutions.* Application Update 107; Literature Product No. 0754, 2003.
15. Dionex Corporation. *Determination of Glycols and Alcohols in Fermentation Broths by Ion-exclusion Chromatography and Pulsed Amperometric Detection,* Application Note 188, LPN 1944, Sunnyvale, CA, 2008.
16. Cheng, J.; Jandik, P. Highly sensitive and direct analysis of chelating agents using integrated pulsed amperometric detection and disposable platinum electrodes, *The Application Notebook, LCGC,* **2006,** 53.
17. Cheng, J.; Jandik, P.; Liu, X.; Pohl, C. Pulsed amperometric detection waveform with disposable thin-film platinum working electrodes in high performance liquid chromatography, *J. Electroanal. Chem.,* **2007,** 608, 117–124.
18. Sebroski, J.; and ASTM D19.06 Cyanide Task Groups. ASTM Cyanide Task Group Research Report, intra-office letter, June 2008.
19. Hach Company. *User Manual for MICRO DIST Operation and Applications,* Catalog Number 01304, Loveland, CO, 2008.
20. Dionex Corporation. Product Manual for IonPac ICE-AS1 Guard and Analytical Columns, Document No. 031181. Sunnyvale, CA, 2006.
21. Weiss, J.; Ion-Exclusion Chromatography (HPICE). *In Handbook of Ion Chromatography, 3rd ed.*; Wiley-VCH Verlag GmbH & Co.: KGaA, Weinheim 2004; pp. 359–391.
22. Fritz, J. S.; Gjerde, D. T. *Ion Chromatography,* Wiley-VCH Verlag GmgH, Weinheim, 2000; pp. 165–186.
23. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). Method 4500-CN- Cyanide, Preliminary Treatment of Samples. In *Standard Methods for the Examination of Water and Wastewater, 20th ed.;* Clesceri, L. S., Greenberg, A. E., Eaton, A. D., Franson, M. A. H.; APHA: Washington, DC, 1998; pp. 35–36.
24. Dionex Corporation. *Determination of inorganic anions in environmental waters using a hydroxide-selective column,* Application Note 154, LPN 1539, Sunnyvale, CA, 2003.
25. Dionex Corporation. *Determination of thiosulfate in refinery and other wastewaters,* Application Note 138, LPN 1237, Sunnyvale, CA, 2001.
26. Dionex Corporation. *Direct determination of cyanate in a urea solution and a urea-containing protein buffer,* Application Note 200, LPN 2034, Sunnyvale, CA, 2008.

Determination of Nitrite and Nitrate in Drinking Water Using Ion Chromatography with Direct UV Detection

INTRODUCTION

The ion chromatographic analysis of nitrite and nitrate in drinking water is accomplished using direct UV detection of the analytes. The method is free from most ionic interferences due to the specificity of UV detection. The method is applicable to all drinking water samples. Bromide may also be separated from other ions and detected using this method.

The method of chemically suppressed conductivity detection of nitrite and nitrate in drinking water (Dionex Application Update #131) is an alternative to this method. Note that if the two methods are combined, chemical suppression will yield additional benefits in the determination of nitrite and nitrate with UV detection. The use of a suppressor (AMMS-II, Dionex P/N 043074) between the column and the detector cell reduces background absorbance and eliminates negative peaks associated with chloride and sulfate in this method (see Fig. 3).

RECOMMENDED EQUIPMENT

Dionex Ion Chromatograph with a UV/Visible absorbance detector

REAGENT AND STANDARD PREPARATION

Sodium carbonate / sodium bicarbonate eluent concentrate (P/N 039513)

Sodium Nitrite, ACS Grade

Sodium Nitrate, ACS Grade

Eluent

To prepare 1.0 L of eluent (1.8 mM sodium carbonate, 1.7 mM sodium bicarbonate), dilute 10.0 mL of eluent concentrate to 1000 mL with deionized water.

Stock Standards

1000 ppm Nitrite: Dissolve 1.499 g $NaNO_2$ in 1.0 L of deionized water

1000 ppm Nitrate: Dissolve 1.371 g $NaNO_3$ in 1.0 L of deionized water

Working Standards

Dilute the stock standards to concentration levels that bracket the concentration level of interest. Prepare working standards from the stock standard just prior to analysis.

CONDITIONS

Column:	IonPac® AS9 analytical column with AG9 guard
Eluent:	1.8 mM Na_2CO_3 / 1.7 mM $NaHCO_3$
Flow Rate:	2.0 mL/min
Sample Volume:	25 µL
Detection:	UV at 210 nm, 0.2 AUFS

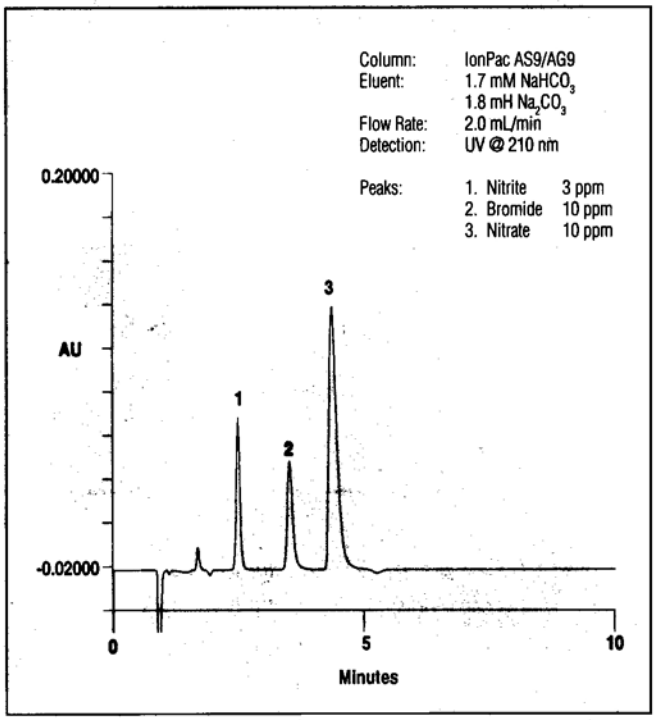


FIGURE 1. DETERMINATION OF NITRITE AND NITRATE WITH DIRECT UV DETECTION

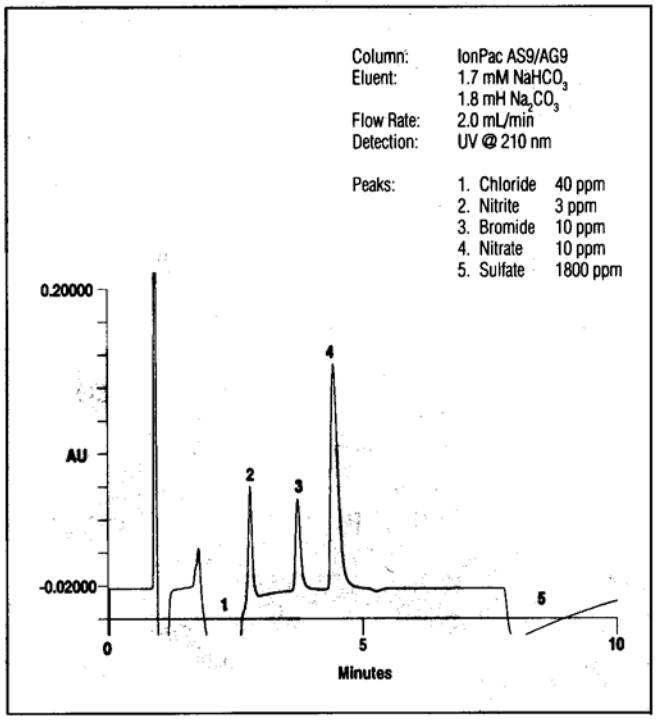


FIGURE 2. DIRECT UV DETECTION OF NITRITE AND NITRATE IN DRINKING WATER (PRESERVED WITH SULFURIC ACID*)

*Presently, the US EPA is evaluating the suitability of acid preservation as described in EPA/570/9-90/008

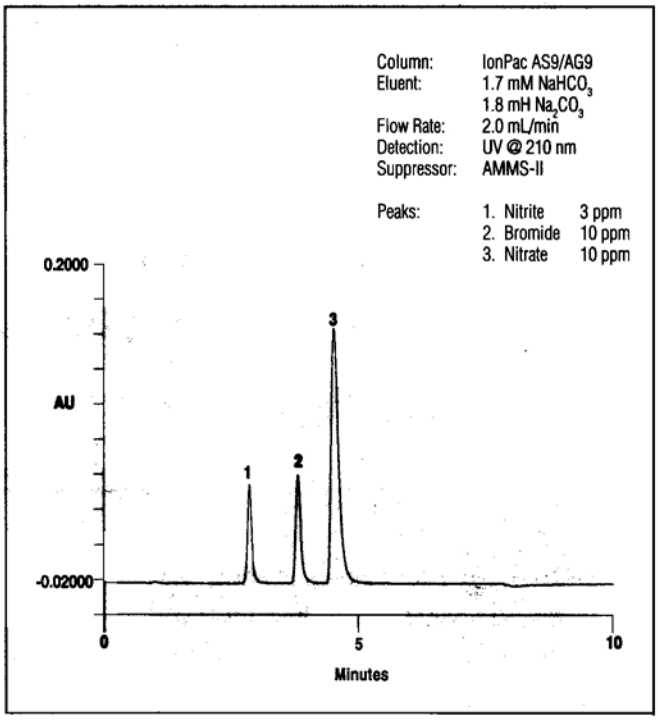


FIGURE 3. DIRECT UV DETECTION OF NITRITE AND NITRATE IN DRINKING WATER (PRESERVED WITH SULFURIC ACID*) WITH CHEMICAL SUPPRESSION OF ELUENT

PERFORMANCE CHARACTERISTICS

The detection limit in drinking water samples using a 25-µL loop is 10 ppb for nitrite and 15 ppb for nitrate. This corresponds to 3.0-ppb nitrogen as nitrite, and 3.5-ppb nitrogen as nitrate. The method is linear for nitrite over the range 10 ppb to 50 ppm. It is linear for nitrate over the range of 15 ppb to 75 ppm.

Determination of Hexavalent Chromium in Drinking Water Using Ion Chromatography

INTRODUCTION

Hexavalent chromium, Cr(VI), is the most toxic form of the metal chromium, a primary drinking water contaminant in the U.S. Dissolved hexavalent chromium can be determined as chromate (CrO_4^{2-}) by ion chromatography in drinking water, groundwater, and industrial wastewater effluents as described in U.S. EPA Method 218.6 and Dionex Technical Note 26.[1,2] Dionex Technical Note 26 uses a 250- L injection onto a high-capacity IonPac® AS7 anion exchange column to separate Cr(III) from Cr(VI) in four minutes. The product of the postcolumn reaction between Cr(VI) and diphenylcarbazide is detected by absorbance at 530 nm, yielding a method detection limit of 0.4 µg/L in reagent water.

The California Department of Health Services (DHS) recently issued a new Public Health Goal (PHG) of 2.5 g/L for total chromium and 0.2 g/L for Cr(VI). In January 2001, California DHS added Cr(VI) to the list of unregulated chemicals that must be monitored. As a result of this regulation, public water systems are now monitoring for Cr(VI) in drinking water.[3]

EPA Method 218.6 does not allow sufficient sensitivity for analysis at the California PHG level of 0.2 µg/L. This application update describes modifications to Method 218.6 that significantly increase sensitivity over the existing method. The modifications include lower eluent and postcolumn reagent (PCR) flow rates, a larger reaction coil, and a larger injection volume. The resulting MDL for Cr(VI) as CrO_4^{2-} of 0.02 g/L is more than sufficient for determinations at the California PHG level.

EQUIPMENT

A Dionex DX-600 chromatography system consisting of:

- GS50 Gradient Pump with Vacuum Degas Option
- AS50 Automated Sampler with Chromatography Compartment
- AD25 UV/Visible Absorbance Detector
- 1.0 mL sample syringe for AS50 (Dionex PN 55066)
- PC10 Postcolumn Pneumatic Delivery Package, 4-mm (Dionex PN 50601)
- 750- L knitted reaction coil (Dionex PN 42631)
- PeakNet® 6.1 Chromatography Workstation
- Syringe filters (Gelman IC Acrodisc 0.2- m, PN 4483)

REAGENTS AND STANDARDS

Prepare all solutions from analytical reagent grade chemicals (when available).

Deionized water, 17.8 MΩ-cm or better
Ammonium sulfate, (Mallinckrodt Gen AR # 7725)
Ammonium hydroxide (Sigma A6899)
1,5-diphenylcarbazide (JT Baker K620-03)
Methanol, HPLC grade (Fisher Optima A454-4)
Sulfuric acid, 95–98% (JT Baker Instra-Analyzed #9673)
Potassium dichromate (JT Baker 4765-01)

CONDITIONS

Guard Column:	IonPac NG1 Guard 4 × 50 mm (Dionex PN 039567)
Analytical Column:	IonPac AS7 Analytical 4 × 250mm (Dionex PN 035393)
Eluent:	250 mM ammonium sulfate/ 100 mm ammonium hydroxide
Eluent Flow Rate:	1.0 mL/min
Sample Volume:	1000 L partial loop with 10 µL cut volume from 1100-µL loop
Postcolumn Reagent:	2 mM diphenylcarbazide, 10% methanol, 1 N sulfuric acid
PCR Flow rate:	0.33 mL/min
Detection:	UV/Vis absorbance, 530 nm
Noise:	25–50 µAU peak-to-peak
Backpressure:	1200–1300 psi
Run Time:	10 min (Retention time = 6–7 min)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent:

250 mM ammonium sulfate
100 mM ammonium hydroxide

Dissolve 66.0 g of ammonium sulfate in about 1 L of reagent water and add 13.0 mL of 29% ammonium hydroxide. Dilute to 2.0 L with water.

Sample Adjustment Buffer:

250 mM ammonium sulfate
1000 mM ammonium hydroxide

Dissolve 3.3 g of ammonium sulfate in about 75 mL of reagent water and add 6.5 mL of 29% ammonium hydroxide. Dilute to 100 mL with water.

Postcolumn reagent:

2 mM diphenylcarbazide
10% methanol
1 N sulfuric acid

Add 28 mL of 98% sulfuric acid to about 500 mL of water in a 1.0 L volumetric flask. (Caution: may get hot.) Mix and allow to cool. Add 0.5 g of 1,5-diphenylcarbazide to about 75 mL of HPLC-grade methanol in a 100 mL volumetric flask, and sonicate to dissolve. Bring to volume with methanol, mix, and add to the cooled sulfuric acid solution. Dilute to 1.0 L with deionized water, mix, and transfer to the pressurized PCR reagent container. The PCR reagent is stable for several days. Prepare fresh as needed.

Standard

Add 0.283 g of potassium dichromate (dried at 100 C to a constant weight) to about 50 mL of deionized water in a 100 mL volumetric flask. Dissolve and bring to volume with deionized water. Or, prepare a 1000 mg/L stock solution of Cr(VI) as CrO_4^{2-} from a commercially available standard (J. T. Baker, Phillipsburg, NJ). Store the stock standard at 4 °C. Prepare working standards fresh daily. Adjust the pH to 9.0–9.5 by adding 1 mL of sample adjustment buffer per 100 mL of final volume before bringing to final volume.

SAMPLE PREPARATION

Clean all sample collection equipment and containers with 1:1 HNO_3 and rinse well with deionized water before use. Collect samples in amber glass bottles with plastic lined caps. Do not filter the samples at the time of collection, but immediately add the sample adjustment buffer dropwise until the sample pH falls in the range of 9.0–9.5. Be careful not to contaminate the sample while measuring the pH. Most drinking water samples can be adjusted to pH 9.0–9.5 by adding 1 mL or less of the adjustment buffer per 100 mL of sample, which introduces an acceptable 1% dilution error. For more difficult samples, start with a known amount of sample and accurately measure the amount of buffer added so that the amount of Cr(VI) as CrO_4^{2-} determined by IC can be corrected for dilution. Cool to 4 °C and hold at 4 °C during transport and storage. Analyze samples within 24 hr of collection to minimize the potential loss of Cr(VI) through chemical reduction.[4]

Filter drinking water samples through 0.2- m Acrodisc IC syringe filters (Gelman, Ann Arbor, MI) just prior to injection. Discard the first 300 µL of filtrate and filter the remainder directly into a clean plastic autosampler vial. Qualify filters by analyzing a reagent water blank and a 10- µg/L Cr(VI) as CrO_4^{2-} standard that has been passed through the filter. The blank should be free of peaks within the retention time window of chromate, and the recovery of the 10- µg/L standard should fall between 80% and 120%.

SYSTEM PREPARATION AND SETUP

Verify that the pump flow rate is within specifications and recalibrate if necessary. A GP50 should deliver water at 1.0 –0.005 mL/min against a constant backpressure of 2000 psi. Verify that the UV/Vis Absorbance Detector wavelength accuracy is within specifications and recalibrate if necessary. It is a good practice to periodically record the visible lamp output (i.e., the reference cell current in nA) and elapsed time as an aid in troubleshooting. Consult the pump or detector manuals for procedural details.

The precision and accuracy of the AS50 will vary depending on the mode of injection. The most accurate and precise injections are made with a calibrated sample loop in the full-loop injection mode, which aspirates a total of four times the sample volume to flush the sample loop and make the injection. The largest full-loop injection possible with the AS50 is 300 µL. To inject 1000 µL, use the partial-loop injection mode with an 1100- µL sample loop, and a programmed sample loop volume of 1100- µL and cut volume of 10 µL. This injection procedure should provide peak area precision of <1% RSD. Refer to the AutoSelect™ AS50 Autosampler operator's manual (Document No. 31169) for a complete discussion of the different injection modes.

Install a 1-mL sample syringe and set the syringe speed to four or five to make faster large-loop injections. Enter the correct sample loop size and sample syringe volume in the AS50 Plumbing Configuration screen.

Configure the IC with the PCR system as depicted in Figure 3 of Dionex Technical Note 26, and as described in the PC10 Postcolumn Delivery System installation instructions. Pump the eluent at 1.0 mL/min and set the PC10 pneumatic pressure to 70 psi. To measure the PCR flow rate, collect the effluent from the detector *(i.e., the total flow from the IC pump and the PCR module)* in a 10-mL graduated cylinder for 5 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 PCR flow rate of 0.33 mL/min is established.

