

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

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## 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.<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>4</sup>

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.<sup>5</sup> 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.<sup>2</sup>



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.<sup>6,7</sup>

U.S. EPA Method 300.0 (Part A) describes the use of a Thermo Scientific™ 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 column, 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 Dionex IonPac AS18 column—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 Thermo Scientific™ Dionex™ ICS-2000 Reagent-Free™ Ion Chromatography (RFIC™) system\* was used in this work. The Dionex ICS-2000 system is an integrated ion chromatograph and consists of:

- Eluent Generator
- Column Heater
- Pump Degas
- Thermo Scientific Dionex EGC-KOH Cartridge
- Thermo Scientific Dionex CR-ATC Continuously Regenerating Anion Trap Column
- Thermo Scientific Dionex AS50 Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Workstation 6.5

\*Equivalent or improved results can be achieved using Thermo Scientific Dionex ICS-2100 or Thermo Scientific Dionex ICS-5000+ system.

## 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
- Fluoride standard 1000 mg/L, 100 mL
- Chloride standard 1000 mg/L, 100 mL
- Sulfate standard 1000 mg/L, 100 mL
- Bromide standard 1000 mg/L, 100 mL

Conditions	
Columns:	Dionex IonPac AS18 Analytical, 4 × 250 mm Dionex IonPac AG18 Guard, 4 × 50 mm
Eluent:	22–40 mM KOH from 7–8 min
Eluent Source:	Dionex ICS-2000 system with Dionex CR-ATC column
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection:	25 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ ULTRA suppressor, 4mm 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 Thermo Scientific 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.

Table 1. Preparation of stock standard solutions.

Anion	Compound	Amount (g)
Fluoride	Sodium fluoride (NaF)	2.210
Chloride	Sodium chloride (NaCl)	1.648
Nitrite	Sodium nitrite (NaNO <sub>2</sub> -N)	4.926
Bromide	Sodium bromide (NaBr)	1.288
Nitrate	Sodium nitrate (NaNO <sub>3</sub> -N)	6.068
Phosphate	Potassium phosphate, monobasic (KH <sub>2</sub> PO <sub>4</sub> -P)	4.394
Sulfate	Sodium sulfate (Na <sub>2</sub> SO <sub>4</sub> )	1.479

## 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.

Table 2. Concentration of MDLs and QCS standards.

Analyte	MDL Calculation Standard ( $\mu\text{g/L}$ )	QCS Used for RSD Calculation ( $\text{mg/L}$ )
Fluoride	10	2
Chloride	10	20
Nitrite-N	6.1 (20 as $\text{NO}_2$ )	2
Bromide	25	2
Nitrate-N	6.8 (30 as $\text{NO}_3$ )	10
Phosphate-P	23 (70 as $\text{PO}_4$ )	2
Sulfate	20	60

## Sample Preparation

All samples were filtered through an appropriate 0.45  $\mu\text{m}$  syringe filter, discarding the first 300  $\mu\text{L}$  of the effluent. The only exception was the domestic wastewater, which was filtered through a 0.20  $\mu\text{m}$  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.<sup>8,9</sup>

## Results and Discussion

Although U.S. EPA Method 300.0 (Part A) specifies the use of a Dionex 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 Dionex IonPac AS18 column, in place of the Dionex IonPac AS4A column, as well as the use of a different eluent, such as hydroxide in place of carbonate/bicarbonate, as in the case of the Dionex IonPac AS18 hydroxide-selective column.

The Dionex IonPac AS18 column is a latex agglomerated column with a 7.5  $\mu\text{m}$  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 Dionex IonPac AS4A column.

The Dionex IonPac AS18 column has a significantly higher capacity (285  $\mu\text{eq/column}$  compared to 20  $\mu\text{eq/column}$  for the Dionex IonPac AS4A column). 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 Dionex IonPac AS18 and Dionex IonPac 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.<sup>10</sup> This greater retention is evident from the separation using the Dionex IonPac 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 Dionex IonPac AS4A column (Figure 1A). Additionally, sulfate elutes between bromide and nitrate on the Dionex IonPac AS18 column, whereas on the Dionex IonPac AS4A column sulfate is the last eluting peak, which is typical for a column using carbonate eluents. Finally, phosphate elutes after sulfate on the Dionex IonPac AS18 column when using the hydroxide eluent conditions in Figure 1B. The higher eluent pH, compared to the Dionex IonPac AS4A column, results in a greater charge on the polyprotic acid species, therefore increasing its retention.

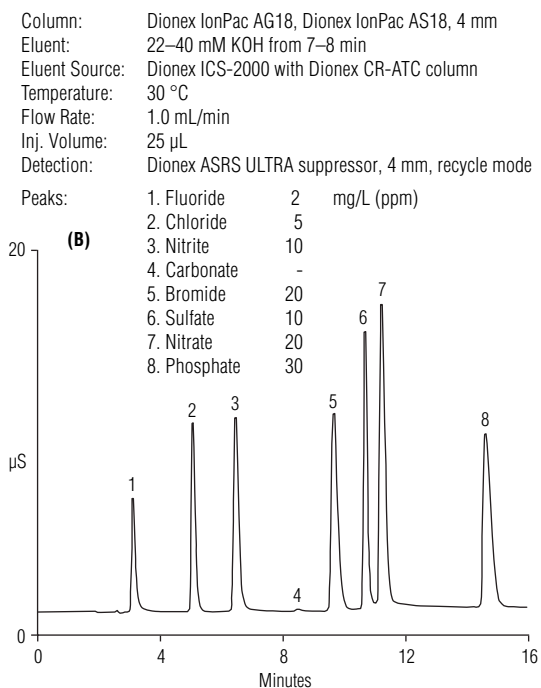
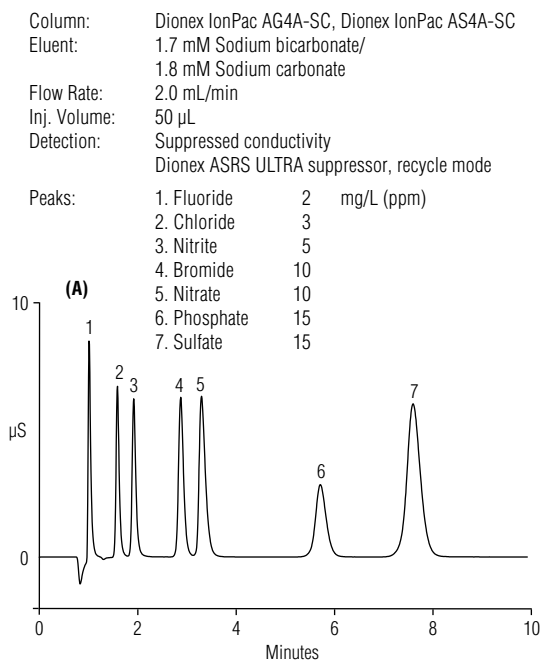


Figure 1. Separation of common inorganic anions using the Dionex IonPac AS4A-SC column (A) and the Dionex IonPac AS18 column (B).

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 preparing hydroxide eluents to minimize contamination by carbonate, which can cause a significant baseline shift during a hydroxide gradient and variation in retention times.<sup>11</sup> 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  $\text{CO}_2$  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 Dionex CR-ATC anion trap column—for removal of carbonate and other anionic contaminants from the source water—is strongly recommended when using hydroxide eluents. The Dionex CR-ATC column minimizes baseline shifts, improves retention time stability, and improves detection limits.<sup>12</sup> In addition, the Dionex CR-ATC column offers several advantages over conventional anion trap columns. The Dionex CR-ATC column eliminates the need for off-line chemical regeneration of the trap, allowing continuous operation and fast IC system startup after shutdown.

Table 3. Linearity, MDLs, and retention time and peak area precisions obtained using the Dionex IonPac AS18 column<sup>a</sup>.