Variations in the PCR flow rate affect the postcolumn reaction time, pH, dilution, mixing rate, and ratio of the reactants. Stable day-to-day results depend on a well-controlled PCR flow rate.

Confirm this flow rate on a daily basis or whenever detector response for a calibration check standard deviates beyond quality control acceptance criteria.

The storage solution that the AS7 is shipped with is 30 mM nitric acid. After equilibrating the column with eluent for 60 min, analyze a system blank of 1000 µL of reagent water. An equilibrated system has a background signal of less than 200 mAU and peak-to-peak noise of less than 50 µAU. There should be no peaks eluting within the retention time window of the chromate anion. The column is equilibrated when two consecutive injections of a standard produce the same retention time for chromate.

RESULTS AND DISCUSSION

Effect of Reaction Coil and Injection Volume

The 375-µL reaction coil and higher flow rates recommended in Dionex Technical Note 26 are adequate for the rapid determination of Cr(VI) as CrO_4^{2-} at the 1- µg/L level, but a standard 375- µL knitted reaction coil does not provide the maximum peak response for chromate.[5] To optimize the sensitivity of this method,

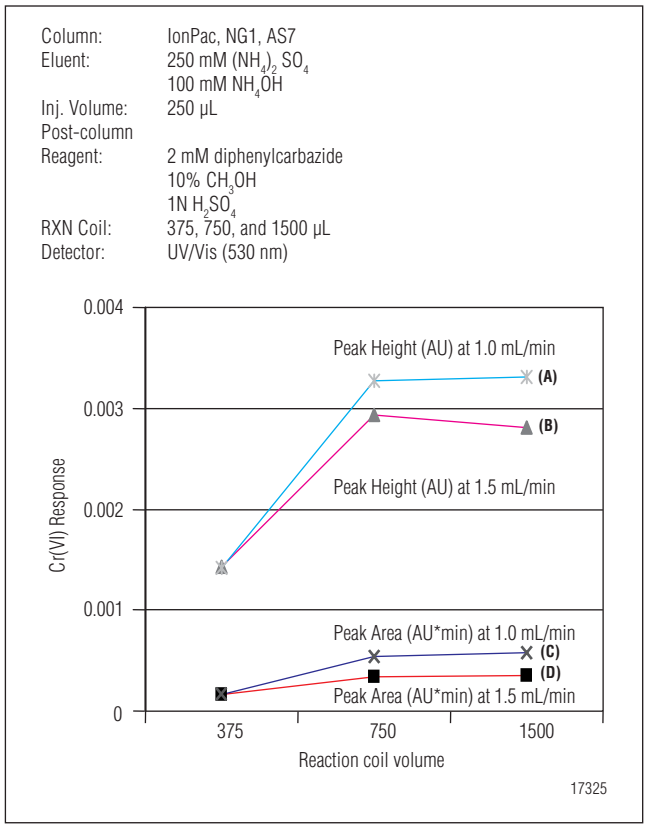


Figure 1. Effect of reaction coil volume on chromate peak response. Conditions: guard column, IonPac NG1; analytical column, IonPac AS7; eluent, 250 mM ammonium sulfate/100 mM ammonium hydroxide; flow rate, 1.0 (A and C) or 1.5 (B and D) mL/min; postcolumn reagent, 2 mM diphenylcarbazide / 10% methanol / 1.0 N sulfuric acid; reaction coil volume, 375–1500 µL as indicated; postcolumn flow rate, 0.33 (A and C) or 0.5 (B and D) mL/min; detection, UV/Vis at 530 nm; injection volume, 250 µL; peaks, chromate (10 µg/L).

the responses obtained with 375-µL, 750-µL, and 1500-µL reaction coils were compared . Figure 1 shows the effect of reaction coil volume on chromate peak height and area at two different flow rates. In both cases, the postcolumn reagent flow rate was adjusted to one-third the eluent flow rate by varying the applied pneumatic pressure. At an eluent flow rate of 1.0 mL/min (A and C) the postcolumn reagent flow rate was 0.33 mL/min. At an eluent flow rate of 1.5 mL/min (B and D) the postcolumn reagent flow rate was 0.50 mL/min.

Increasing the reaction coil volume from 375 µL to 750 µL significantly increases the peak response, while the change from 750 µL to 1500 µL only marginally increases the response. Larger coil sizes require greater pneumatic pressure to deliver the necessary PCR flow rate. Also, higher eluent flow rates require greater pneumatic pressure to deliver the necessary PCR flow

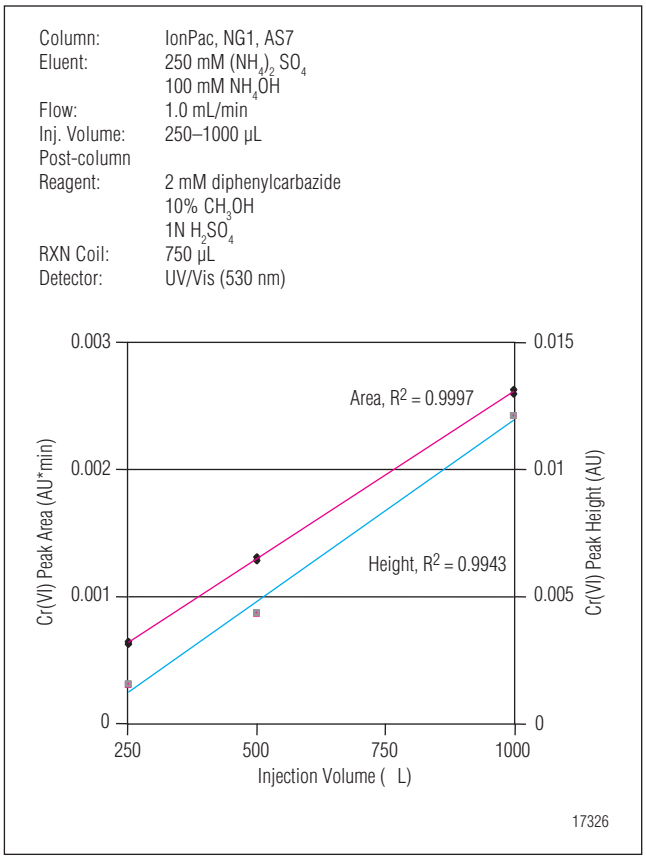


Figure 2. Effect of injection volume on chromate peak response. Conditions: as for Fig. 1, except; flow rate, 1.0 mL/min; postcolumn flow rate, 0.33 mL/min; reaction coil volume, 750 µL; injection volume, 250–1000 µL as indicated.

rate. For example, a pneumatic pressure of over 100 psi was required to deliver the PCR reagent at a flow rate of 0.5 mL/min against an eluent flow rate of 1.5 mL/min through a 1500- µL reaction coil. We recommend using a 750- µL reaction coil with an eluent flow rate of 1.0 mL/min and a PCR flow rate of 0.33 mL/min. This combination provides nearly the maximum peak response while requiring a modest pneumatic pressure of about 70 psi to deliver the postcolumn reagent at the necessary flow rate.

Greater sensitivity can be gained by increasing the volume of sample injected, but too large a sample can cause retention time shifts or loss of efficiency when column overloading leads to excessive peak distortion. The effect of injection volume on chromate peak response was studied using the conditions described above. Figure 2 shows a linear increase in both chromate peak height and area as the injection volume is increased from 250 µL to 1000 µL. We did not test larger injection volumes because a 1000 µL injection volume is the largest that can be made with the AS50 Autosampler.

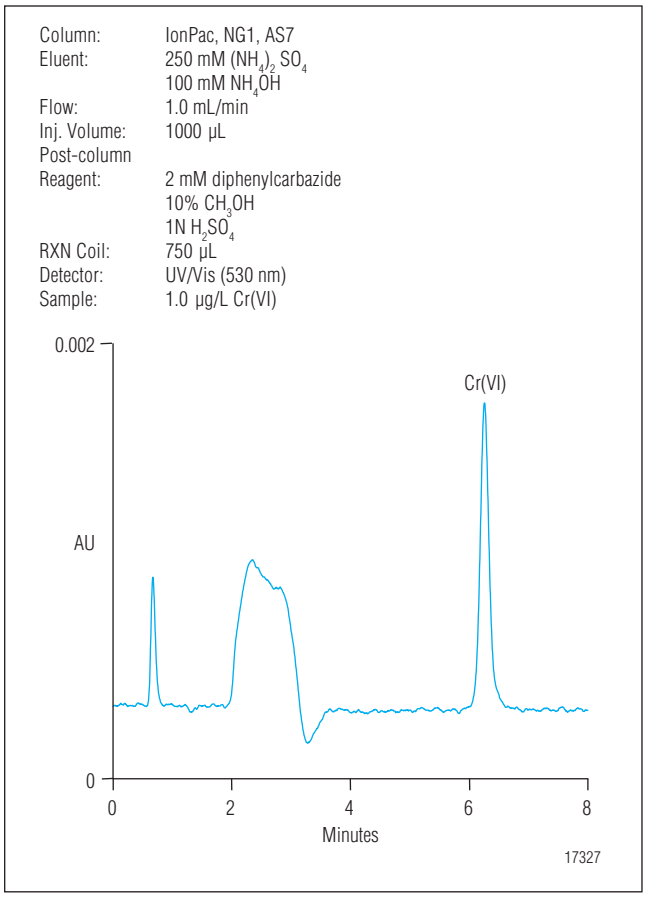


Figure 3. Determination of chromate using optimized EPA Method 218.6. Conditions: as for Fig. 2, except injection volume, 1000 µL; peaks, chromate (1.0 µg/L).

Sample Adjustment Buffer

Method 218.6 requires a solution consisting of 330 g/L ammonium sulfate and 65 mL/L ammonium hydroxide to adjust the sample pH. However, the large-loop injection used in this application update increases the possibility of overloading the analytical column if the sample ionic strength is too high. A solution consisting of 33 g/L ammonium sulfate and 65 mL/L ammonium hydroxide is a suitable substitute to adjust the sample pH and provides results comparable to those obtained by using the Method 218.6 buffer (data not shown).

Optimized Method Performance

By using a larger volume reaction coil, lower eluent flow rate and increased injection volume, this updated method provides greater than a 10-fold increase in the chromate peak area compared to the response obtained by using the standard conditions specified in Method 218.6. Figure 3 shows a chromatogram of a 1.0- µg/L

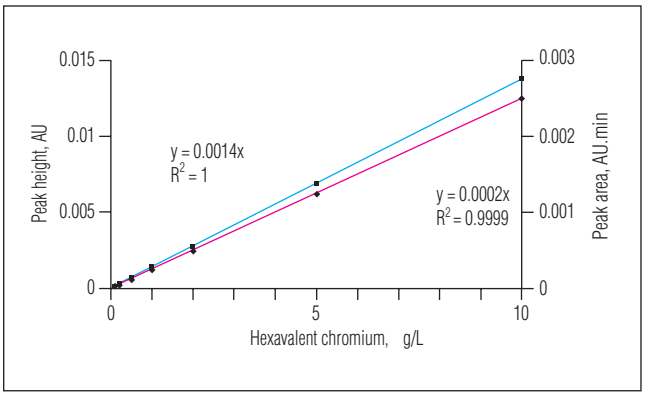


Figure 4. Calibration curve for Cr(VI) as chromate.

Cr(VI) as CrO_4^{2-} standard obtained by using the optimized conditions described above.

Figure 4 summarizes the calibration data for chromate anion obtained by using the partial-loop injection mode to make 1000- L injections of Cr(VI) as CrO_4^{2-} standards at 0, 0.04, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 g/L. The calibration curve is linear over the calibration range of 0.1–10 µg/L for Cr(VI) as CrO_4^{2-} with a coefficient of determination of 0.9999.

Table I. Method Detection Limits for Chromate Based on a 1000- µL Injection

Chromate Conc. (µg/L)	Std. Dev. (g/L)	RSD (%)	MDL* (µg/L)
0.1	0.0060	6.986	0.018
0.2	0.0056	3.193	0.018

* MDL = (Std. Dev.) x ($t_{s, 99}$), where $t_{s, 99}$ = 3.14 for n = 7.

Method Detection Limits (MDLs) for Cr(VI) as CrO_4^{2-} are summarized in Table 1. The MDL is a measure of the precision of replicate injections of a low-level solution and is defined as the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.[6] We determined the MDL for Cr(VI) as CrO_4^{2-} by analyzing seven replicates of reagent water fortified with Cr(VI) as CrO_4^{2-} at two concentrations levels of 0.1 and 0.2 µg/L (i.e., about 3–5 times the estimated instrument detection limit). Both levels produced a calculated MDL value of 0.018 µg/L. This permits a minimum limit (ML) for quantitation of 0.06 µg/L for Cr(VI) as CrO_4^{2-}, which is

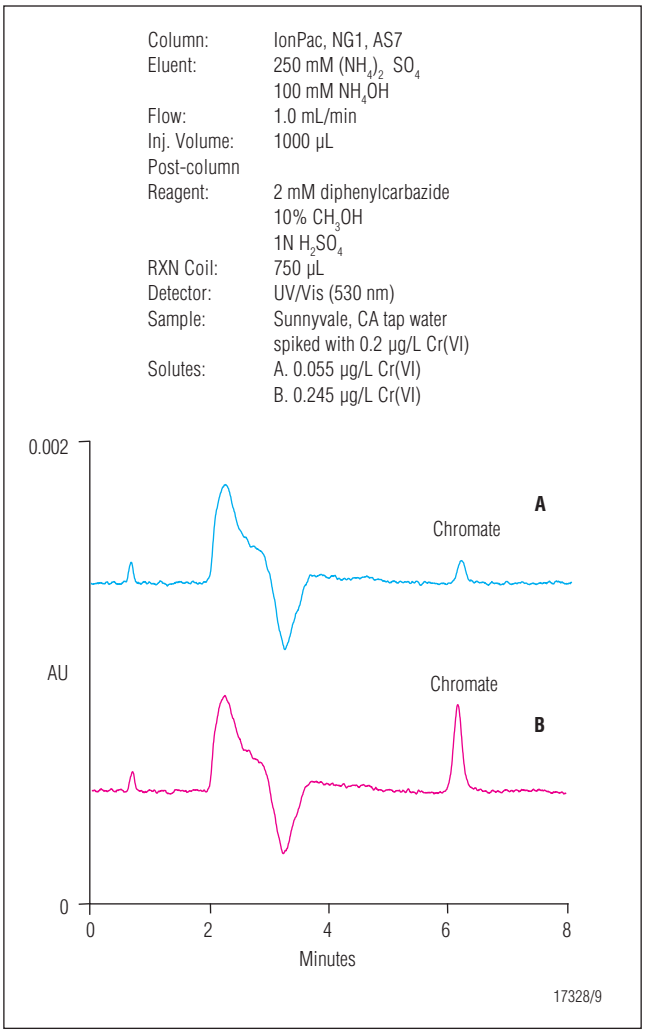


Figure 5. Determination of chromate in drinking water. Conditions: as for Fig. 3, except sample, buffered Sunnyvale, CA drinking water (A) and drinking water spiked with 0.2 µg/L chromate (B); peaks, (A) chromate (0.055 µg/L) and (B) chromate (0.245 µg/L)

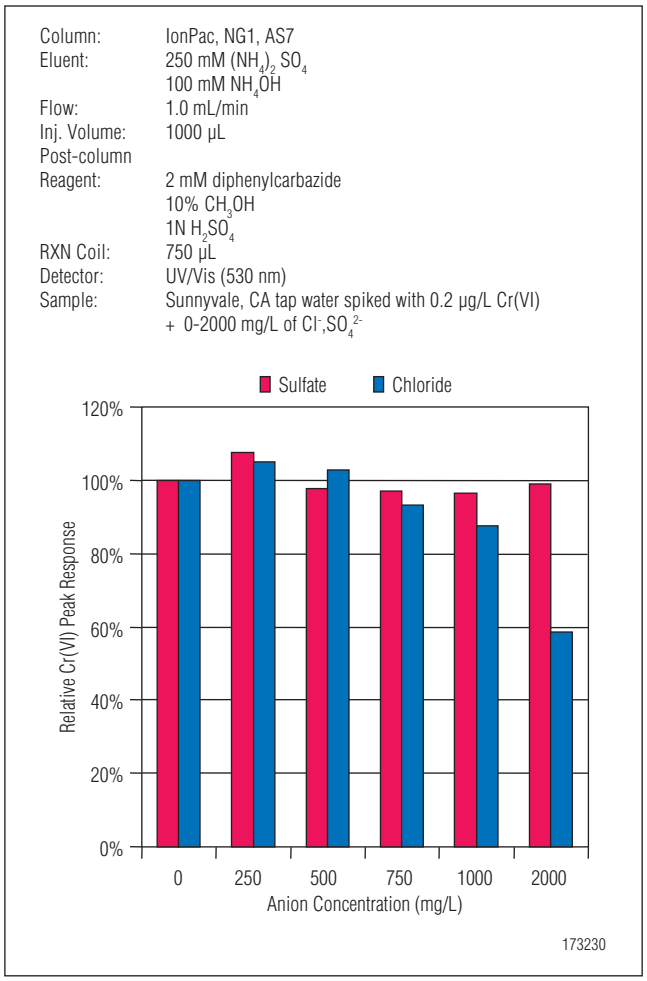


Figure 6. Effect of common anions on chromate peak response. Conditions: as for Fig.3, except sample, buffered Sunnyvale, CA drinking water spiked with 0.2 µg/L chromate to which 0–2000 mg/L of sulfate or chloride was added, as indicated.

adequate for routine analysis at the California PHG level of 0.2 µg/L.

Fig. 5 shows chromatograms obtained by using the optimized conditions of a Sunnyvale, CA, tap water blank (A) and tap water sample spiked with Cr(VI) as CrO_4^{2-} at the PHG level of 0.2 µg/L (B). In both cases, the sample was adjusted to pH 9 by using the solution consisting of 33 g/L ammonium sulfate and 65 mL/L ammonium hydroxide. The presence of the ammonium sulfate and ammonium hydroxide in the sample did not adversely affect the chromate peak shape and a recovery of 96% was obtained for the Cr(VI) as CrO_4^{2-} spike at this level. The tap water blank contained a background level of 0.06 µg/L chromate.

Although the IonPac AS7 column specified in Method 218.6 has a relatively high capacity of 100 µ equivalents/column, the large injection volume used in this application update increases the possibility of interference from other anions in the sample. Hence, the effect of chloride and sulfate on Cr(VI) as CrO_4^{2-} response was investigated, as some drinking and ground waters can contain elevated levels of these common anions. Increasing concentrations of sulfate or chloride were added to a series of tap water samples that had been adjusted to pH 9 with the recommended buffer and spiked with 0.2 µg/L Cr(VI) as CrO_4^{2-}. Figure 6 shows the effect of common anions on chromate peak response, relative to the peak area for 0.2 µg/L Cr(VI) as CrO_4^{2-} spiked in tap water containing no added sulfate or chloride. This method provides acceptable performance, for example greater than 80% recovery in the presence of up to 1000 mg/L chloride or 2000 mg/L sulfate. Typical ground and drinking waters are not expected to contain more than 200 mg/L chloride or 500 mg/L sulfate.

CONCLUSIONS

U.S. EPA Method 218.6, as published, does not allow sufficient sensitivity to determine hexavalent chromium (i.e., Cr(VI) as CrO_4^{2-}) at the California PHG level of 0.2 µg/L. Modifications to the method, including the use of a lower eluent flow rate and larger reaction coil (to increase reaction time) and a larger injection volume, significantly increase the sensitivity of Method 218.6, resulting in an MDL for Cr(VI) as CrO_4^{2-} of 0.02 µg/L. These modifications allow a minimum limit (ML) of quantitation for Cr(VI) as CrO_4^{2-} of 0.06 µg/L, which is more than sufficient for analysis at the California PHG level. Calibration was linear over the range of 0.1–10 µg/L, and quantitative recoveries were obtained for Cr(VI) as CrO_4^{2-} spiked at 0.2 µg/L in drinking water. The modified method provides acceptable performance, in terms of peak shape and recovery, in the presence of up to 1000 mg/L chloride or 2000 mg/L sulfate.

REFERENCES

1. *Determination of Dissolved Hexavalent Chromium in Drinking Water, Groundwater, and Industrial Wastewater Effluents by Ion Chromatography;* U.S. Environmental Protection Agency, Method 218.6; Cincinnati, OH (1991).
2. Dionex Corporation. Determination of Cr(VI) in Water, Waste Water, and Solid Waste Extracts; Technical Note 26; Sunnyvale, CA.
3. Governor Signs Bill Speeding Water Testing, *The Los Angeles Times,* Valley Ed., September 30, 2000, p. 1.
4. *Chromium-6 (Hexavalent Chromium) in Drinking Water,* California Department of Health Services, Update, August 22, 2001.
5. Raimund Roehl, California Department of Health Services, personal communication, May 2001.
6. *The Determination of Inorganic Anions in Water by Ion Chromatography;* U.S. Environmental Protection Agency, Method 300.0; Cincinnati, Ohio (1993).

SUPPLIERS

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

Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219 USA, Tel. 800-766-7000, www.fishersci.com.

Gelman Sciences, 600 S. Waygner Road, Ann Arbor, MI 48106-1448 USA, 800-521-1520 www.pall.com/gelman.

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

Mallinckrodt Baker, 222 Red School Lane, Phillipsburg, NJ 08665 USA, Tel. 800-582-2537, www.jtbaker.com.

Determination of Perchlorate in Drinking Water Using Reagent-Free Ion Chromatography

INTRODUCTION

Perchlorate (ClO_4^-) is an environmental contaminant and has been found in drinking, ground, and surface waters in several states in the U.S.[1] However, most contaminated sites appear to be geographically confined, particularly in the western U.S., and linked to identifiable sources, such as military installations and manufacturing sites.[2] Because perchlorate targets the thyroid gland at sufficiently high concentrations,[3] in 1998 the EPA's Office of Groundwater and Drinking Water placed this anion on its Contaminant Candidate List (CCL) for drinking water. Currently, the EPA has not established any enforceable health regulations for perchlorate in drinking water or related matrices, although some states have set individual action levels. For example, the California Department of Health Services (CDHS) has adopted an action level of 4 µg/L perchlorate.[4] If concentrations are detected above this level, then the CDHS recommends that utilities remove the drinking water source from service for proper treatment.

Dionex Application Note 134 describes the determination of perchlorate in environmental waters to 2 µg/L using a large-loop injection with an IonPac® AS16 column and suppressed conductivity detection with an ASRS® ULTRA operating in external water mode.[5] However, the U.S. EPA is currently investigating different analytical approaches that can improve the sensitivity and selectivity for perchlorate. The sensitivity for perchlorate can be improved by coupling a mass spectrometer (MS) to an ion chromatograph or by using a 2-mm IonPac AS16 column.[6,7]

Further improvements to the existing chromatographic conditions for determining perchlorate as outlined in U.S. EPA Method 314.0 can be accomplished by decreasing the baseline noise. In this application update, we describe a minor modification to EPA Method 314.0 that replaces the standard ASRS ULTRA with an improved ASRS ULTRA II suppressor. The ASRS ULTRA II can routinely produce peak-to-peak noise in the range of 1–2 nS when operated in external water mode, compared to 9–10 nS for the ASRS ULTRA. Nearly an order of magnitude decrease in baseline noise significantly enhances the detection of perchlorate to 1 µg/L or less. As an additional benefit, the ASRS ULTRA II does not require chemical regenerates, such as the sulfuric acid required for the AMMS III suppressor as described in AU 145.[7] This application update describes the determination of perchlorate using the procedure outlined in EPA Method 314.0.[8] This application used an integrated ion chromatography system with a 4-mm IonPac AS16 column, an EGC II KOH cartridge, a 1000-µL injection, and suppressed conductivity detection with an ASRS ULTRA II operated in external water mode. This application update evaluates and describes the linear range, initial demonstration of capability (EPA Method 314.0,[9] Section 9.2), matrix conductivity threshold (MCT) (EPA Method 314.0, Section 9.2.8), method detection limits (MDLs) (EPA Method 314.0 Section 9.2.6), and recovery of perchlorate in typical environmental matrices (EPA Method 314.0, Section 9.4).

EQUIPMENT

A Dionex ICS-2000 Reagent-Free Ion Chromatography (RFIC) System was used in this work. The ICS-2000 is an integrated ion chromatograph that includes:

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

AS50 Autosampler

Chromeleon® 6.5 Chromatography Workstation

Suppressor External Regen Installation Kit for External Water Mode (P/N 038018)

Conductivity Meter (Thermo Orion, Model 105)

This application update is also applicable to other RFIC systems.

REAGENTS AND STANDARDS

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

Sodium Perchlorate ($NaClO_4$) (Aldrich 41,024-1)

Sodium Chloride (NaCl) (J. T. Baker; VWR P/N JT3625-1)

Sodium Sulfate (Na_2SO_4) (Aldrich 29,931-3)

Sodium Carbonate Monohydrate ($Na_2CO_3 \cdot H_2O$) (Fisher S262-3)

CONDITIONS

Columns:	IonPac AS16 Analytical, 4 × 250 mm (P/N 055376) IonPac AG16 Guard, 4 × 50 mm (P/N 055377)
Eluent:	65 mM potassium hydroxide
Eluent Source:	ICS-2000 EG with CR-ATC
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Injection:	1000 µL (with 10-µL cut volume from a 1100-µL sample loop)
Detection:	Suppressed conductivity, ASRS ULTRA II (4 mm), Autosuppression external water mode, power setting, 193 mA
System Backpressure:	~2500 psi
Background Conductance:	~1–2 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	15 min

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Perchlorate Standard Solution

Dissolve 0.1231 g of sodium perchlorate in 100 mL of deionized water for a 1000-mg/L standard solution. This stock standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Prepare working standards at lower concentrations by diluting the appropriate volumes of the 1000-mg/L stock standard with deionized water. In this application, calibration standards were prepared at 1, 2, 10, 25, 50, and 100 µg/L perchlorate, with each standard injected in duplicate.

Mixed Common Anion Stock Solutions (EPA Method 314.0, Section 7.4.1)

Prepare 25 mg/mL (25,000 mg/L) each of chloride, sulfate, and carbonate. Dissolve 4.1213 g of sodium chloride in deionized water and dilute to 100 mL. Dissolve 3.6965 g of sodium sulfate in deionized water and dilute to 100 mL. Dissolve 5.1658 g of sodium carbonate monohydrate (4.416 g of sodium carbonate) in deionized water and dilute to 100 mL. These solutions were used to prepare 50, 100, 200, 400, 600, 800, and 1000 mg/L (ppm) of mixed anion (MA) standards of chloride, sulfate, and carbonate. These standards were used to determine the matrix conductivity threshold (MCT) (EPA Method 314.0, Section 9.2.8) and the MDLs (Section 9.2.6[8]).

Sample Preparation

Measure the conductance of the samples with a calibrated conductivity meter that has a minimum measuring range of 1–10,000 µS/cm. Verify the conductivity meter calibration by measuring the conductance of a commercially available reference solution or a prepared 745-mg/L KCl standard (EPA Method 314.0 Section 7.5) with a conductance of 1410 µS/cm at 25 °C. The conductivity meter must yield a value between 1380 and 1440 µS/cm to be considered calibrated.

Compare the sample conductivity to the MCT determined in your laboratory, as explained in EPA Method 314.0, Section 11. Filter all samples with a 0.2-µm syringe filter. Use a hydrophilic polypropylene or polyethersulfonate filter; do not use polyvinylidene fluoride (PVDF). Discard the first 300 µL of the filtrate and filter the remainder directly into a clean plastic autosampler vial. Qualify filters by analyzing a deionized water blank and a 10-µg/L perchlorate standard that has been passed through the filter. The blank should be free of peaks within the retention time window of perchlorate, and the recovery of the 10-µg/L standard should fall within 80–120%.

Samples that exceed the MCT can often be analyzed after an appropriate dilution followed by filtration with a 0.2-µm filter. EPA Method 314.0 Section 11.1.3 explains how to determine the sample's dilution factor based on the MCT. For diluted samples, the minimum reporting level (MRL) **must** be raised by a proportion equivalent to the dilution.

If sample dilution does not yield the desired results—or to avoid diluting samples—the concentration of the matrix ions can be reduced by treating the sample with Dionex OnGuard® cartridges. This procedure is explained in further detail in EPA Method 314.0, Section 11.1.4 and in Dionex Application Update 145.[7] In this application, no pretreatment or dilution was required for the samples analyzed.

SYSTEM PREPARATION AND SETUP

Install backpressure tubing in place of the column set to produce a total system pressure between 2000 and 2500 psi at a flow rate of 1 mL/min. Install an EGC II KOH cartridge (Dionex P/N 058900). Condition the cartridge as directed in the *EGC II Cartridge Quickstart Guide* (Document No. 031909) by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After completing the cartridge conditioning process, disconnect the backpressure tubing that was temporarily installed in place of the column set. Install a CR-ATC between the EGC II KOH cartridge and EGC degas. For more information on installing the CR-ATC, consult the *EGC II Cartridge Quickstart Guide.*

Install and configure the AS50 Autosampler. The precision and accuracy of the autosampler will vary depending on the injection mode. The most accurate and precise injections are made with a calibrated sample loop, flushed with about five times the loop volume. The largest full-loop injection possible with the AS50 is 300 µL. To inject 1000 µL, use the partial-loop injection mode with an 1100 µL sample loop, and a programmed "Sample Loop Volume" of 1100 µL and a "Cut Volume" of 10 µL. This injection procedure should provide peak area precision of <1% RSD. Install a 1-mL sample syringe and set the syringe speed to 4 or 5 to make faster large-loop injections. Enter the correct "Sample Loop Size" and "Sample Syringe Volume" in the AS50 Plumbing Configuration Screen. Refer to the *Autoselect AS50 Autosampler Operator's Manual* (Document No. 31169) for details.

Install a 4 × 50 mm IonPac AG16 and a 4 × 250 mm IonPac AS16 column in the column oven. Make sure the system pressure is 2300 ± 200 psi when 65 mM KOH is delivered at 1.2 mL/min to allow the degas assembly to effectively remove electrolysis gases from the eluent. If necessary, install additional backpressure tubing between the degas assembly and the injection valve to adjust the system pressure to 2100–2500 psi. Do not allow the pressure to reach 3000 psi. Therefore, monitor the pressure periodically because pressure can gradually rise over time. To reduce pressure, trim the backpressure tubing.

Unlike the ASRS ULTRA suppressor, the ASRS ULTRA II does not require any *Quick Start* using acid regenerants, and the suppressor can be installed after hydration with deionized water. Configure the suppressor for external water mode according to the directions provided in the *ASRS ULTRA II Operator's Manual* (Document No. 031956).

The storage solution of the AS16 column is 35 mM NaOH; equilibrate the column with 65 mM KOH eluent at 1.2 mL/min for approximately 60 min, then analyze a system blank of deionized water. An equilibrated system has a background signal of less than 2 µS and peak-to-peak noise of less than 2 nS. No peaks should elute within the same retention time window as perchlorate. Inject a 25-µg/L perchlorate standard. The column is equilibrated when two consecutive injections of the standard produce the same retention time for perchlorate.

RESULTS AND DISCUSSION

U.S. EPA Method 314.0 specifies the use of an IonPac AS16 column with an eluent of 50 mM NaOH at a flow rate 1.5 mL/min, followed by suppressed conductivity detection with an ASRS ULTRA operated in the external water mode and a 1000-µL large-loop injection. However, Section 6.1.3 of the method states that "An equivalent suppressor device may be utilized provided that comparable conductivity detection limits are achieved and adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 5 nS per minute over the background." Section 9.4.3 further states that, "In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns (which meet the criteria in Section 6.1.2.2), injection volumes, and/or eluents, to improve the separations or lower the cost of measurements." Therefore, a different eluent concentration, flow rate, and suppressor may be used for U.S. EPA Method 314.0, provided that the quality control parameters are met. We replaced the ASRS ULTRA specified in the method with an improved ASRS ULTRA II suppressor. The ASRS ULTRA II provides significantly lower noise of 2 nS/min or less, and therefore improves the detection limits for perchlorate. In addition, the KOH concentration was increased from 50 mM to 65 mM, and the flow rate was proportionally adjusted to 1.2 mL/min, as specified in Dionex Application Note 134.

Calibrate the system by injecting one blank and at least five standards to cover two orders of magnitude concentration range. Section 10.2.2 of the method states that the linear calibration range "should not extend over more than two orders of magnitude in concentration." Tabulate the peak area response against the perchlorate concentration injected using a linear regression fit.

Table 1 summarizes the calibration data from duplicate injections of 1, 2, 10, 25, 50, and 100 µg/L perchlorate standards. The calibration curve is linear over two orders of magnitude with a correlation coefficient of 0.9998. Figure 1 shows a chromatogram of a 1-µg/L perchlorate standard using the conditions described in this application

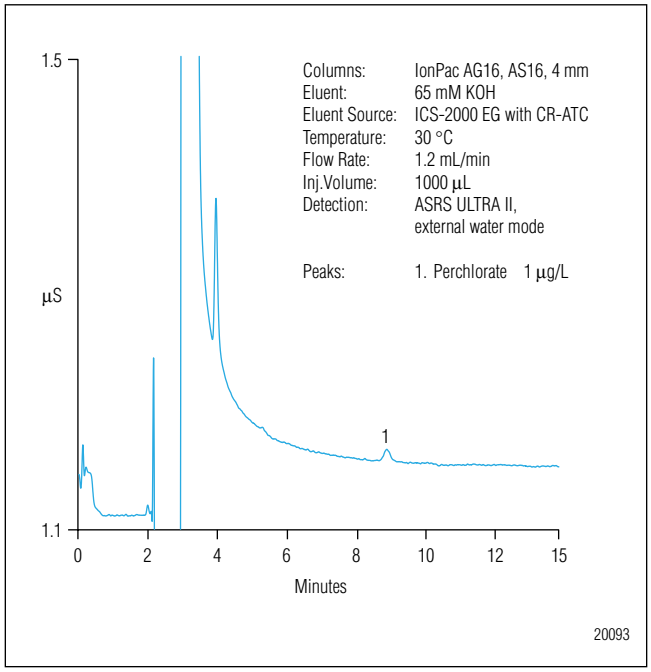


Figure 1. Determination of 1 µg/L perchlorate in deionized water.

update. After establishing the calibration curve, a quality control standard (QCS) of 50 µg/L perchlorate was analyzed resulting in a recovery of 103.8%. This recovery meets the criteria outlined in Section 9.2.5 of the method that states the recovery of the QCS **must** be within ±10% of the stated value.

U.S. EPA Method 314.0 requires an initial demonstration of capability (IDC), as described in Section 9.2. The IDC is used to characterize the instrument and laboratory performance prior to performing any sample analyses by the method. This performance is determined by demonstrating an initial demonstration of accuracy (IDA) and an initial demonstration of precision (IDP) by performing seven replicate injections of a laboratory fortified blank (LFB) fortified with 25 µg/L perchlorate. To meet the requirements of the IDA and IDP, the recovery **must** be within ±10% and the percent RSD **must** be less than 10%, respectively. As shown in Table 2, our results for the IDA and IDP met the requirements described in Sections 9.2.3 and 9.2.4.

Table 1. Calibration Data for Perchlorate

Analyte	Range (µg/L)[a]	Linearity
Perchlorate	1–100	0.9998

[a] Calibration standards were 1, 2, 5, 10, 25, 50, and 100 µg/L, each injected in duplicate

Table 2. Initial Demonstration of Capability for Perchlorate

	EPA Method 314.0 Performance Requirements			Experimental Values	
Requirement	**Reference**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
IDA	9.2.3	90–110		103.1	
IDP	9.2.4		<10		0.5

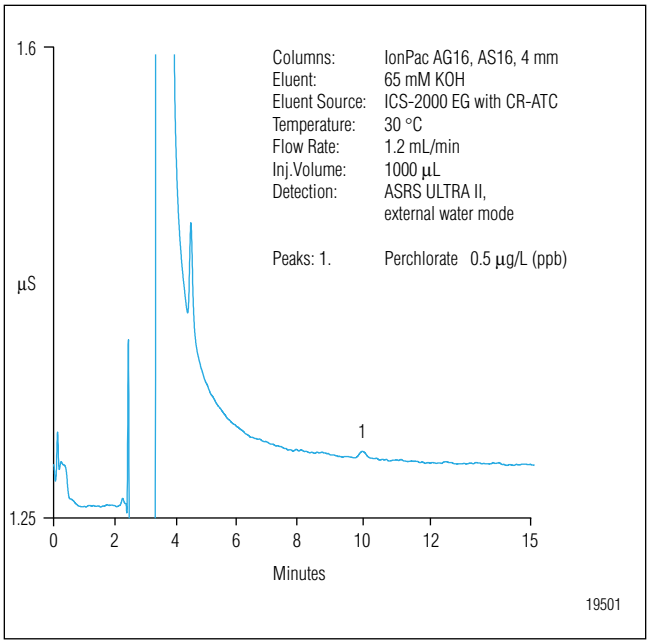


Figure 2. Determination of 0.5 µg/L perchlorate in deionized water.