Analyte	Range (mg/L)	Linearity ( $r^2$ )	Calculated MDL <sup>b</sup> ( $\mu\text{g/L}$ )	Retention Time Precision (RSD <sup>c</sup> ) %	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 $\text{NO}_2$ )	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 $\text{NO}_3$ )	<0.05	0.19
Phosphate-P	0.1–100	0.9999	5.3 (16.3 as $\text{PO}_4$ )	<0.05	0.63
Sulfate	0.2–200	0.9998	5.1	<0.05	0.19

<sup>a</sup> Dionex ICS-2000 Reagent-Free IC System

<sup>b</sup>  $\text{MDL} = \sigma t_{5,99}$  where  $t_{5,99} = 3.14$  for  $n = 7$

<sup>c</sup> RSD = Relative Standard Deviation,  $n = 7$

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 Dionex IonPac AS18 column 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 Dionex IonPac AS18 column using electrolytic generation of potassium hydroxide with an Dionex 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 Dionex ICS-2000 system.

The data in Table 3 represent the typical results expected when using the Dionex IonPac AS18 column for routine analyses of common inorganic anions with U.S. EPA Method 300.0. These results demonstrate that the Dionex IonPac AS18 column 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 Dionex IonPac AS4A column 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 Dionex CR-ATC column requires no off-line regeneration using chemical reagents, and the Dionex ASRS suppressor electrolytically generates the hydronium ion used for suppression.

The performance of the Dionex IonPac AS18 column 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 Dionex IonPac AS18 column for common inorganic anions spiked into drinking water, raw (unfinished) drinking water, and other environmental water matrices.

Table 4. Anion recoveries for spiked water samples obtained using the Dionex IonPac AS18 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	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
Anion	Domestic Wastewater		Industrial Wastewater		Well Water	
	Amount Added (mg/L)	Amount Added (mg/L)	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

Column: Dionex IonPac AG18, Dionex IonPac AS18, 4 mm  
 Eluent: 22–40 mM KOH from 7–8 min  
 Eluent Source: Dionex ICS-2000 system with Dionex CR-ATC column  
 Temperature: 30 °C  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 25 µL  
 Detection: Dionex ASRS ULTRA suppressor, 4 mm, recycle mode

Peaks: 1. Fluoride 0.07 mg/L (ppm)  
 2. Chloride 45.3  
 3. Nitrite-N 0.02  
 4. Carbonate -  
 5. Bromide 0.03  
 6. Sulfate 58.7  
 7. Nitrate-N 0.65  
 8. Phosphate-P 0.47

Column: Dionex IonPac AG18, Dionex IonPac AS18, 4 mm  
 Eluent: 22–40 mM KOH from 7–8 min  
 Eluent Source: Dionex ICS-2000 system with Dionex CR-ATC column  
 Temperature: 30 °C  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 25 µL  
 Detection: Dionex ASRS ULTRA suppressor, 4 mm, recycle mode

Peaks: 1. Fluoride 0.14 (A) 1.26 mg/L (B)  
 2. Chloride 28.8 58.4  
 3. Nitrite-N - 2  
 4. Carbonate - -  
 5. Bromide 0.03 2.0  
 6. Sulfate 99.5 179.3  
 7. Nitrate-N 0.89 5.88  
 8. Phosphate-P - 9.24

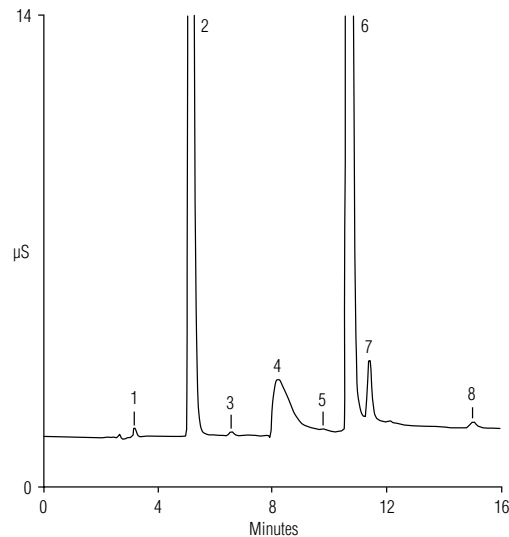


Figure 2. Determination of inorganic anions in Sunnyvale, CA, drinking water using the Dionex IonPac AS18 column.