We determined the method detection limit (MDL), as described in Section 9.2.6, by performing seven replicate injections of deionized water fortified with perchlorate at a concentration of three to five times the estimated instrument detection limit. The concentration values determined from the calibration curve were used to calculate the MDL. Figure 2 shows a chromatogram of a 0.5 µg/L perchlorate MDL standard fortified in deionized water. This MDL value is only valid for perchlorate in a "clean" matrix, such as deionized water. The MDL is expected to change as the ionic strength of the sample increases. Therefore, in addition to deionized water, we determined the MDL in MA(50), MA(100), MA(200), MA(400), and MA(600), where MA indicates a mixed common anion solution of chloride, sulfate, and carbonate included in the sample matrix at the parenthetical mg/L concentration for each anion. Table 3 summarizes the results of this study. Because the MDL is based on precision and not accuracy, the determined MDL value for high-ionic-strength matrices, such as MA(400) and MA(600), do not meet the requirement of a fortified perchlorate concentration of three to five times the estimated instrument detection limits. Meeting this condition would typically require the analyst to repeat the MDL at a lower fortified perchlorate concentration. However, determining the MDL using lower perchlorate concentrations is not feasible because high concentrations of common anions interfere with the determination of perchlorate.

Table 3. Perchlorate MDLs

Matrix	MDL Standard (µg/L)	Retention Time RSD (%)	Calculated MDL[a] (µg/L)
Deionized Water	0.5	0.10	0.10
MA(50)[b]	0.5	0.20	0.10
MA(100)	0.5	0.05	0.13
MA(200)	1.0	0.27	0.24
MA(400)	2.0	0.07	0.18
MA(600)	5.0	0.07	0.24

[a]The MDLs were calculated as MDL = $(t) \times (SD)$ 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 replicate injections for the MDL standard) and SD = standard deviation of the replicate analyses.
[b]MA indicates a mixed common anion solution of chloride, sulfate, and carbonate included in the sample matrix at the parenthetical mg/L concentration for each anion.

Section 9.2.8 describes the matrix conductivity threshold (MCT) as "an individual laboratory defined value" determined by preparing a series of sequentially increasing concentrations of chloride, sulfate, and carbonate fortified with a constant perchlorate concentration. Deionized water fortified with a recommended perchlorate concentration of 25 µg/L must be initially analyzed and followed by a series of increasing anionic solutions of chloride, sulfate, and carbonate, each containing 25 µg/L perchlorate. The recommended 25 µg/L perchlorate assumes that the MRL has been set between 3 µg/L and 5 µg/L. However, if an MRL of 1 µg/L is required, then the MCT should be determined at a perchlorate concentration of 5 µg/L. We determined the MCT using 5 µg/L and 25 µg/L perchlorate. To determine the MCT with 25 µg/L perchlorate, a standard was prepared in deionized water and injected in triplicate. Next, standards containing MA(50), MA(100), MA(200), MA(400), MA(600), MA(800), and MA(1000) were prepared by adding 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, and 4 mL of each common anion from the stock solution (see the section "Preparation of Solutions and Reagents") to separate 120-mL polypropylene bottles. Then, 2.5 mL of perchlorate was added from a 1-mg/L secondary stock dilution standard to each MA solution and dilute each standard to a final volume of 100 mL. A calibrated conductivity meter measured and recorded the conductance for each of these prepared solutions. Section 9.2.8.5 states that the MA(400) solution "should display a conductance of between 3200 µS/cm and 3700 µS/cm."

Table 4 shows the results from this study. Based on multiple determinations, our laboratory determined the MCT with 25 µg/L perchlorate was a value varying from ~4500 µS/cm to 5330 µS/cm. However, individual results may vary within or between laboratories and analysts. The same procedure also determined the MCT using 5 µg/L perchlorate. In this study, the mixed anion solution did not exceed MA(600) because of a significant increase in the percent difference (PD) in the area to height (A/H) ratio. Table 5 shows the results for the MCT study using 5 µg/L perchlorate. Figure 3A and 3B show chromatograms of 5 µg/L and 25 µg/L perchlorate fortified in MA(200), respectively.

Table 4, MCT Study—Perchlorate Fortified at 25 µg/L

Sample	Conductivty (µS/cm)	Measured ClO_4^- (µg/L)	Percent Recovery	Peak Area	Peak Height	A/H Ratio	PD(A/H) (%)
LFB	<1	25.84	102.6	0.0511	0.223	0.229	0.00
MA(50)	568	25.21	99.9	0.0493	0.211	0.234	2.01
MA(100)	1089	25.25	100.4	0.0494	0.207	0.239	4.25
MA(200)	1979	24.93	99.6	0.0487	0.196	0.249	8.71
MA(400)	3590	24.85	100.2	0.0486	0.182	0.268	16.8
MA(600)	4890	24.30	96.8	0.0475	0.170	0.279	22.0
MA(800)	6070	23.96	95.9	0.0456	0.158	0.288	25.7
MA(1000)	7380	22.76	91.5	0.0454	0.148	0.306	33.6

Table 5. MCT Study—Perchlorate Fortified at 5 µg/L

Sample	Conductivty (µS/cm)	Measured ClO_4^- (µg/L)	Percent Recovery	Peak Area	Peak Height	A/H Ratio	PD(A/H) (%)
LFB	<1	5.09	101.3	0.0102	0.046	0.222	0.00
MA(50)	588	5.03	100.8	0.0101	0.045	0.224	1.22
MA(100)	1116	5.02	99.1	0.0101	0.045	0.226	1.90
MA(200)	1977	4.76	96.1	0.0094	0.040	0.233	4.93
MA(400)	3660	4.57	90.8	0.0090	0.035	0.259	16.6
MA(600)	4900	3.97	78.1	0.0075	0.026	0.284	28.1

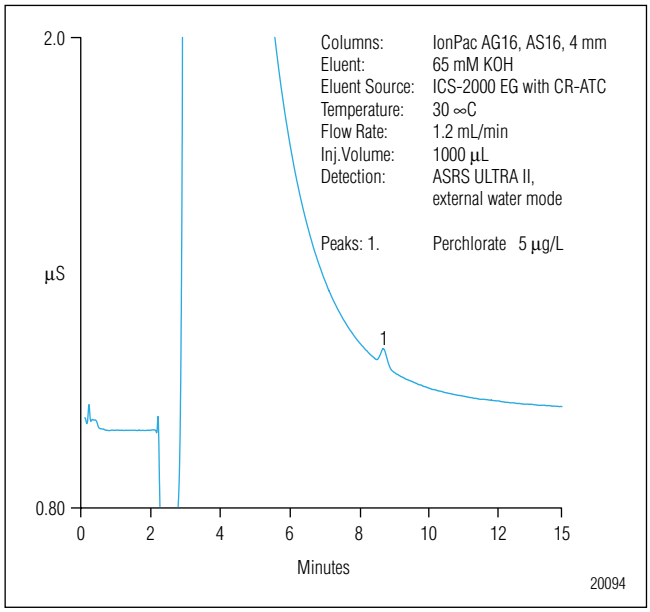


Figure 3A. Determination of 5 µg/L perchlorate in 200 mg/L each of chloride, sulfate, and carbonate.

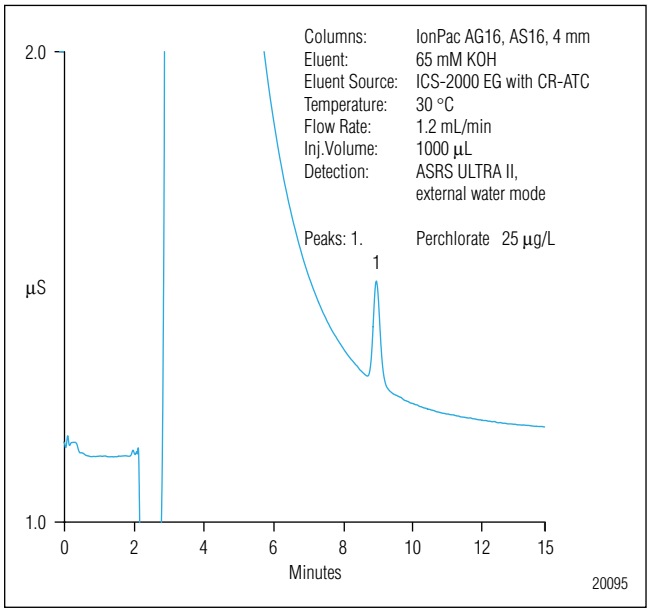


Figure 3B. Determination of 25 µg/L perchlorate in 200 mg/L each of chloride, sulfate, and carbonate.

Chromatographic performance of perchlorate can deteriorate at high ionic concentrations, primarily due to the presence of high concentrations of chloride, sulfate, and carbonate. Before samples are analyzed, the conductance **must** be determined. If the conductance is greater than the determined MCT, the samples should either be appropriately diluted or pretreated to reduce the common anion concentrations. One way to assess matrix effects is to prepare a laboratory fortified matrix (LFM). An LFM is accomplished by spiking the sample with a known amount of analyte and then determining the percent recovery from the amount added. This application analyzed four matrices: deionized water, drinking water, raw (untreated) drinking water, and surface water. Each matrix was spiked with 1 or 2 µg/L perchlorate and the recoveries were calculated with the equation provided in Method 314.0, Section 9.4.1.3. Table 6 shows the results of this study. The calculated perchlorate recoveries were ~97–108%, which was well within the 80–120% (Section 9.4.1.4) range specified by the method. Figure 4 shows a chromatogram of surface water spiked with 1 µg/L perchlorate.

Table 6. Perchlorate Recoveries from Laboratory Fortified Matrices (LFM)
Fortified Matrices (LFM)

Matrix	Conductivty (µS/cm)	Amount Added (µg/L)	Number of Replicates	Precision (% RSD)	Recovery (%)
Deionized water	<1	1.0	7	2.76	99.3
Drinking water	130	1.0	8	10.0	106.8
Raw (untreated) Drinking water	467	2.0	8	5.26	97.6
Surface water	670	1.0	8	12.6	108.2

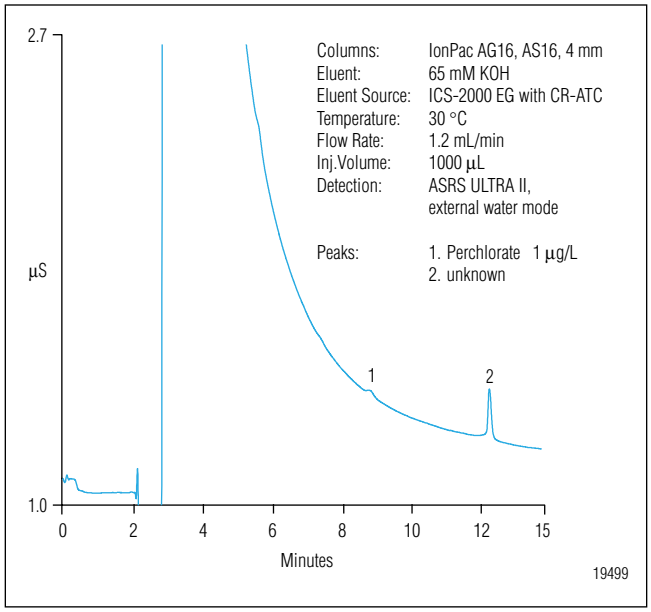


Figure 4. Trace-level perchlorate spiked into surface water.

CONCLUSION

This application update demonstrates an approved approach compared to Dionex Application Note 134 for the determination of perchlorate in environmental samples using U.S. EPA Method 314.0. The lower baseline noise from an ASRS ULTRA II compared to the ULTRA I suppressor improved the limit of detection and quantification of perchlorate resulting in a calculated MDL of 0.1 µg/L in deionized water. The MDLs in high-ionic-strength matrices containing up to 600 ppm each of chloride, sulfate, and carbonate ranged from 0.1 µg/L to 0.24 µg/L perchlorate. Calibration is linear over the range of 1–100 µg/L in deionized water, and acceptable recoveries were obtained for perchlorate spiked at 1–2 µg/L in typical environmental samples. The MCT determined in our lab using 5 µg/L perchlorate was ~3900 µS/cm and the MCT ranged from ~4500 to 5300 µS/cm using 25 µg/L perchlorate. However, results from individual laboratories or analysts may vary. The results presented in this application update meet or exceed the performance requirements specified in U.S. EPA Method 314.0.

REFERENCES

1. Jackson, P. E.; Gokhale, G. T.; Streib, T.; Rohrer, J. S.; Pohl, C. A. *J. Chromatogr. A* **2000**, 888, 151.
2. Urbansky, E. T.; Collette, T. W. *J. Environ. Monit.* **2001**, 3, 454.
3. Urbansky, E. T. *Biorem. J.* **1998**, 2, 81.
4. *Perchlorate and Drinking Water: Action Level and Public Health Goal*, Updated September, 2003, California Department of Health Services: www.dhs.ca.gov/ps/ddwem/chemicals/perchl/actionlevel.htm.
5. Dionex Corporation. *Determination of Low Concentrations of Perchlorate in Drinking and Groundwaters Using Ion Chromatography*. Application Note 134; Sunnyvale, CA.
6. Dionex Corporation. *Determination of Perchlorate in Environmental Waters by Ion Chromatography Coupled with Electrospray Mass Spectrometry (IC-MS).* Application Note 151; Sunnyvale, CA.
7. Dionex Corporation. *Determination of Perchlorate in Drinking Water by Ion Chromatography*. Application Update 145; Sunnyvale, CA.
8. *Determination of Perchlorate in Drinking Water Using Ion Chromatography*. Method 314.0; U.S. Environmental Protection Agency; Cincinnati, Ohio, 1999.

Sensitive Determination of Microcystins in Drinking and Environmental Waters

INTRODUCTION

Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the primary toxins produced by these species growing in lakes, ponds, and rivers used as drinking water sources. Microcystins (structures shown in Figure 1) are hepatotoxins that exhibit tumor-promoting activity and are among the most commonly found cyanobacteria toxins. Microcystin contamination of drinking water at low nanomolar concentrations is considered a risk factor for cancer, and microcystin-LR has been associated with most of the incidents of toxicity involving microcystins. Therefore, the World Health Organization (WHO) has proposed a provisional guideline concentration of 1.0 µg/L for microcystin-LR in drinking water.[1]

The analytical approaches commonly used for microcystins include bioassay, chemical, and biochemical methods. Bioassays have been used in screening but were found to be non-specific and/or more time consuming. Biochemical methods, such as enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PPIA), are advantageous as screening methods due to their high sensitivity and ability to quickly treat a large number of samples; the disadvantage of these methods, however, is that they provide poor identification and have the potential for false positives. Reversed-phase high-performance liquid chromatography (HPLC) with UV detection, liquid chromatography mass spectrometry (LC-MS), and capillary electrophoresis are chemical methods that have been used for the identification and quantification of microcystins.[2]

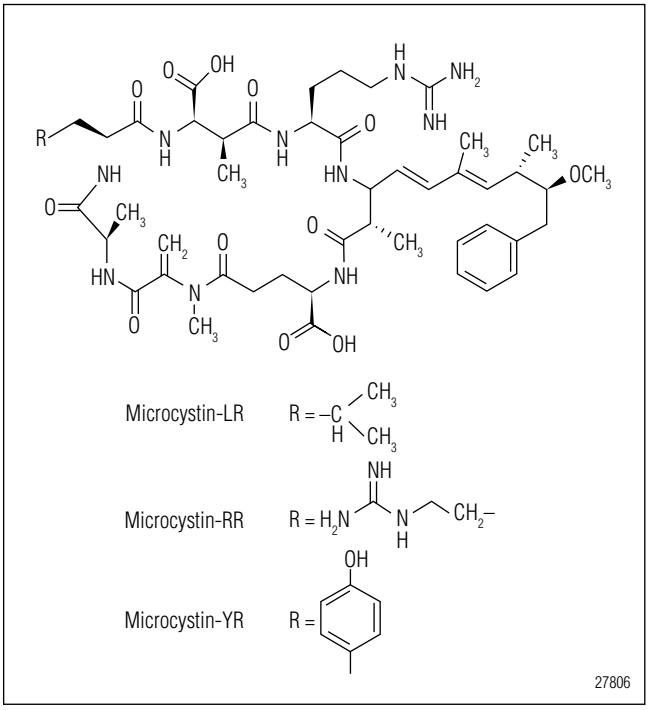


Figure 1. Structures of microcystins.

The control of microcystins at 1.0 µg/L levels requires sensitive analytical methods and HPLC methods have been widely used for this purpose. Solid-phase extraction (SPE) is one of the main methods for sample extraction and preconcentration; however, the authors of Reference 3 suggest that the typically used SPE stationary phase (C18) does not supply good selectivity for trace analysis.[3] Immunoaffinity columns (IAC) modified with anti-microcystin-LR monoclonal antibodies on polypropylene stationary phases have been used for

extraction with good selectivity for the HPLC analysis of microcystins,[3-5] but extensive use of this method is limited because an IAC is not commercially available for this application.

The authors have reported a simple, fast, and effective target-cut on-line SPE method followed by HPLC with UV detection on an UltiMate® 3000 HPLC system consisting of a dual gradient pump, autosampler, and column oven equipped with one 2p–6p valve for the determination of trace amounts of vitamin B_{12} added to beverages.[6, 7] This on-line SPE method is different from the typical one. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. This reduces the number of interferences for sample analysis. While the SPE process is running, the analytical column is equilibrating. Just before the front portion of the analyte peak elutes from the SPE column, the SPE column is switched into the analytical flow path. As soon as the analyte is completely eluted from the SPE column, the SPE column is switched out of the analytical flow path and back to the SPE flow path. Therefore, only those interferences co-eluting with the analytes will enter the analytical column; thus, more interferences are removed. The volume of analyte cut from the SPE column is separated on the analytical column and detected by the UV detector. This target-cut on-line SPE method with dual function (analyte capture and partial separation) operates under automatic control of Chromeleon® Chromatography Data System (CDS) software and offers full automation, absence of operator influence, and strict process control, compared to a typical off-line SPE method.[8]

Here, the target-cut on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking, tap, and lake water. The three target analytes were co-eluted from the first column using chromatographic conditions that eliminated as many interferences as possible; then the analytes were sent to the analytical flow path and separated on the second column using the same type of stationary phase under different chromatographic conditions. This design takes advantage of the separation power of both columns and may eliminate interferences more efficiently than typical on- and off-line SPE methods. An additional dual-valve design is easy to use and convenient for method development.

The UltiMate 3000 ×2 Dual HPLC system provides an efficient platform to fulfill the requirements of these designs. Sub-µg/L concentrations of microcystins-LR, -RR, and -YR spiked in water samples were determined, which exceeds the WHO requirement.

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

DGP-3600A pump with SRD 3600 solvent rack with degasser

WPS-3000TSL semiprep autosampler (with 2500 µL sample loop)*

TCC-3200 Thermostatted Column Compartment equipped with two 2p–6p valves

VWD-3400RS UV-vis detector

Chromeleon software

Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL Autosampler can also be converted and used for large-volume injection for on-line SPE. The procedure is the same as specified in Reference 6.

REAGENTS

Deionized water, Milli-Q® Gradient A10, Millipore Corporation

Acetonitrile (CH_3CN) and methanol (CH_3OH), HPLC grade, Fisher

Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and phosphoric acid (H_3PO_4), 85% (analytical grade), SCRC, China

STANDARDS

100 µg of microcystins-LR (CAS 101043-37-2), -RR (CAS 111755-37-4), and -YR (CAS 101064-48-6), respectively, ≥ 95% (HPLC), Alexis Corporation

Prepare stock standard solutions with 50 µg/mL concentrations by dissolving the standards with 2000 µL of methanol. Prepare the standard solutions used for the calibration curve by making appropriate dilutions of the stock standard solutions with water.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. The lake water sample was collected at Zhangjiang High-Science and Technology Park located in the Pudong District of Shanghai, China. Bottled spring water samples were purchased from a supermarket in Shanghai. These samples were filtered through a 0.45 µm membrane (Millex-HN) prior to injection.

CHROMATOGRAPHIC CONDITIONS

On-Line SPE

Column: Acclaim® PA2, 3 µm, 3.0 × 33 mm (P/N 066276)

Analytical

Column: Acclaim PA2, 3 µm, 3.0 × 150 mm (P/N 063705)

Column Temp.: 40 °C

Mobile Phase: For SPE:
A: 22.5 mM KH_2PO_4–2.5 mM K_2HPO_4 buffer (dissolve ~ 3.1 g of KH_2PO_4 and 0.44 g of K_2HPO_4 in 1 L of water)
B: CH_3CN
In gradient (Table 1)

For separation:
A: 0.05% (v/v) H_3PO_4 (dilute 0.6 mL of 85% H_3PO_4 to 1 L with water)
B: CH_3CN
In gradient (Table 1)

Valve-Switching: Table 1

Flow Rate: 0.7 mL/min for both SPE and separation

Injection Vol.: 2500 µL on the SPE column

UV Detection: Absorbance at 240 nm

Table 1. Gradients and Valve Switching for Target-Cut On-Line SPE and Separation

Time (min)	Right Pump (for Separation)			Left Pump (for On-Line SPE)			Valve Switching	
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Left	Right
0.00	0.7	85	15	0.7	80	20	6-1	1-2
5.00		—	—		80	20		—
6.95		—	—		—	—		6-1
7.00		85	15		65	35		—
7.35		—	—		—	—		1-2
7.50		—	—		20	80		—
8.50		—	—		20	80		—
8.60		—	—		80	20		—
12.0		41	59		—	—		—
12.1		85	15		—	—		—
15.0		85	15		80	20		—

Table 2. Gradient and Valve Switching for Traditional On-Line SPE and Separation

Time (min)	Right Pump (for Separation)			Left Pump (for On-Line SPE)			Valve Switching
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B CH_3CN (%)	
0.00	0.7	80	20	0.7	80	20	1-2
5.00		80	20				6-1
6.00		—	—				1-2
9.00		50	50				
9.10		25	75				
11.0		25	75				
11.1		80	20				
12.0		80	20				

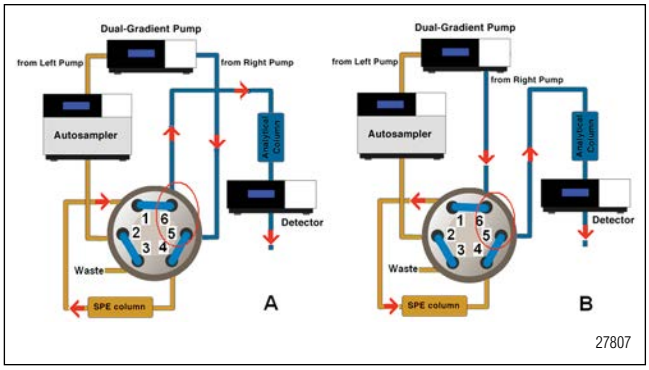


Figure 2. Flow schematics for A) traditional and B) target-cut on-line SPE methods equipped with one 2p–6p valve for sample preparation and analysis.

RESULTS AND DISCUSSION

Retention Behavior of Microcystins-RR, -YR, and -LR on the Acclaim PA2 Column

The Acclaim Polar Advantage II (PA2) is a polar-embedded column designed for enhanced hydrolytic stability within a wide range of pH values (pH 1.5 to 10), and compatibility with 100% aqueous mobile phases, overcoming the limitations of conventional C8 and C18 reversed-phase columns.

Effect of Buffer pH Value

The pH value of the mobile phase buffer may affect the retention of microcystins-RR, -YR, and -LR. Changes in their retention behavior on the Acclaim PA2 stationary phase were investigated. Experiments showed that when the buffer pH value decreased from pH 6.5 to 2.7, the retention time of microcystins-YR and -LR increased and the resolution between them improved, whereas the retention time of microcystin-RR did not change. The three microcystins were separated at a pH value lower than 2.5. They co-eluted at approximately pH 6.0.

Thus, for the requirements addressed here, the PA2 column is a good choice as an SPE column for concentrating the three microcystins from large-volume water samples (tap water and beverages) and co-eluting them using mobile phase buffer with a high pH value (~ 6.0). The PA2 column is also a good choice as an analytical column for the separation using a mobile phase buffer with a low pH value.

Effect of Column Temperature

The effect of column temperature on the retention of microcystins-RR, -YR, and -LR on the Acclaim PA2 stationary phase was investigated. Increasing column temperature may shorten the retention time, and is a benefit to the separation of microcystins-YR and -LR, which have close retention times. For example, resolution (R_s) between the two compounds increased from 0.50 to 1.94 when the column temperature increased from 25 to 40 ºC.

Comparison of Traditional and Target-Cut On-Line SPE Methods

The commonly used on-line SPE flow scheme (Figure 2A) couples the SPE column directly with the analytical HPLC column using one six-port (2p–6p) column valve. The filtered sample is injected directly onto the system and delivered to the SPE column for enrichment (1-2 position) using the left pump; the analytical column is equilibrated with the right pump at the same time. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6-1 position), then the analytes are separated on the analytical column and detected by the UV detector.

For the target-cut on-line SPE method, a small change in the flow scheme of the traditional on-line SPE mode reverses the flush direction on the SPE column (Figure 2B) and creates an on-line SPE system that can have a dual function to eliminate interferences more efficiently. The SPE process in this mode is different from that described in the traditional method. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. As the SPE process (position 1-2) is running, the analytical column is equilibrating. Just before the front portion of the analyte peak elutes from the SPE column, the SPE column is switched into the analytical flow path (position 6-1). As soon as the analyte is completely eluted from the SPE column, the SPE column is switched out of the analytical flow path and back to SPE flow path (position 1-2). Therefore, only those interferences co-eluting with the analytes will enter the analytical column; thus, more interferences are removed.

SPE Column:	Acclaim PA2, 3 µm, 3.0 × 33 mm
Anal. Column:	Acclaim PA2, 3 µm, 3.0 × 150 mm
Column Temp.:	40 °C
Eluent for SPE:	CH_3CN–phosphate buffer (22.5 mM KH_2PO_4–2.5 mM K_2HPO_4) A. Isocratic: 20% CH_3CN: 80% phosphate buffer (v/v) B. In gradient: CH_3CN, 0–5 min, 20%; 7.0 min, 35%; 7.5–8.5 min, 80%; 8.6–15 min, 20%
Eluent for Separation:	CH_3CN–0.05% (v/v) H_3PO_4, in gradient A. CH_3CN, 0–5.0 min, 20%; 9.0 min, 50%; 9.1–11 min, 75%; 11.1–12 min, 20% B. CH_3CN, 0–7.0 min, 15%; 12 min, 59%; 12.1–15 min, 15%
Flow Rates:	0.7 mL/min for SPE and separation
Injection Vol.:	2500 µL on SPE column
Detection:	UV at 240 nm

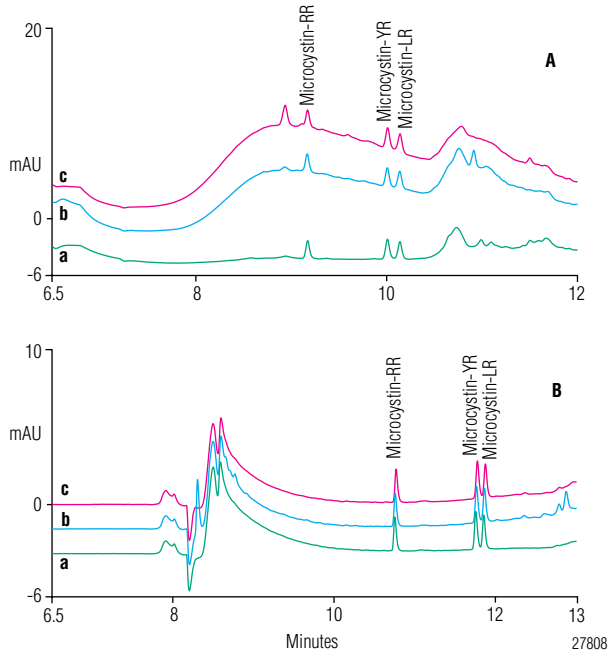


Figure 3. Chromatograms of a) bottled spring water, b) tap water, and c) lake water spiked with 1 µg/L each of microcystin-RR, -YR, and -LR standard using A) traditional and B) target-cut on-line SPE methods.

Figure 3 shows chromatograms of three types of water samples spiked with 1.0 µg/L each of microcystin-RR, -YR, and -LR standard using the traditional and target-cut on-line SPE methods, respectively. Tables 1 and 2 list the gradients and valve-switching times. Comparison of the two on-line SPE methods for analysis of different water samples demonstrates that the target-cut method may flush far fewer interferences to the analytical flow path, which is more efficient for analysis of the three microcystins in different water samples, whereas the traditional on-line SPE method is merely acceptable for the water samples.

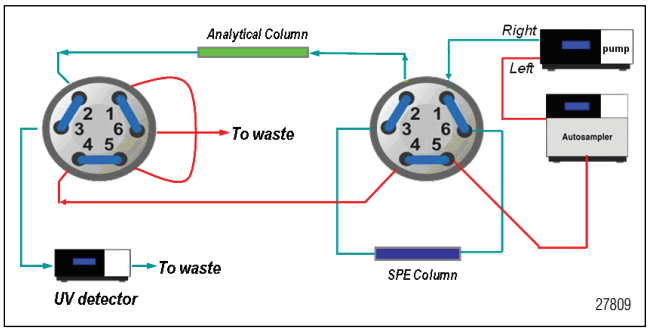


Figure 4. Flow schematic for the target-cut on-line SPE method equipped with two 2p–6p valves.

Position of Left Valve	Position of Right Valve	Description
1-2	1-2	Determine switching time of right valve during method development
6-1	1-2	Load sample and analysis
6-1	6-1	Transfer analytes from SPE column to analytical column

In practice, an additional 2p–6p valve may be used to construct a two-valve (2p–6p) system for convenient method development. The flow schematic of the two-valve configuration is shown in Figure 4. The left valve can be used to switch the SPE column or separation column into the flow path of the detector.

Evaluation of Microcystins Extraction Using the Target-Cut On-Line SPE Method

Configuration of Target-Cut Method

This newly developed on-line SPE method with dual function (analyte capture and partial separation) automatically controlled by Chromeleon software was used for analysis of vitamin B_{12}.[6,7] In that application, it was easy to configure the instrument and set the method parameters for target-cut mode because there was only a single target analyte and the same mobile phases were used for SPE and separation.

For samples containing more than one target analyte, the choice of target-cut method parameters is important for the success of the on-line SPE method. In theory, the ideal approach would be to cut the analytes one by one from the first stationary phase (SPE column) to the second stationary phase (analytical column), thereby minimizing the interferences entering the analytical flow path. This approach is not recommended, however, because it may result in a complicated valve-switching process and affect the separation on the analytical column.

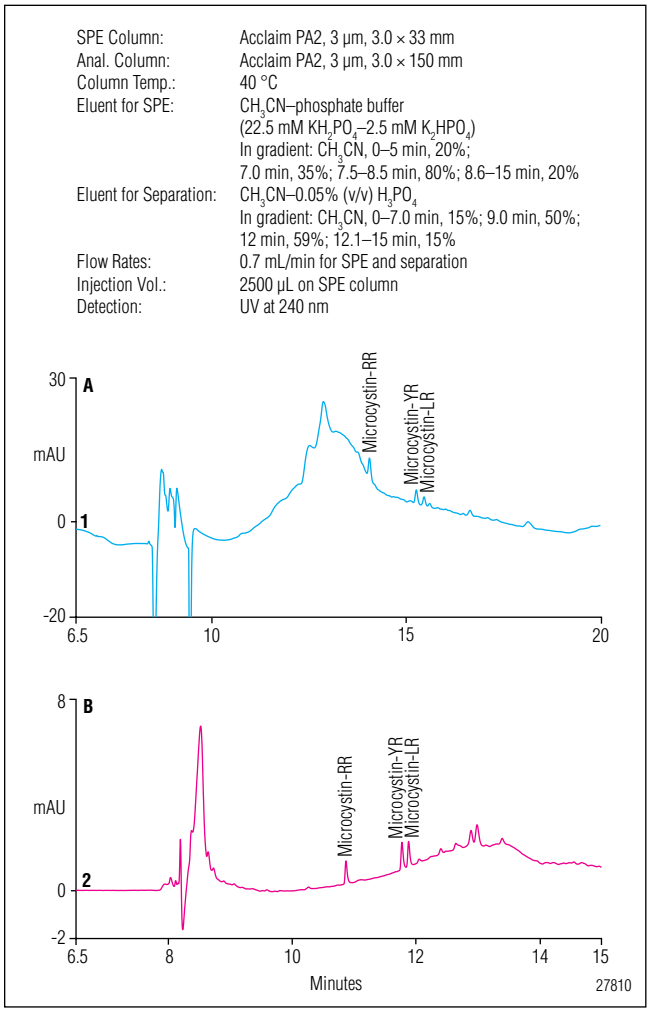


Figure 5. Chromatograms of a tap water sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using different target-cut modes. A) Valve-switching starts from microcystin-RR and ends at microcystin-LR when they are eluted from the SPE column. B) The three microcystins elute together from the SPE column.

A simpler approach is to start the target-cut when the front shoulder of the first analyte peak is just eluting from the SPE column, then end when the tail of the last analyte peak elutes from the SPE column. This target-cut method is suitable for analytes with similar retention on the SPE column. For example, on the Acclaim PA2 SPE column, the retention times of microcystins-YR and -LR are similar but significantly different from that of microcystin-RR. For the determination of microcystins-RR, -YR, and -LR in a spiked tap water sample, the volume of cut analytes separated on the analytical column (Acclaim PA2 column) was large. As shown in Figure 5A, with the target-cut method, a large amount of interferences were still cut to the analytical flow path, which resulted in interference with the determination of microcystins at sub-µg/L concentrations.

The appropriate target-cut method for a sample containing several target analytes is to use a mobile phase that will elute the analytes together (as one chromatographic peak) from the SPE column and then send them to the analytical flow path. Because the volume of cut target analytes is much smaller than that obtained by the alternate method, the co-eluted interferences may be much less; if so, the elimination of interferences will be more efficient.

Using the same determination of microcystins-RR, -YR, and -LR in a spiked tap water sample, Figure 5B shows the target-cut method with a CH_3CN–phosphate buffer (pH 6.0) mobile phase to elute analytes from the SPE column, and the analytical column using CH_3CN–0.05% H_3PO_4 (v/v, pH 2.2) mobile phase. Figure 5B shows that this approach does, in fact, have fewer interferences. Note that if the valve-switching times are inaccurate, the difference between the two mobile phases may affect separation of the three microcystins. Therefore, correctly setting valve-switching times is key to success of the target-cut on-line SPE method.

Determination of Valve-Switching Times

Based on the target-cut method in which all three analytes are eluted from the SPE column together, the valve-switching times for the extraction of microcystins-RR, -YR, and -LR can be estimated using the following equation, which was applied to vitamin B_{12} analysis.[6]

$$t_{valve\text{-}switching\ 2} = t_{valve\text{-}switching\ 1} + (v_1/v_2) \times w_h$$

Where $t_{valve\text{-}switching\ 1}$ represents the first valve-switching time when the front shoulder of the analyte peak is just eluting from the SPE column at the flow rate for SPE; $t_{valve\text{-}switching\ 2}$ represents the second valve-switching time when the SPE column is switched out of the analytical flow path; v_1 and v_2 represent the flow rates for SPE and separation, respectively; and w_h represents baseline peak width (min) of analytes on the SPE column.