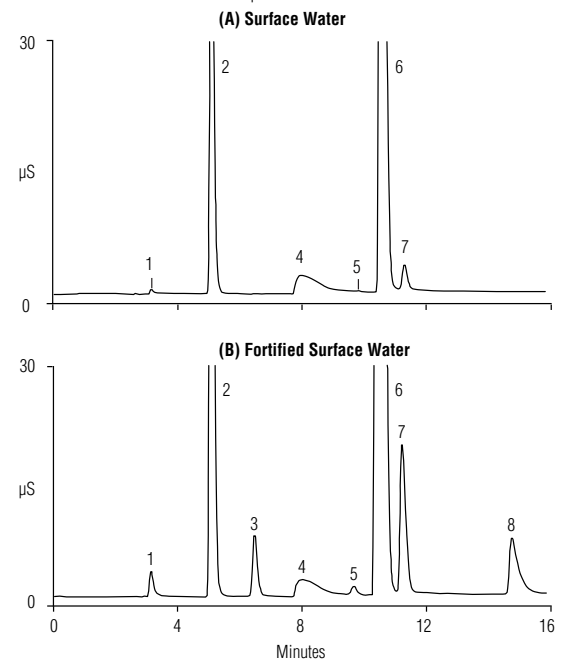


Figure 3. Determination of inorganic anions in (A) surface water and (B) fortified surface water using the Dionex IonPac AS18 column.



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 Dionex IonPac 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.

### Summary

The use of a Reagent-Free ion chromatograph with a Dionex IonPac AS18 column and electrolytic eluent generation is an improved approach to the routine determination of inorganic anions in environmental waters. The Dionex IonPac AS18 column provides improved retention for fluoride from the column void volume, overall improved selectivity, and a significantly higher capacity compared to the Dionex IonPac 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 Dionex IonPac 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.<sup>13</sup>

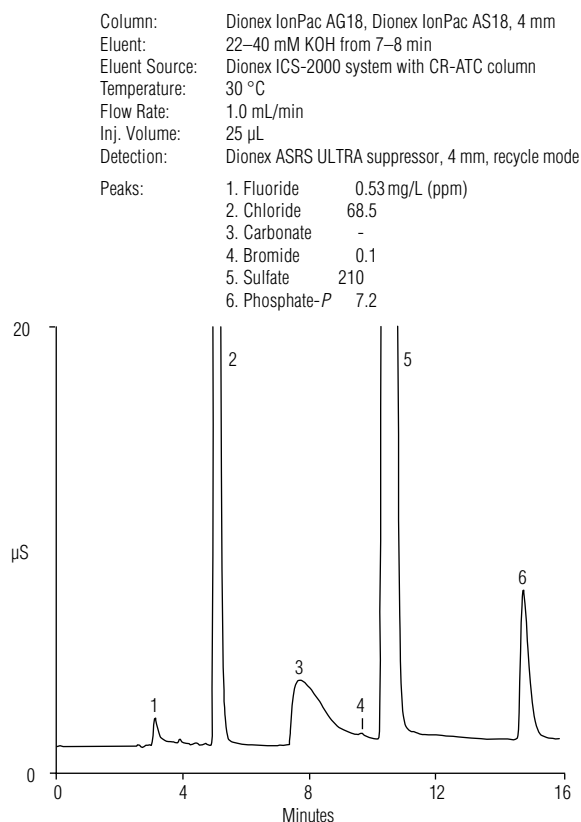


Figure 4. Determination of inorganic anions in domestic wastewater using the Dionex IonPac AS18 column.

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