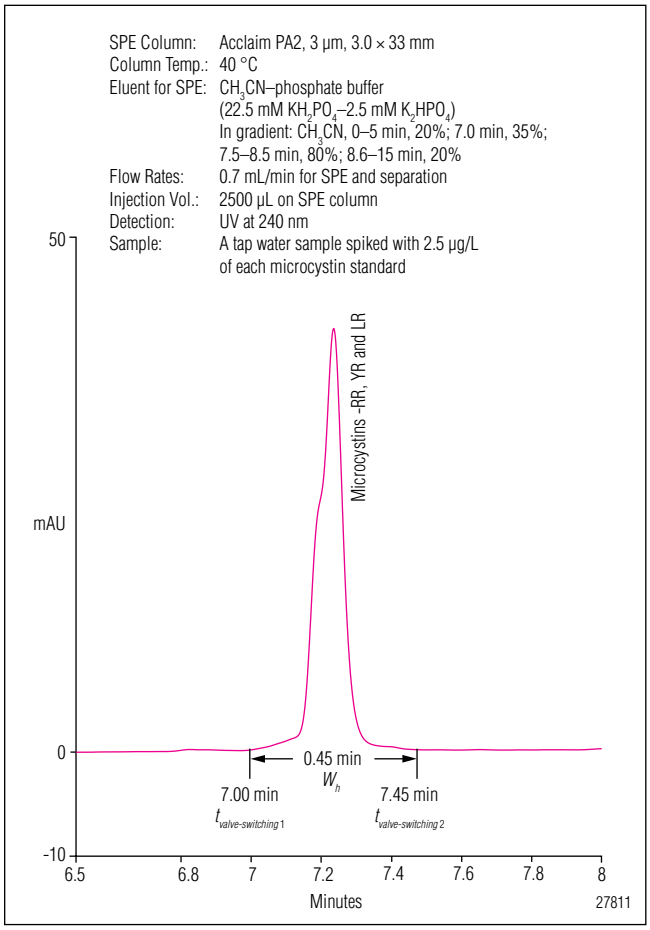


Figure 6. Chromatography to determine valve-switching time for the target-cut on-line SPE method based on the configuration showed in Figure 4.

Figure 6 shows the chromatogram of co-eluted microcystins-RR, -YR, and -LR on the SPE column. The front shoulder of the peak eluting from the SPE column at 0.7 mL/min (v_1) appears at 7.00 min ($t_{valve\text{-}switching\ 1}$). The peak is detected by the UV detector and the baseline peak width on the SPE column is 0.45 min (w_h). When the flow rate for the separation on the analytical column is also 0.7 mL/min (v_2), the second valve-switching time ($t_{valve\text{-}switching\ 2}$) calculated using the equation is 7.45 min.

The authors tried to have a slightly earlier $t_{valve\text{-}switching\ 1}$ and a delay in $t_{valve\text{-}switching\ 2}$ (0.10 min) to avoid losing microcystins when using different mobile phases for SPE and separation. Experiments showed that a 0.10 min delay in $t_{valve\text{-}switching\ 2}$ had no obvious effect, but 0.10 min earlier in $t_{valve\text{-}switching\ 1}$ resulted in the loss of microcystin-RR.

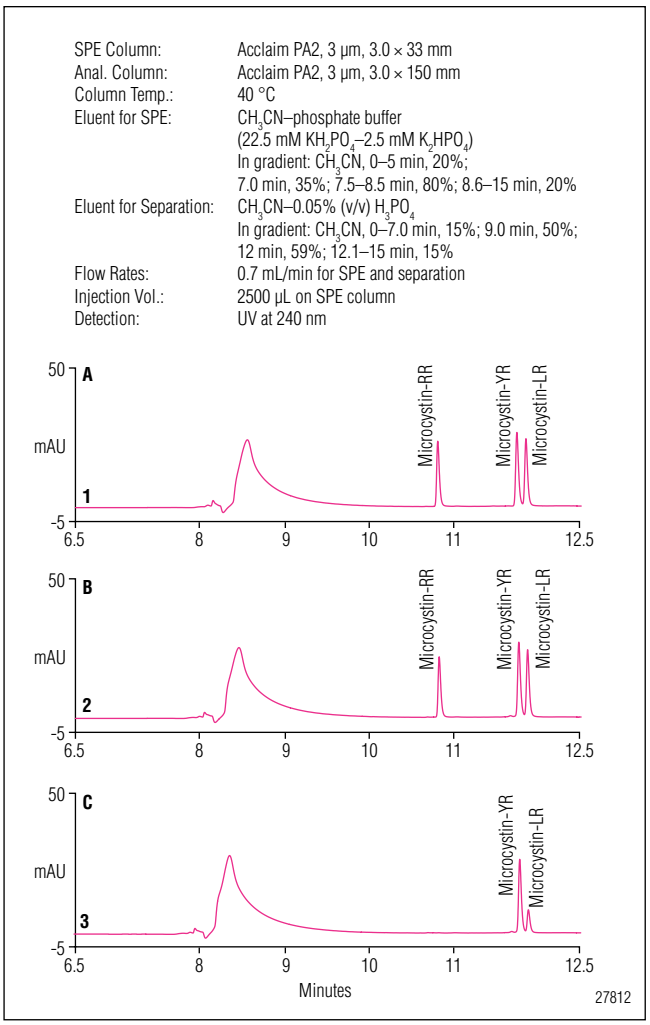


Figure 7. Chromatograms of a mixture of microcystin-RR, -YR, and -LR standards with concentration 1.0 µg/L for each extracted at different valve-switching times: A) $t_{valve\text{-}switching\ 1}$ = 7.00 min, B) $t_{valve\text{-}switching\ 1}$ = 6.90 min, and C) $t_{valve\text{-}switching\ 1}$ = 6.80 min.

As shown in Figure 7, when $t_{valve\text{-}switching\ 1}$ = 7.00 min, all three microcystins were well retained; with 0.10 min earlier ($t_{valve\text{-}switching\ 1}$ = 6.90 min), a small part of microcystin-RR was lost; and with just 0.20 min earlier ($t_{valve\text{-}switching\ 1}$ = 6.80 min), microcystin-RR was lost completely, and more than half of microcystin-LR was lost as well. The authors hypothesize that this analyte loss was due to the cut volume obtained by using the slightly earlier time (0.2 min), which brought mobile phase of higher pH value (pH 6.0) and higher proportion of organic solvent (CH_3CN) to the analytical flow path before the analytes; this resulted in a change of the intrinsic equilibrium of the analytical column that significantly affected analyte retention. Therefore, the control of valve-switching time $t_{valve\text{-}switching\ 1}$ must be accurate.

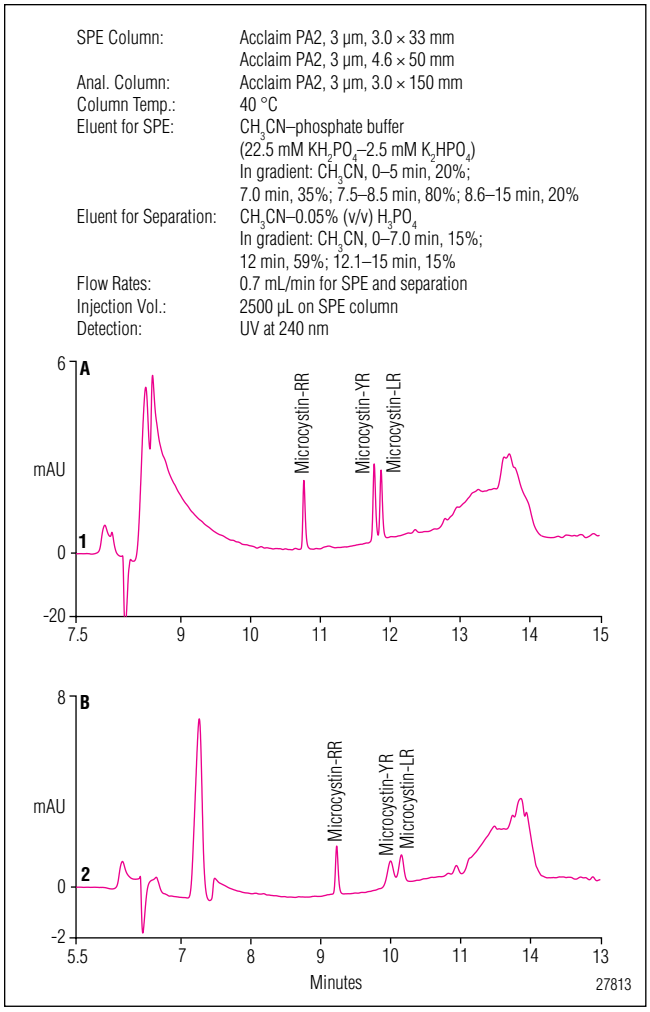


Figure 8. Chromatograms of a lake water sample spiked with 1.0 µg/L each of microcystin-RR, -YR, and -LR standard using different size SPE columns: A) Acclaim PA2, 3 µm, 3.0 × 33 mm column, and B) Acclaim PA2, 3 µm, 4.6 × 50 mm column with the target-cut on-line SPE method in Table 1.

Selection of SPE Column Format

The effect of SPE column size on elimination of impurities using the target-cut on-line SPE method was investigated. Two Acclaim PA2 columns with different sizes, 4.6 × 50 mm and 3.0 × 33 mm, were used for SPE. As shown in Figure 8, interference elimination was slightly better on the larger column, which can be attributed to separation on the larger column being more efficient than that on the smaller one; therefore, fewer impurities enter the analytical flow path. However, the larger column did have a significant effect on separation on the analytical column due to more mobile phase being cut from SPE to the analytical flow path, which resulted in poor peak shape and less detection sensitivity for microcystins-YR and -LR. Therefore, the 3.0 × 33 mm Acclaim PA2 column was selected as the SPE column for this application.

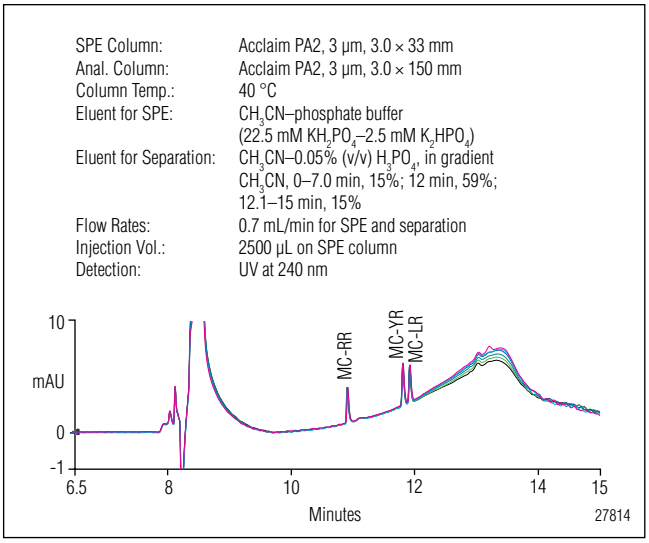


Figure 9. Overlay of chromatograms of six consecutive injections of a drinking water sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Table 3. Reproducibility for Peak Retention Time and Area

Microcystins	Retention Time RSD	Peak Area RSD	Concentration of standard (µg/L)
RR	0.037	1.53	0.5
YR	0.028	1.59	0.5
LR	0.029	1.13	0.5

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making six consecutive 2500 µL injections of a drinking water sample spiked with 0.5 µg/L of each microcystin standard. Retention time and peak area reproducibilities are summarized in Table 3 and show good precision. Figure 9 shows an overlay of chromatograms for the six consecutive injections.

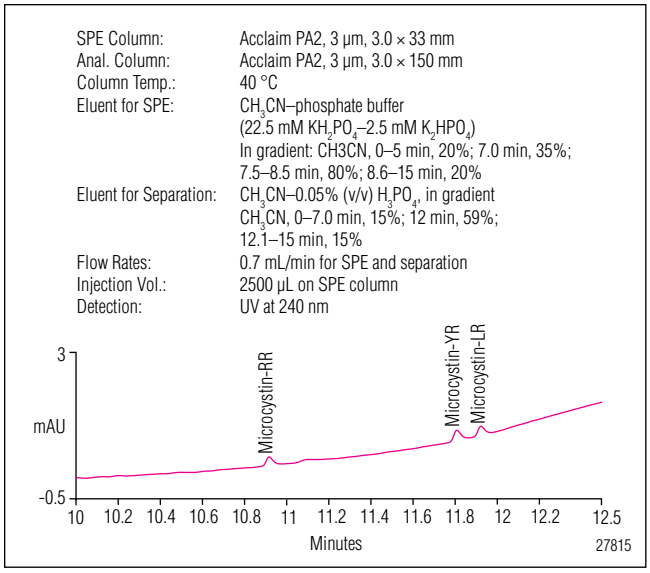


Figure 10. Chromatogram of a mixed solution with concentrations of 0.1 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Calibration linearity for microcystins-RR, -YR, and -LR was investigated by making three consecutive injections of a mixed standard prepared at five different concentrations. The external standard method was used to establish the calibration curve and to quantify these microcystins in samples. Excellent linearity was observed from 0.1 to 10 µg/L when plotting concentration versus peak area. Figure 10 shows a chromatogram of the three microcystins with concentrations of 0.1 µg/L each. Table 4 reports the data from the calibration as calculated by Chromeleon software.

Detection limits were calculated using the equation:

$$\text{Detection limit} = S_{t(n-1,\ 1-\alpha=0.99)}$$

Where S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, $t_{(n-1,\ 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom.

Method detection limits (MDL) were estimated using six consecutive injections of drinking water sample spiked with 0.5 µg/L of each microcystin standard to determine S (Table 4).

Table 4. Calibration Data and MDLs for Microcystins-RR, -YR, and -LR

Microcystin	Regression Equations	r (%)	Range of Standards µg/L	RSD for Calibration Curve	MDL* (µg/L)
RR	A = 0.0844 c - 0.0027	99.997	0.1–10	0.91	0.028
YR	A = 0.1054 c - 0.0022	99.994		1.25	0.028
LR	A = 0.0942 c + 0.0030	99.994		1.21	0.019

Note. * The single-sided Student's test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of six injections is multiplied by 4.03 to yield the MDL.

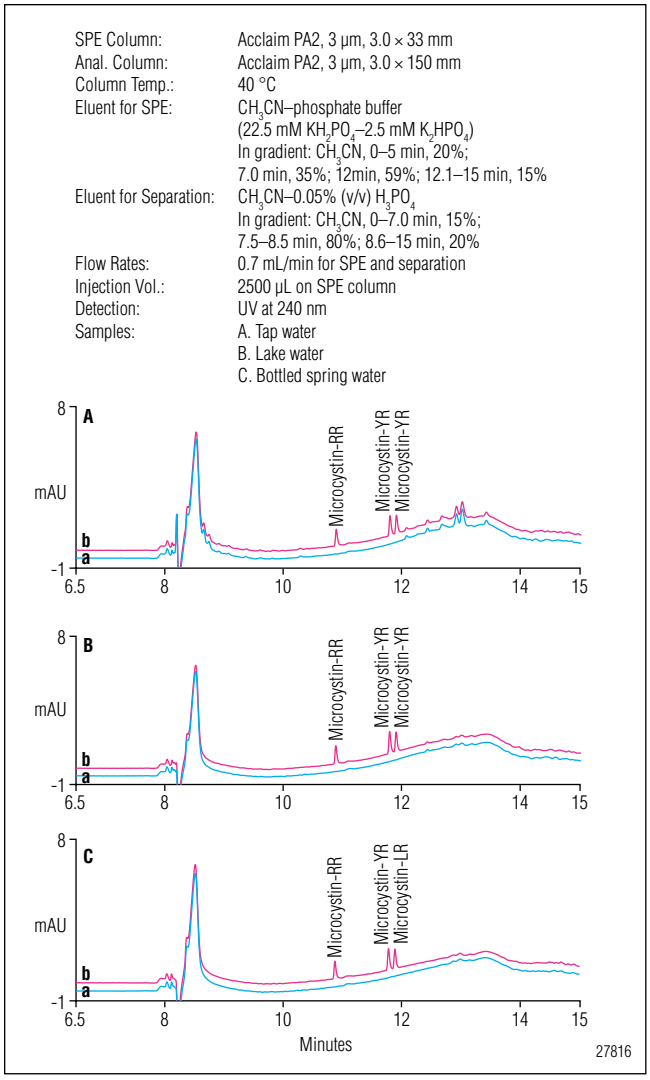


Figure 11. Overlay of chromatograms for a) water sample and b) the same sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using the target-cut on-line SPE method in Table 1.

Sample Analysis

Figure 11 shows chromatograms of tap water, lake water, and bottled spring water samples, as well as the same samples spiked with 0.5 µg/L of each microcystin standard. None of the three samples had detectable microcystins. Recoveries for each standard in all three samples ranged from 92 to 100%, thus indicating that the analysis method is accurate (Table 5).

CONCLUSION

This work describes a target-cut on-line SPE method that can fully recover low concentrations (< 1 µg/L) of three microcystins (-RR, -YR, and -LR) when added to three different water samples. These concentrations are less than the maximum concentrations recommended by WHO. This method is fully automated and easily configured on an UltiMate 3000 ×2 Dual HPLC system.

Table 5. Analysis Results of Microcystins-RR, -YR, and -LR in the Samples

Sample	Tap Water				Lake Water				Bottled Spring Water			
Microcystin	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)
RR	ND	0.50	0.48	96	ND	0.50	0.55	110	ND	0.50	0.49	98
YR	ND	0.50	0.46	92	ND	0.50	0.51	102	ND	0.50	0.48	96
LR	ND	0.50	0.48	96	ND	0.50	0.51	102	ND	0.50	0.49	98

Note: * ND = not detected

REFERENCES

1. *Cyanobacterial Toxins: Microcystin-LR in Drinking-Water;* Background Document for Development of WHO Guidelines for Drinking-Water Quality: World Health Organization, 2003.
2. Lee, H.S.; Jeong, C.K.; Lee, H.M.;Choi, S.J.; Do, K.S.; Kim, K.; Kim, Y.H. On-Line Trace Enrichment for the Simultaneous Determination of Microcystins in Aqueous Samples Using High-Performance Liquid Chromatography with Diode-Array Detection. *J. Chromatogr. A* **1999**, *848,* 179.
3. Aguete, E.C.; Gago-Martínez, A.; Leão, J.M.; Rodríguez-Vázquez, J.A.; Menàrd, C.; Lawrence, J.F. HPLC and HPCE Analysis of Microcystins RR, LR, and YR Present in Cyanobacteria and Water by Using Immunoaffinity Extraction. *Talanta,* **2003**, *59,* 697.
4. Kondo, F.; Matsumoto, H.; Yamada, S.; Tsuji, K.; Ueno, Y.; Harada, K. Immunoaffinity Purification Method for Detection and Quantification of Microcystins in Lake Water. *Toxicon.* **2000**, *38,* 813.
5. Tsutsumi, T.; Nagata, S.; Hasegawa, A.; Ueno, Y. Immunoaffinity Column as Clean-Up Tool for Determination of Trace Amounts of Microcystins in Tap Water. *Food Chem. Toxicol.* **2000,** *38,* 593.
6. Dionex Corporation, *Determination of Vitamin B_{12} in Beverages Using On-Line SPE Followed by HPLC with UV Detection.* Application Note 256, LPN 2574, 2010, Sunnyvale, CA.
7. Dionex Corporation, *Determination of Water- and Fat-Soluble Vitamins by HPLC.* Technical Note 89, LPN 2598, 2010, Sunnyvale, CA.
8. Sun, Q.L; Zhuang, S.Q. *Determination of Vitamin B_{12} Using UPLC;* Application Summary for Waters Corporation: Milford, MA, 2010.

Determination of Aniline and Nitroanilines in Environmental and Drinking Waters by On-Line SPE

INTRODUCTION

Aniline is an organic compound widely used in the polymer, rubber, pharmaceutical, and dye industries. Aniline and its derivatives (e.g., nitroanilines) are suspected carcinogens and are highly toxic to aquatic life. Therefore, it is necessary to establish sensitive, efficient, and simple methods for the determination of aniline and its derivatives in drinking and environmental waters.

The most common techniques for the determination of aniline and its derivatives in environmental and drinking waters are gas chromatography (GC)[1,2] and high-performance liquid chromatography (HPLC).[3–5] Capillary zone electrophoresis (CZE)[6] and spectrophotometric methods[7] have been reported as well. Because these compounds are thermolabile and polar, a derivatization step prior to GC analysis is often required, and most of these procedures are time consuming and complicated. Therefore, HPLC analysis is a good alternative to GC analysis because derivatization is not needed.

Normally, extraction processes for aniline and its derivatives from environmental and drinking water samples prior to HPLC analysis are required due to the limited sensitivity of direct injection for these samples, which have low concentrations of anilines. The typical extraction techniques are liquid-liquid extraction[8] and solid-phase extraction (SPE),[9] with SPE gaining favor either in the on-line or off-line mode. Compared to off-line SPE, on-line SPE offers the advantages of full automation, absence of operator influence, time savings, and strict process control.[10–12]

Here, an on-line SPE HPLC system is used to fulfill the simple and sensitive determination of aniline and four nitroanilines—*o*-nitroaniline, *m*-nitroaniline, *p*-nitroaniline, and *o,p*-dinitroaniline—in tap and pond water. The analyte structures are shown in Figure 1.

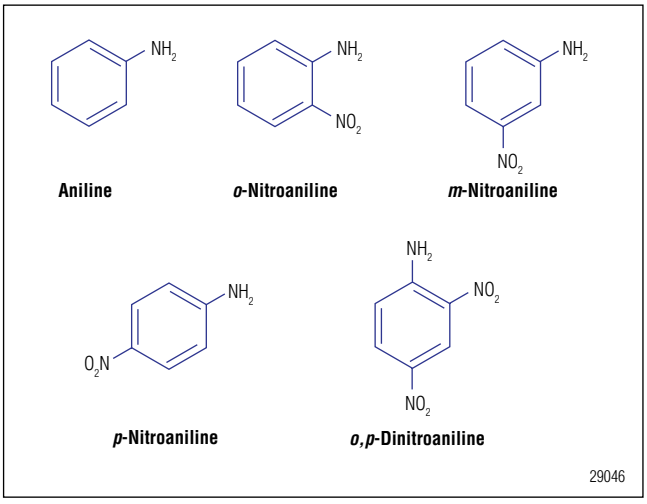


Figure 1. Structures of aniline and nitroanilines.

This on-line SPE HPLC system uses a Thermo Scientific Dionex SolEx™ HRP cartridge for the enrichment and a Thermo Scientific Acclaim™ 120 C18 column for the separation. The Thermo Scientific Dionex UltiMate™ 3000 Dual HPLC system provides an efficient platform to fulfill the on-line SPE and separation, and the system operates under automatic control of Thermo Scientific Dionex Chromeleon™ Chromatography Data System (CDS) software. The complete analysis requires only 15 min, and method detection limits (MDL) for these compounds are all less than 0.2 µg/L, which meets the requirement of United States Environmental Protection Agency (EPA) Method 8131 (GC method, MDLs range from 1.0 to 11 µg/L).[13]

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

- DGP-3600A pump with SRD-3600 solvent rack with degasser
- WPS-3000TSL semiprep autosampler with 2500 µL sample loop*
- TCC-3200 thermostatted column compartment equipped with one 2p–6p valve
- DAD-3000RS UV-vis detector

Chromeleon CDS software, Version 6.80, SR9

Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL autosampler can also be converted to the semipreparative version by installing the Semipreparative Conversion Kit (P/N 6822.2450) for large-volume injections for on-line SPE.

REAGENTS

Deionized water, Milli-Q® Gradient A10, Millipore Corporation

Methanol (CH_3OH), HPLC grade (Cat.# AC610090040) Fisher Chemical

Acetonitrile (CH_3CN), HPLC grade (Cat.#AC610010040) Fisher Chemical

Phosphoric acid (H_3PO_4), analytical grade, SCRC, China

Dipotassium hydrogen phosphate (K_2HPO_4), analytical grade, SCRC, China

STANDARDS

Aniline, analytical standard, Fluka

o-Nitroaniline, 98%, Aldrich

m-Nitroaniline, 98%, Aldrich

p-Nitroaniline, 99%, Aldrich

o,p-Dinitroaniline, 98%, Aldrich

Accurately weigh ~50 mg of a standard and dilute in a 50 mL volumetric flask with methanol. The concentration of the standard is 1000 mg/L (stock standard solution 1). Pipet 50 µL of stock standard 1 into a 50 mL volumetric flask and dilute to the mark with methanol. The concentration of the standard is 1000 µg/L (stock standard solution 2). Prepare four working standard solutions for the calibration with 1, 10, 50, and 100 µg/mL concentrations by adding the proper amount of stock standard solution 2 and making dilutions with methanol.

Note: The concentration of the stock standard solution 1 is not 1000 mg/L because of the < 100% purity for the standards. So, the actual volume taken for the preparation of stock standard solution 2 must be, for example, 51 µL for *o*-nitroaniline with 98% purity.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. Pond water samples were collected at Zhangjiang High-Tech Park located in the Pudong District of Shanghai, China.

These samples were filtered through a 0.45 µm membrane (Millex®-HN) prior to injection.

CHROMATOGRAPHIC CONDITIONS

SPE Cartridge:	Dionex SolEx HRP Cartridge, 12–14 µm, 2.1 × 20 mm (P/N 074400) Use V-3 Holder (P/N 074403)*
Analytical Column:	Acclaim 120 C18, 3 µm, 4.6 × 150 mm (P/N 059133)
Mobile Phase:	For on-line SPE: A: 10 mM phosphate buffer (pH 6.5) B: CH_3OH In gradient (Table 1)
For Separation:	A: H_2O B: CH_3CN In gradient (Table 1)
Valve-Switching:	Table 1
Flow Rate:	2.0 and 0.5 mL/min for on-line SPE 1.0 mL/min for separation
Inj. Volume:	5000 µL on the on-line SPE cartridge*
Column Temp.:	30 °C
UV Detection:	Absorbance at 230 nm

*Two consecutive injections of 2500 µL using the User Defined Program (UDP) injection mode controlled by Chromeleon CDS software

Table 1. Elution and Valve Switching for On-Line SPE and Separation							
Time (min)	Left Pump (for On-Line SPE)			Right Pump (for Separation)			Valve Switching
	Flow Rate (mL/min)	Solvent A 10 mM Phosphate Buffer (pH 6.5) (%)	Solvent B Methanol (%)	Flow Rate (mL/min)	Solvent A H_2O (%)	Solvent B Acetonitrile (%)	
0	2	90	10	1.0	70	30	1–2
2		90	10		70	30	6–1
3	0.5	30	70		—	—	1–2
10		30	70		45	55	—
11	2	90	10		30	70	—
13	—	—	—		30	70	—
15	—	—	—		70	30	—

RESULTS AND DISCUSSION

Selection of SPE Column

Considering the tolerance to large-volume injection of water samples, and the relative ease or difficulty of retention/elution of aniline and nitroanilines by SPE, two types of silica-based stationary phases (the Acclaim Mixed-Mode WCX-1 Guard and the Acclaim PA2 Guard) and two types of polymeric sorbents (the Dionex SolEx HRP Cartridge and the Thermo Scientific Dionex IonPac™ NG1 Guard) were evaluated as SPE columns. This evaluation followed the typical on-line SPE flow schematic shown in Figure 2. The chromatograms of aniline, *p*-nitroaniline, *m*-nitroaniline, *o*-nitroaniline, and *o,p*-dinitroaniline are shown in Figure 3.

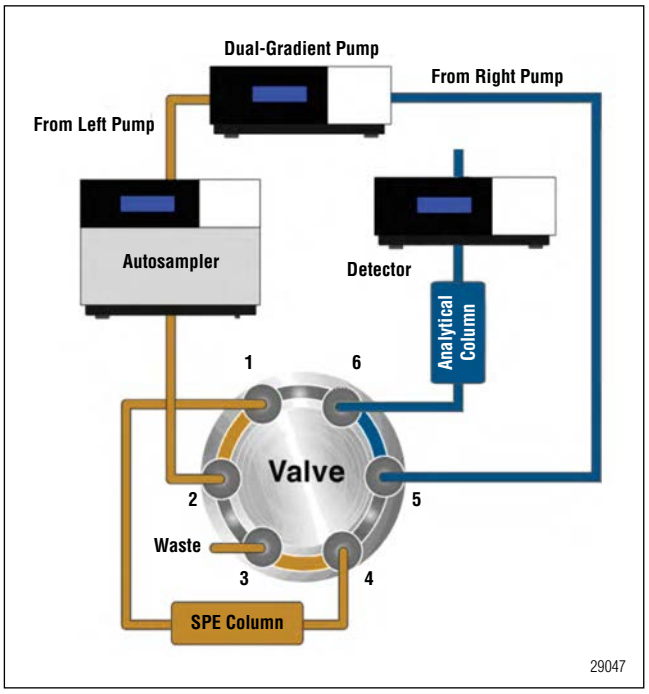


Figure 2. Flow schematic of on-line SPE.

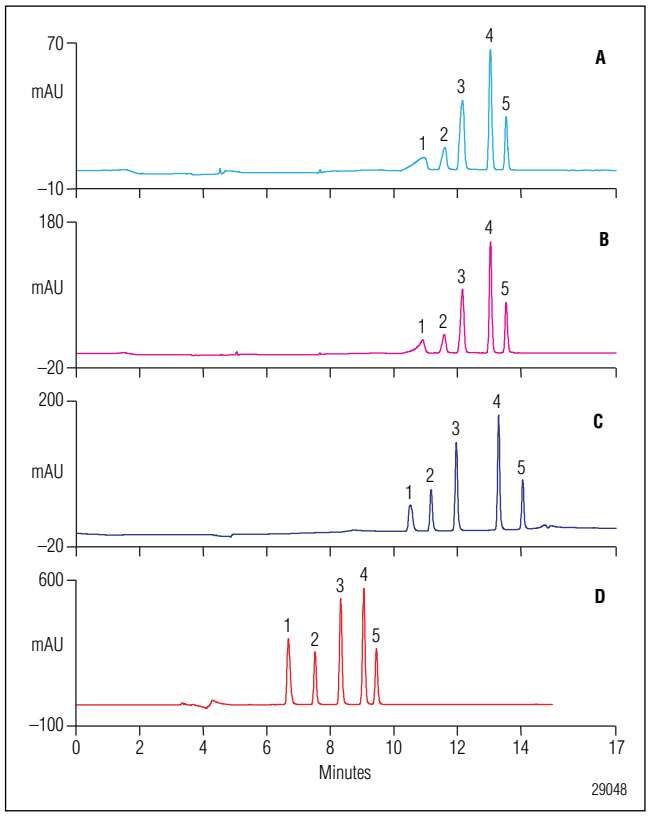


Figure 3. Chromatograms of aniline and nitroanilines (100 µg/L each) using different on-line SPE stationary phases (A) Dionex IonPac NG1 Guard, (B) Acclaim PA2 Guard, (C) Acclaim Mixed-Mode WCX-1 Guard, and (D) Dionex SolEx HRP Cartridge. See Table 2 for conditions.

As shown in Figure 3 A and B, severe band spreading for aniline (peak 1) was observed when using the Dionex IonPac NG1 Guard and the Acclaim PolarAdvantage II (PA2) Guard. This can be attributed to aniline's weak retention on these stationary phases, even using water as the mobile phase. During its enrichment in on-line SPE, aniline diffused on these SPE columns, resulting in severe band spreading on the analytical column even if using a reversed flush with organic mobile phase. Meanwhile, the weak retention of aniline on these stationary phases may result in its loss during the course of enrichment. Poor extraction efficiency, low to about 50%, was estimated by comparing the peak area obtained with on-line SPE to that obtained without SPE.

Although the peak shape improved using the Acclaim Mixed-Mode WCX-1 Guard (Figure 3C), a stationary phase that combines cation-exchange and RP properties, there was not a significant improvement in extraction efficiency. The Dionex SolEx HRP cartridge, packed with a divinylbenzene polymer with a hydrophilic bonded layer,[14] was thus selected based on its excellent retention properties of the analytes with different polarities. As shown in Figure 3D, good peak shape of aniline was observed; and the estimated extraction efficiency was > 95%. The peak shape and efficiency of *p*-nitroaniline were also improved using the Dionex SolEx HRP cartridge.

Table 2. Chromatographic Conditions for Figure 3

On-Line SPE Stationary Phase	**Dionex IonPac NG1 Guard (10 µm, 4 × 35 mm) and Acclaim PA2 Guard (5 µm, 4.6 × 10 mm)**		**Acclaim Mixed-Mode WCX-1 Guard (5 µm, 4.6 × 10 mm) and Dionex SolEx HRP Cartridge (12–14 µm, 2.1 × 20 mm)**	
Analytical Column		Acclaim 120 C18 (3 µm, 3.0 × 150 mm)	Acclaim 120 C18 (3 µm, 4.6 × 150 mm)	
Mobile Phase	For on- line SPE	50 mM NH_4 Ac-HAc (pH 4.6)/CH_3OH Gradient: CH_3OH, 0~2 min, 1%; 6~11 min, 70%; 11~17 min, 1.0%	10 mM phosphate buffer (pH 6.5/ CH_3OH Gradient: CH_3OH, 0~3 min, 0%; 7~14.5 min, 70%; 15.1~18 min, 0%	10 mM phosphate buffer (pH 6.5/ CH_3OH Gradient: CH_3OH, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%
	For separation	H_2O/CH_3OH Gradient: CH_3OH, 0~4 min, 5%; 10~17 min, 60%	H_2O/CH_3OH Gradient: CH_3OH, 0 min,10%; 2.5 min, 10%; 13~18 min, 70%; 23 min, 10%	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 30%; 10 min, 55%; 11~13 min, 70%; 15 min, 30%
Flow Rate	For on- line SPE	0~2 min, 1.5 mL/min; 2.1~15 min, 0.5 mL/min; 17 min, 1.5 mL/min	0~3 min, 0.5 mL/min; 7~18 min, 1.0 mL/min; 18.1 min, 0.5 mL/min	0~2 min, 2.0 mL/min; 3~10 min, 0.5 mL/min; 11~15 min, 2 mL/min
	For separation	0.5 mL/min	1.0 mL/min	
Inj. Volume	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)			
Column Temp.		30 °C	30 °C	
UV Detection	285 nm		230 nm	
Sample	Tap water spiked with anilines standards (100 µg/L each)			
Peaks	1) Aniline, 2) *p*-nitroaniline, 3) *m*-nitroaniline, 4) *o*-nitroaniline, 5) *o,p*-dinitroaniline			

Effect of Mobile Phase for On-Line SPE

The effect of mobile phase on on-line SPE was investigated. As shown in Figure 4, when using either water or phosphate buffer mobile phase containing 10% methanol for sample enrichment on the Dionex SolEx HRP cartridge, no difference was observed for the *p*-nitroaniline, *m*-nitroaniline, *o*-nitroaniline, and *o,p*-dinitroaniline peaks on the Acclaim 120 C18 analytical column. A tailing aniline peak was observed when using water; however, the peak became sharp and symmetrical when using phosphate buffer. So, a 10 mM phosphate buffer (pH 6.5) mobile phase was used for on-line SPE.

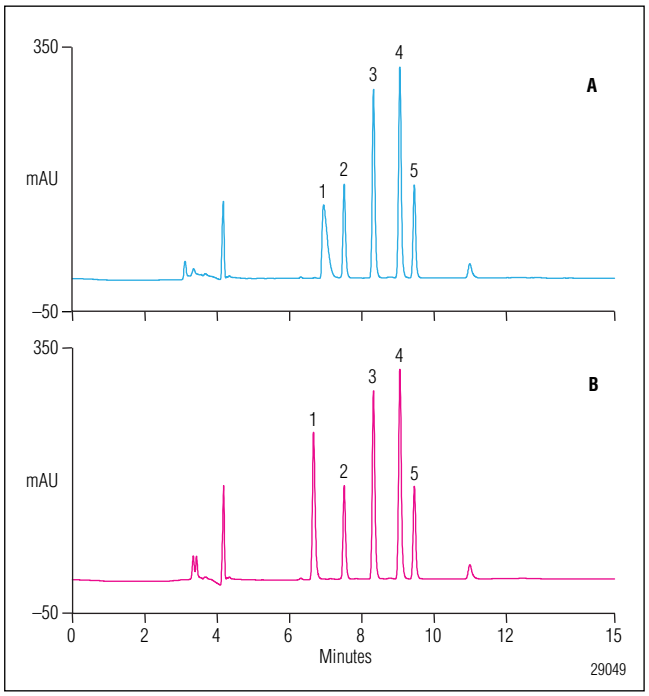


Figure 4. Chromatograms of aniline, p-*nitroaniline,* m-*nitroaniline,* o-*nitroaniline, and* o,p-*dinitroaniline using (A) H_2O/CH_3OH and (B) 10 mM phosphate buffer (pH 6.5)/CH_3OH mobile phases for on-line SPE. See Table 3 for conditions.*

Table 3. Chromatographic Conditions for Figure 4

On-Line SPE Cartridge	Dionex SolEx HRP		
Analytical Column	Acclaim 120 C18		
Mobile Phase	For on-line SPE	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%	10 mM phosphate buffer (pH 6.5/CH_3OH Gradient: CH_3OH, 0~2 min, 10%; 3~10 min, 70%; 11~15 min, 10%
	For separation	H_2O/CH_3CN Gradient: CH_3CN, 0~2 min, 30%; 10 min, 55%; 11~13 min, 70%; 15 min, 30%	
Flow Rate	For on-line SPE	0~2 min, 2.0 mL/min; 3~10 min, 0.5 mL/min; 11~15 min, 2 mL/min	
	For separation	1.0 mL/min	
Inj. Volume	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)		
Column Temp.	30 °C		
UV Detection	230 nm		
Sample	Tap water spiked with aniline standards (100 µg/L for each)		
Peaks	1) Aniline, 2) *p*-nitroaniline, 3) *m*-nitroaniline, 4) *o*-nitroaniline, 5) *o,p*-dinitroaniline		

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making five consecutive 5000 µL injections of mixed standards with a 10 µg/L concentration of each. Retention time and peak area reproducibilities are summarized in Table 4 and show good precision.

Table 4. Reproducibility for Peak Retention Time and Area

Analyte	Retention Time RSD	Peak Area RSD	Concentration of Standard (µg/L)
Aniline	0.022	0.300	10
p-Nitroaniline	0.031	0.183	
m-Nitroaniline	0.028	0.051	
o-Nitroaniline	0.026	0.123	
o,p-Dinitroaniline	0.039	0.160	

Table 5. Method Linearity Data and Method Detection Limits (MDL)					
Analyte	**Regression Equation**	**r**	**Range of Standards (µg/L)**	**MDL, µg/L**	
				Current Data	**Data Reported in EPA Method 8131**
Aniline	$A = 0.3686\ c - 0.1530$	0.9999	1–100	0.2	2.3
p-Nitroaniline	$A = 0.2290\ c - 0.0830$	1.0000		0.2	1.0
m-Nitroaniline	$A = 0.4770\ c + 0.0302$	1.0000		0.1	3.3
o-Nitroaniline	$A = 0.5286\ c - 0.0194$	1.0000		0.1	11.0
o,p-Dinitroaniline	$A = 0.2432\ c - 0.0252$	1.0000		0.2	8.9

Calibration linearity for aniline and nitroanilines was investigated by making three consecutive injections of a mixed standard prepared at four different concentrations. The external standard method was used to establish the calibration curve and to quantify these compounds in samples. Excellent linearity was observed from 1 to 100 µg/L when plotting concentration versus peak area, and the correlation coefficient was ≥ 0.9999 for each plot. The MDLs of each compound for UV detection were calculated using S/N = 3 (signal to noise), and all were ≤ 0.2 µg/L. Table 5 summarizes the method linearity and MDL data, which show excellent method linearity and sensitivity, with detection limits well below those defined in the EPA method.[13]

Sample Analysis

Chromatograms of tap and pond water samples, as well as the same samples spiked with aniline and related standards (1.0 µg/L each and 10 µg/L each, respectively), are shown in Figures 5 and 6, and the related data are summarized in Table 6. Recoveries for each standard in both sample sets ranged from 98 to 108% for the 10 µg/L standard spiked samples, and ranged from 93 to 147% for the 1 µg/L standard spiked samples. None of the samples had detectable aniline or nitroanilines.

The real samples may sometimes yield a false positive for aniline and/or one of the nitroanilines. An efficient and convenient way to determine if the peak is a target analyte is to compare the peak's UV spectrum to that of standards. Therefore, using a photodiode array detector for this analysis will help reduce the possibility of false positives.

When the pond water sample was analyzed, a small peak with retention time near that of aniline was found and labeled as aniline with a concentration 0.3 µg/L, similar to the estimated MDL of aniline (0.2 µg/L).

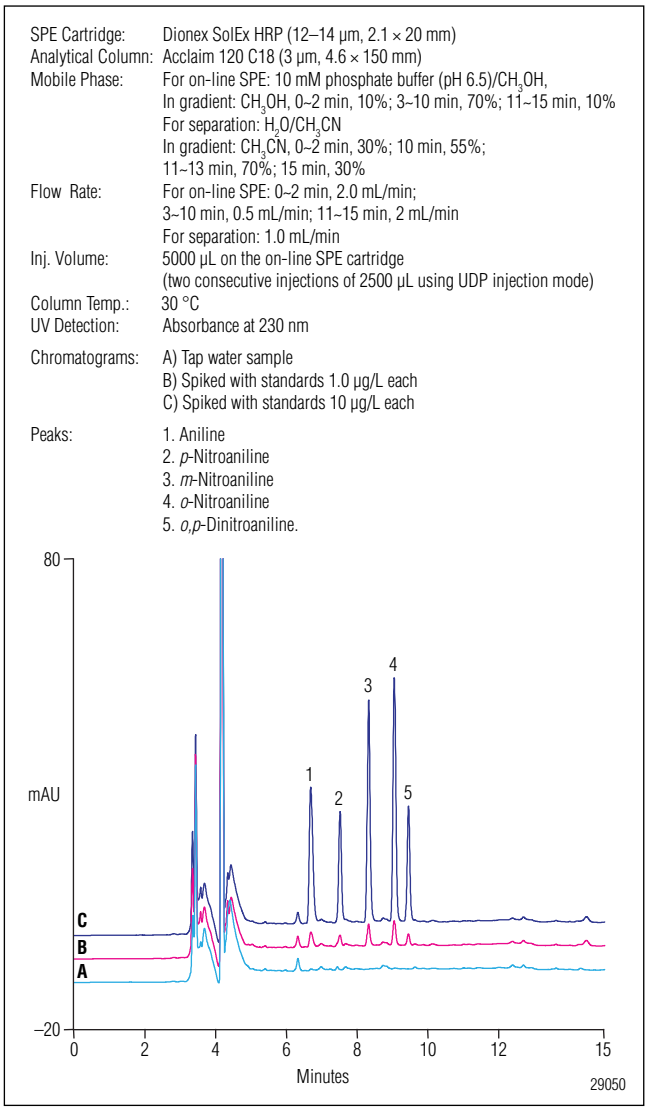


Figure 5. Chromatograms of (A) tap water sample, (B) the same sample spiked with 1.0 µg/L aniline and nitroanilines standard, and (C) spiked with 10 µg/L.

Comparison of the UV spectra shown in Figure 7 revealed that the peak was not aniline. The spike-recovery of aniline at 1.0 µg/L level in pond water, 147%, also suggests that there is interference.

SPE Cartridge:	Dionex SolEx HRP (12–14 µm, 2.1 × 20 mm)
Analytical Column:	Acclaim 120 C18 (3 µm, 4.6 × 150 mm)
Mobile Phase:	For on-line SPE: 10 mM phosphate buffer (pH 6.5)/CH_3OH, In gradient: CH_3OH, 0–2 min, 10%; 3–10 min, 70%; 11–15 min, 10% For separation: H_2O/CH_3CN In gradient: CH_3CN, 0–2 min, 30%; 10 min, 55%; 11–13 min, 70%; 15 min, 30%
Flow Rate:	For on-line SPE: 0–2 min, 2.0 mL/min; 3–10 min, 0.5 mL/min; 11–15 min, 2 mL/min For separation: 1.0 mL/min
Inj. Volume:	5000 µL on the on-line SPE cartridge (two consecutive injections of 2500 µL using UDP injection mode)
Column Temp.:	30 °C
UV Detection:	Absorbance at 230 nm
Chromatograms:	A) Pond water sample B) Spiked with standards 1.0 µg/L each C) Spiked with standards 10 µg/L each
Peaks:	1. Aniline 2. *p*-Nitroaniline 3. *m*-Nitroaniline 4. *o*-Nitroaniline 5. *o,p*-Dinitroaniline.

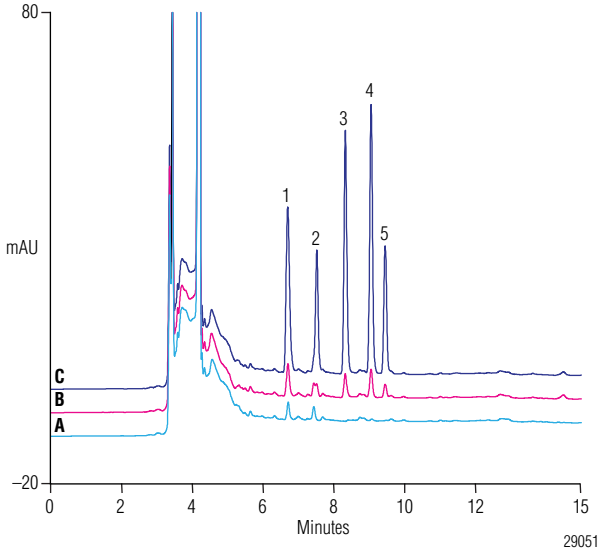


Figure 6. Chromatograms of (A) pond water sample, (B) the same sample spiked with 1.0 µg/L aniline and nitroanilines standard, and (C) spiked with 10 µg/L.

In addition, as shown in Figures 5 and 6, interference with retention time near that of *p*-nitroaniline (peak 2) was found. Although it was not labeled as *p*-nitroaniline, its presence affects the spike-recoveries of *p*-nitroaniline at the 1.0 µg/L level in both pond and tap waters samples (140% and 127%, respectively). This demonstrates that the limits of detection are often set by matrix interference instead of instrumental uncertainties in the analysis of environmental samples.

Table 6. Analysis Results of Anilines in Water Samples

Sample	**Pond Water**				
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Recovery (%)**	**Added (µg/L)**	**Recovery (%)**
Aniline	ND	1.0	147	10	104
p-Nitroaniline	ND		140		101
m-Nitroaniline	ND		94.2		99.7
o-Nitroaniline	ND		105		101
o,p-Dinitroaniline	ND		101		98.8
Sample	**Tap Water**				
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Recovery (%)**	**Added (µg/L)**	**Recovery (%)**
Aniline	ND	1.0	103	10	100
p-Nitroaniline	ND		127		108
m-Nitroaniline	ND		93.1		100
o-Nitroaniline	ND		109		102
o,p-Dinitroaniline	ND		103		100

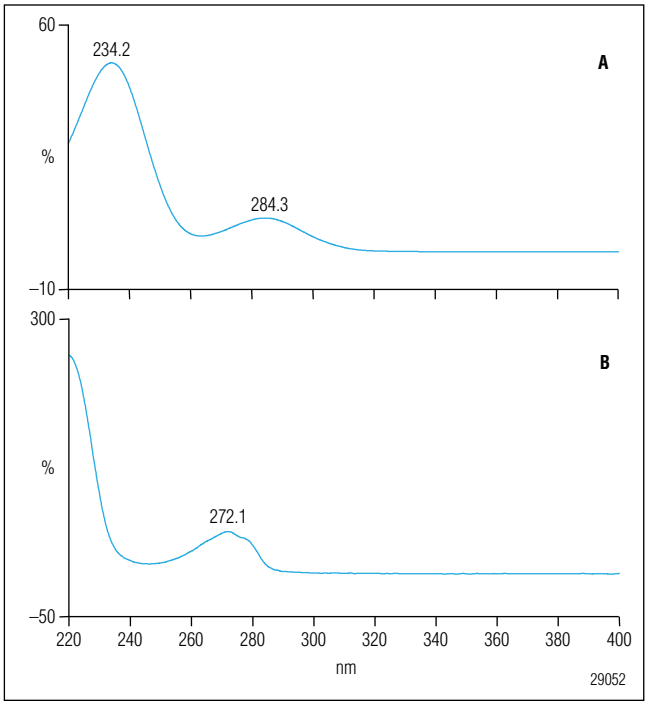


Figure 7. UV spectra of (A) aniline standard and (B) the putative aniline peak in a pond water sample.

CONCLUSION

This work describes an on-line SPE system using the Dionex SolEx HRP cartridge to enrich aniline and nitroanilines followed by HPLC with UV detection. The enrichment of aniline and nitroanilines in tap and pond water is sufficient, and baseline separation on the Acclaim 120 C18 column is achieved. The Dionex UltiMate 3000 Dual HPLC system provides an efficient platform to fulfill this on-line SPE, and the system operates under automatic control of Chromeleon CDS software. The determination of aniline and nitroanilines in tap and pond water is simple, rapid, and sensitive, and meets the MDL requirement of the EPA Method 8131. Although this work cannot be a substitute for the EPA method, it does demonstrate that these analytes can be determined by on-line SPE-HPLC while meeting the performance criteria of the EPA method.

REFERENCES

1. Brede, C.; Skjevrak, I.; Herikstad, H. Determination of Primary Aromatic Amines in Water Food Simulant Using Solid-Phase Analytical Derivatization Followed by Gas Chromatography Coupled with Mass Spectrometry *J. Chromatogr., A* **2003,** *983,* 35.
2. Chiang, J. S.; Huang, S. D. Simultaneous Derivatization and Extraction of Anilines in Waste Water with Dispersive Liquid–Liquid Microextraction Followed by Gas Chromatography–Mass Spectrometric Detection *Talanta* **2008,** *75,* 70.
3. Jen, J. F.; Chang, C. T.; Yang, T. C. On-Line Microdialysis–High-Performance Liquid Chromatographic Determination of Aniline and 2-Chloroaniline in Polymer Industrial Wastewater *J. Chromatogr., A* **2001,** *930,* 119.
4. Sarafraz-Yazdi, A.; Es'haghi, Z. Liquid–Liquid–Liquid Phase Microextraction of Aromatic Amines in Water Using Crown Ethers by High-Performance Liquid Chromatography with Monolithic Column *Talanta* **2005,** *66,* 664.
5. Zhao, L. M.; Zhu, L. Y.; Lee, H. K. Analysis of Aromatic Amines in Water Samples by Liquid–Liquid–Liquid Microextraction with Hollow Fibers and High-Performance Liquid Chromatography *J. Chromatogr., A* **2002,** *963,* 239.
6. Li, J.; Yuan, Z. B. Separation of Aniline Derivatives by Micellar Electrokinetic Capillary Chromatography *Chin. Chem. Lett.* **2004,** *15,* 947.
7. Gu, X. X.; Li, C. Y.; Qi, X.; Zhou, T. Z. Determination of Trace Aniline in Water by a Spectrophotometric Method After Preconcentration on an Organic Solvent-Soluble Membrane Filter *Anal. Lett.* **1997,** *30,* 259.
8. Wu, X. H.; Lei, Z. G.; Li, Q. S.; Zhu J. Q.; Chen, B. H. Liquid-Liquid Extraction of Low-Concentration Aniline from Aqueous Solutions with Salts *Ind. Eng. Chem. Res.* **2010,** *49,* 2581.
9. Patsias, J.; Papadopoulou-Mourkidou, E. Development of an Automated On-Line Solid-Phase Extraction–High Performance Liquid Chromatographic Method for the Analysis of Aniline, Phenol, Caffeine and Various Selected Substituted Aniline and Phenol Compounds in Aqueous Matrices *J. Chromatogr., A* **2000,** *904,* 171.
10. Thermo Fisher Scientific. *Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection.* Dionex Application Note 191, LPN 1949, 2007, Sunnyvale, CA.
11. Thermo Fisher Scientific. *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection.* Dionex Application Note 196, LPN 1998, 2008, Sunnyvale, CA.
12. Thermo Fisher Scientific. *Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections.* Dionex Application Note 213, LPN 2126, 2009, Sunnyvale, CA.
13. *niline and Selected Derivatives by Gas Chromatography;* U.S. EPA Method 8131, U.S. Environmental Protection Agency: Cincinnati, OH, 1996.
14. Thermo Fisher Scientific. *SolEx HRP On-Line Sample SPE Concentration Cartridges.* LPN 2565, 2010, Sunnyvale, CA.

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																														
	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												√	√						√	√	√					√	√			

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	Hydrophobicity
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										

AccuStandard is a trademark of AccuStandard Inc.
Acrodisc is a registered trademark of Pall Corporation.
Anke is a registered trademark of Anting Scientific Instrumental Factory.
Anotop is a trademark of Whatman.
BD and Bacto are trademarks of Becton, Dickinson, and Company.
Bigelow is a registered trademark of R.C. Bigelow, Incorporated.
Diet Rite is a registered trademarks of Royal Crown Company, Inc.
Enfamil is a registered trademark of Bristol-Myers Squibb Co., Mead Johnson Nutritionals.
Good Start and Alsoy are registered trademarks of Nestle USA, Inc., Nutrition Division.
IKA is a registered trademark of IKA Works.
Kudos is a registered trademark of Kudos Ultrasonic Instrumental Company.
Lipton is a registered trademark of the Unilever Group of Companies.
MICRO DIST is a trademark of Lachat Instruments, a Hach Company.
Milli-Q and Millex are registered trademarks of Millipore Corporation.
Minute Maid is a registered trademark of the Coca-Cola Company.
PEEK is a trademark of Victrex PLC.
Phenomenex is a trademark of Phenomenex, Inc.
Pyrex is a registered trademark of Corning Glass Works.
Similac and Isomil are registered trademarks of Abbott Laboratories, Ross Products Division.
Splenda is a registered trademark of McNeil-PPC, Inc.
Strata is a trademark of Shine Technology.
Sunett is a registered trademark of Nutrinova.
Supelcosil is a trademark of Sigma-Aldrich Co.
SUPELCOWAX 10 is a trademark of Supelco, Inc.
Teflon is a registered trademark of E. I. du Pont de Nemours.
TurboVap is a registered trademark of Caliper Life Sciences.
ULTREX is a registered trademark of J.T. Baker.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Dionex Products: 1228 Titan Way, PO Box 3603, Sunnyvale, CA 94088-3603, (408) 737-0700
North America: U.S./Canada (847) 295-7500
South America: Brazil (55) 11 3731 5140
Europe: Austria (43) 616 51 25, Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90, France (33) 1 39 30 01 10, Germany (49) 61125 991 0
Ireland (353) 644 0064, Italy (39) 02 51 62 1267, Sweden (46) 8 473 3380,
Switzerland (41) 62 205 9966, United Kingdom (44) 1276 691722
Asia Pacific: Australia (61) 2 9420 5233, China (852) 2428 3282, India (91) 22 2764 2735,
Japan (81) 6885 1213, Korea (82) 2 2653 2580, Singapore (65) 6289 1190,
Taiwan (886) 2 875 6655

www.thermoscientific.com/dionex

LPN 3088 02/12

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC