

Fast Anion Determinations in Environmental Waters Using a High-Pressure Compact Ion Chromatography System

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Key Words

Standard Bore, Integrin, HPIC, Fast IC, IonPac AS18-4 μm , RFIC, Reagent-Free IC

Introduction

Ion chromatography (IC) is 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} Inorganic anions are regulated in drinking water as primary contaminants (fluoride, nitrate, nitrite, and disinfection byproducts) for health reasons, as secondary contaminants affecting taste, color, or odor, or for aesthetic reasons, as in the case of chloride or sulfate contamination. Many of these same inorganic anions are regulated through the CWA industrial permitting process.

In the U.S., compliance monitoring of inorganic anions in drinking water and wastewater has been required since 1992 to follow U.S. EPA Method 300.0, updated in 1997 to U.S. EPA Method 300.1.^{3,4} Other industrial countries, such as Germany, France, Italy, Japan, and China, have similar requirements (ASTM, EU International Organization for Standardization (ISO), China EPA). In U.S. EPA Method 300.0 (Part A) and 300.1 (Part A), inorganic anions are separated by anion-exchange chromatography on the Thermo Scientific™ Dionex™ IonPac™ AS4A and Dionex IonPac AS14A anion exchange columns, respectively, using manually prepared carbonate-based eluents and detected by suppressed conductivity detection. However, there have been significant advances in technology since 1997. In accordance with the rapid technology advancements, the standard methods allow for comparable results using alternative columns, eluents, suppression devices, and detectors.



As such, this application has been updated numerous times. In the latest iteration, Thermo Fisher Scientific Application Note 154 (AN154)⁵, demonstrated increased sensitivity, peak retention time, and peak area precision using hydroxide eluents over the previous application (AN133)⁶, which used manually prepared carbonate eluents.

In AN154, the effectiveness of electrolytic eluent generation of hydroxide eluent combined with a hydroxide-optimized, high-capacity anion-exchange column (Dionex IonPac AS18) was demonstrated on a Thermo Scientific™ Dionex™ ICS-2000 Integrated IC system. In AN154 (originally published in 2003), seven anions were eluted within 16 min using an electrolytically generated hydroxide gradient and detected by suppressed conductivity detection with the continuously regenerated Thermo Scientific™ Dionex™ ASRS™ ULTRA Anion Self-Regenerating Suppressor.

This document updates AN154 with fast separations (12 min run time) on the higher pressure Dionex IonPac AS18-4 μ m column, reducing the run time by 8 min. This column is optimized with the same selectivity using the 4 μ m resin particles but with a shorter format (150 mm versus 250 mm length). The Dionex IonPac AS18-4 μ m allows for faster run times while maintaining highly efficient separations, thereby yielding good quantification accuracy and consistently reliable results. This application is demonstrated on the high-pressure-capable Thermo Scientific™ Dionex™ Integrion™ HPIC™ compact IC system.

The Dionex Integrion HPIC system includes the recent advances in IC instrument technology, including high-pressure capabilities for Reagent-Free™ IC (RFIC) (up to 5000 psi), column heater control, and many new features designed to increase customer ease-of-use. These features include:

- A compact, fully integrated system design
- Easy access to eluent generator and electrolytic trap column
- Separate compartments for pump, column heater with injection valve, and detection-suppressor to provide separate temperature control and faster equilibration
- Thermo Scientific™ Dionex™ IC PEEK Viper™ fittings replacing standard fitting connections in specified positions to minimize void volume problems, improve chromatography, and ensure accurate reporting
- Components tracked by consumables device monitoring for GMP compliance tracking, which prompts the user to install compatible devices and reduces the likelihood of an improper set-up
- Independent tablet control for convenient, continuous full-screen monitoring, independent manual control, and the online instrument manual and troubleshooting guides
- New Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System (CDS) software features that provide easy instrument configuration, monitoring of consumable devices, and online video instructions for conditioning columns, suppressors, and other electrolytic devices

The Dionex Integrion HPIC system can also be configured for electrochemical detection of electroactive ions and mono- and disaccharides. Another model of the Dionex Integrion HPIC system, without RFIC capabilities, can be configured with or without conductivity detection.

Equipment

- Thermo Scientific Dionex Integrion HPIC High-Pressure IC system includes:
 - CD conductivity detector
 - Column oven temperature control
 - Detector compartment temperature control
 - Tablet control
 - Consumables device monitoring capability
 - Eluent generation capabilities
- Thermo Scientific™ Dionex™ AS-AP Autosampler with 10 mL trays

Software

Thermo Scientific Dionex Chromeleon CM 7.2 SR4 CDS software was used.

Table 1 lists the consumable products recommended for the Dionex Integrion HPIC system, configured for suppressed conductivity detection.

Table 1. Consumables list for the Dionex Integrion HPIC System.

Product Name	Product Details	Part Number
Dionex IC PEEK Viper Fitting Tubing Assembly Kits	Dionex IC PEEK Viper fitting assembly kit for the Dionex Integrion HPIC system includes one each of P/Ns: 088805-088808, 088810, 088811	088798
Dionex IC PEEK Viper Fitting Tubing Assemblies, Included in Kit, P/N 088798	Guard to separator column: 0.007 × 4.0 in. (102 mm)	088805
	Injection valve, Port C (Port 2) to guard column: 0.007 × 5.5 in. (140 mm)	088806
	EGC Eluent Out to CR-TC Eluent In: 0.007 × 6.5 in. (165 mm)	088807
	Separator column to Suppressor Eluent In: 0.007 × 7.0 in. (178 mm)	088808
	Suppressor Eluent Out to CD In: 0.007 × 9.0 in. (229 mm)	088810
	CR-TC Eluent Out to Degasser Eluent In: 0.007 × 9.5 in. (241 mm)	088811
Dionex AS-AP Autosampler Vials	Package of 100, polystyrene vials, caps, blue septa, 10 mL	074228
Thermo Scientific™ Dionex™ EGC™ 500 KOH Eluent Generator Cartridge*	Eluent generator cartridge	075778
Thermo Scientific™ Dionex™ CR-ATC™ 600 Electrolytic Trap Column*	Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge	088662
HP EG Degasser Module*	Degasser installed after Dionex CR-TC trap column and before the injection valve. Used with eluent generation	075522
Thermo Scientific™ Dionex™ AERS™ 500 Suppressor	Suppressor for 4 mm and 5 mm columns, using recycle mode	082540
Dionex IonPac AG18-4µm Column	Anion guard column, 4 × 30 mm	076035
Dionex IonPac AS18-4µm Column	Anion separation column, 4 × 150 mm	076034
Thermo Scientific™ Nalgene™ Syringe Filter	Syringe filters, 25 mm, PES membrane, 0.2 µm. This type is compatible with IC analysis	Thermo Scientific 7252520 / Fisher Scientific 09-740-113

* High-pressure device recommended for 4 µm particle resin columns.

Chromatographic Conditions

Columns:	Thermo Scientific Dionex IonPac AG18-4 μ m guard (4 \times 30 mm) and Dionex IonPac AS18-4 μ m separation (4 \times 150 mm)
Eluent:	15–44 mM KOH (0.2–6 min, 44 mM KOH (6–9 min, 15 mM KOH (9–12 min)
Eluent Source:	Thermo Scientific Dionex EGC 500 KOH cartridge with Thermo Scientific Dionex CR-ATC 600 trap column and high-pressure EG degasser
Flow Rate:	1.0 mL/min
Column Temperature:	35 $^{\circ}$ C
Detector Compartment Temperature:	15 $^{\circ}$ C
Injection Volume:	10 μ L, in Push-Full mode
Detection:	Suppressed conductivity, Thermo Scientific Dionex AERS 500 suppressor, 4 mm, recycle mode
Run Time (min):	12 min
Background Conductance (μ S):	< 1
Typical Noise (nS):	< 1
System Backpressure (psi):	~ 2200

Samples and Sample Preparation

Samples were municipal drinking water, surface water, wastewater, and well water.

Drinking water samples were analyzed with minimal dilution (with deionized water). All surface, wastewater, and well water samples were filtered (0.2 μ m) prior to injection.

Instrument Setup and Installation

The Dionex Integrion HPIC system is a high-pressure-capable integrated Reagent-Free IC (RFIC) system. The Dionex Integrion HPIC system, Dionex EGC 500 KOH cartridge, and Dionex CR-ATC 600 consumable products are designed for high-pressure conditions up to 5000 psi.

To set up this application, connect the Dionex AS-AP autosampler and the Dionex Integrion HPIC system modules according to Figure 1. Note that the injection valve is plumbed through different ports than previous Dionex IC systems.

Connect the USB cables from the Dionex Integrion HPIC system to the Dionex AS-AP autosampler and to the computer. Connect the power cables and turn on the IC instrument and the autosampler.

Configuring the Modules in the Chromeleon CDS Software

To configure the IC system, first start the Chromeleon CDS software Instrument Controller program and then select the link *Configure Instruments* (opens the Chromeleon Instrument Configuration Manager). Right-click on computer name, select *Add an Instrument*, and enter an appropriate name (for example: Integrion_EPA300_1). Select *Add a Module, IC: Dionex Integrated Modules*, and *Integrion HPIC System*. The instructions to configure each module are summarized at the end of this section in Table 2.

After the multi-tabbed program opens, select the Model Serial No. The Chromeleon CDS software will automatically detect all Dionex Integrion HPIC system devices: the electrolytic devices, detectors, pump degasser, and seal wash requiring minimal data entry during instrument configuration. The Chromeleon CDS software automates the system configuration process by automatically detecting the installed devices. To add pressure monitoring capabilities in the configuration, right-click and select *Add a Module, IC: Dionex Integrated Modules, Integrion HPIC Pump (Wellness)* module and then select the USB address to link the module to the configuration. Select the Devices tab and select the Pressure Signal(s) checkbox (Figure 2).

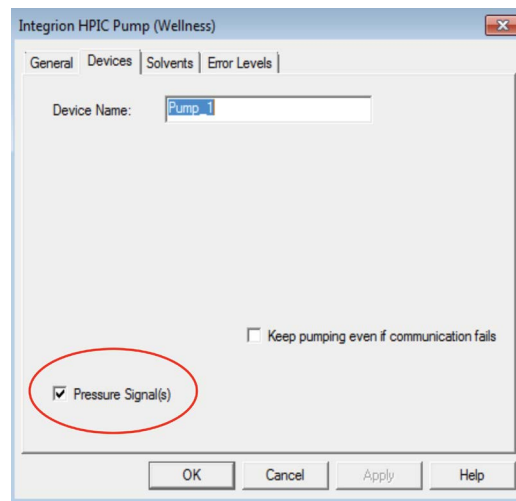


Figure 2. Adding the Dionex Integrion HPIC Pump Wellness module to the instrument configuration.

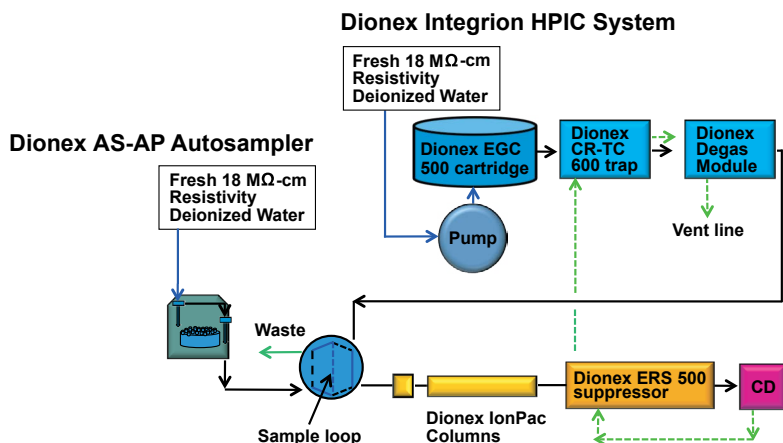


Figure 1. Flow diagram for the Dionex Integrion HPIC system.

Add the Dionex AS-AP Autosampler to the Configuration

Add the Dionex AS-AP autosampler as a module, and select the USB address. In the Segments/Pump Link tab, select the appropriate vial trays for each color zone. In the Options tab, select Push, installed syringe size, 1.2 mL for buffer line, and enter the sample loop volume. Save the configuration, select Check the Configuration, and then close the Chromeleon CDS software Instrument Configuration program.

Table 2. Summary of system configuration for high-pressure Dionex Integrion HPIC system.

Tab	Action	Result
Dionex Integrion HPIC Module		
General	Link to USB address	
Pump		Flow rate and pressure limitations are displayed
Detectors		Automatically detected
Electrolytics		Automatically detects Dionex eluent generator cartridges and Dionex CR-TC trap columns
Inject Device		Automatically detected
Thermal Controls		Automatically detects thermal control options for column, detector, and suppressor
High-Pressure Valves		Automatically detected
Low-Pressure Valves		Automatically detected
Options		Automatically detects pump degasser and seal wash pump
Pump Wellness Module		
Devices	Select Pressure Signal checkbox	Activates pressure monitoring feature (Figure 2)
Add Dionex AS-AP Autosampler		
Add Module	Link to USB address	
Sharing		Only if more than one instrument is detected. If this option is present, select Instrument
Segments / Pump Link		Select 10 mL polystyrene vials or 1.5 mL vials for "Red", "Blue", and "Green"
Options		Select Push, select syringe size, select 1.2 mL buffer line, enter the loop size

Plumbing the High-Pressure Dionex Integrion HPIC System

Tip: To achieve the best chromatography, it is important to gently tighten the IC PEEK Viper fittings to finger-tight plus 1/8 clockwise turn for the first installation, and 1/16 turn the second use. Use the IC PEEK Viper fitting assemblies:

- Dionex EGC 500 KOH eluent generator cartridge—*Eluent Out to Eluent In* on Dionex CR-ATC 600 trap column
- Dionex CR-ATC 600 trap column—*Eluent Out to Eluent In* on the Dionex Degas Module
- Injection Valve—*Port 2* (Column) to the guard column
- Between the guard and separation columns
- Separation column to *Eluent In* on the Dionex AERS 500 Suppressor
- Dionex AERS 500 Suppressor—*Eluent Out to Eluent In* on CD Conductivity Cell

First, loosen the waste lines, including the metal-wrapped waste line in the back of the instrument, and direct the free ends to a waste container. To plumb the system, first connect the pump eluent line to the eluent bottle containing previously degassed (vacuum filtration and ultrasonic agitation) deionized water. Prime the pump by opening the priming knob $\frac{1}{4}$ turn and press the priming button. Prime the pump until no bubbles are visible and water is flowing at a steady rate out of the pump waste line. Close priming knob to finger-tight. For more information, review the product manual by selecting “?” on the tablet.

Conditioning Electrolytic Devices and Columns

Tip: Do not remove RFIC tags on the columns and consumable devices. These tags are required for RFID monitoring functionality.

Install the Dionex EGC 500 KOH cartridge and Dionex CR-ATC 600 Continuously Regenerating Anion Trap Column in reservoir tray compartment. Condition the devices according to instructions in the drop-down menu under Consumables > Install (Figure 3).

(This information is also available in the product manuals and the system installation manual.⁷⁻⁹) Install one black PEEK (0.010 in i.d. tubing) backpressure loop (exerting an additional ~40 psi) at the cell outlet.

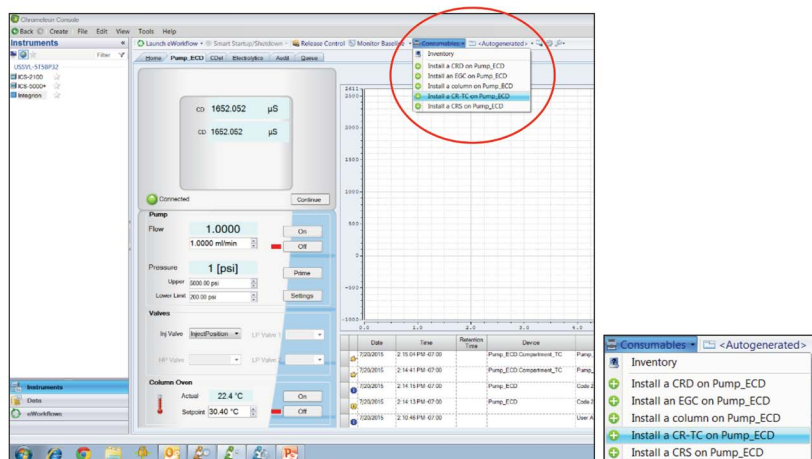


Figure 3. Consumables online installation instructions.

To hydrate the Dionex ERS 500 suppressor, follow the QuickStart Instructions received with the suppressor and in the product manual.¹⁰ Wait for 20 min for the suppressor to fully hydrate before installing the suppressor in the detector compartment. Install the backpressure loop between the CD outlet and the suppressor *Regen In* port.

Condition the columns for 30 min according to the instructions in the *Consumables, Install Column* section (Figure 3). The general practice is to follow the eluent and flow rate conditions listed in the QAR report while directing the eluent exiting the column to a waste container.¹¹ Complete the installation according to the Figure 1 flow diagram.

Installing and Optimizing the Dionex AS-AP Autosampler

The Dionex AS-AP autosampler needle must be aligned to the injection port. To align the autosampler needle, first select the Sampler tab on the instrument panel and press the Alignment button. Follow the commands to align the autosampler needle to the Injection Port and Wash Port (Section B.12 in the Operator's Manual).¹² Then, connect the autosampler syringe line to wash container containing degassed water to the syringe. Prime the syringe to flush out any air in the Buffer Wash line and syringe. Initially select a 5000 μ L wash volume until a steady flow of water is observed at the Wash Port. Then, calibrate the transfer line volume by following the prompts on the TLV Calibration button. This volume will be recorded automatically. For more information, review Section 5.9 in the Dionex IC Series AS-AP Autosampler Operator's Manual.¹²

Starting the Dionex Integrion HPIC System

To start the system, turn on the pump and immediately turn on both the Dionex EGC 500 cartridge and the Dionex CR-ATC 600 trap when liquid is flowing through the device. The system backpressure is dependent on the flow rate and type of column, but the system must be above 2000 psi to support the Dionex EGC cartridges. Typically, columns with 4 μ m resin particles operate, as is, above 2000 psi and therefore do not require backpressure tubing. However, if additional pressure is needed to achieve system pressures > 2000 psi, install yellow PEEK backpressure tubing (yellow PEEK, 0.076 mm i.d., 0.003 in i.d.) between the Dionex HP EG Degasser module and the injection port (Pump position). Set the eluent concentration, column oven, compartment oven, and cell temperatures as shown in the Conditions section in the application. Allow the system to equilibrate for 30 min. For optimum chromatography equilibrate until the total background is stable, 1–2 μ S.

Creating a Instrument Method

To create a new instrument method using the Chromleon Wizard, select *Create, Instrument Method*, and select *Instrument*. Enter the values from the Chromatographic Conditions section. Save the instrument method.

Consumables Device Monitoring

Tip: An action (approve or correct an incompatibility between devices) is required to start a sequence after installing any new consumable device.

A new feature of the Dionex Integrion HPIC system is consumables device monitoring and tracking, which automatically detect the electrolytic devices and the columns. Review and approval of the devices is required to start the first sequence on the Dionex Integrion HPIC system and after installing new consumable devices. To access this approval, select *Consumables* and select *Inventory* (Figure 4).

Device monitoring shows the device history, tracking, part number, size, chemistry, serial numbers, manufacture lot, installed location (On Device), and best if used by date (Figure 4, top). Additionally, the device monitoring will provide warnings if there is incompatibility in the devices installed (Figure 4, bottom left). To start the sequence, review the list of consumables listed as inventory, correct any errors, approve, and close page (Figure 4, bottom right). Then, select the Instrument Queue tab, and conduct a *Ready Check* on the sequence and press *Start*.

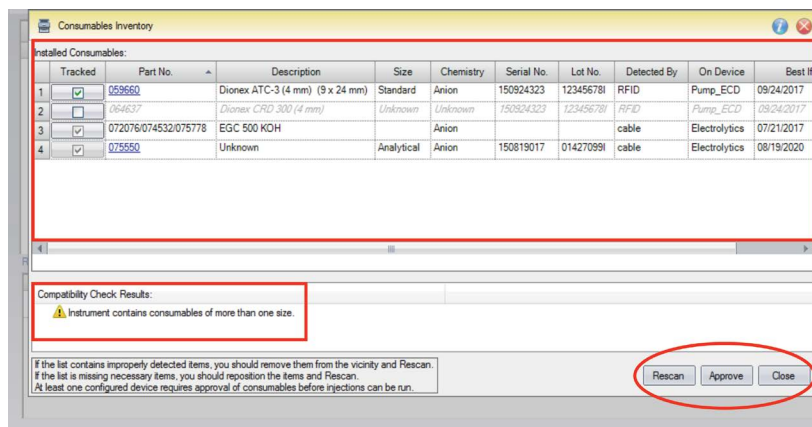


Figure 4. Consumables tracking.

Results and Discussion

Seven anions, including phosphate, were separated using an electrolytically generated hydroxide gradient from 14 mM to 44 mM KOH (0.2 to 9 min) on the Dionex IonPac AS18-4 μ m, 4 \times 150 mm, high-capacity, 4 μ m resin particle anion-exchange column. All anions were eluted within 9 min and detected by suppressed conductivity with the latest innovation in suppressors (Dionex AERS-500) using the high-pressure capable Dionex Integriion HPIC system. This method demonstrates shorter run times than those in AN154 using the 4 \times 250 mm, 7.5 μ m column. Faster runs are made possible using the shorter, 150 mm length columns, while the 4 μ m resin particles provide highly efficient separations that allow for the reduction in column length. The improved efficiency also allows the column temperature to be increased five degrees to help shorten the analysis time. Additionally, the Dionex Integriion HPIC system has a separate suppressor-detector compartment that can be set to 15 $^{\circ}$ C, which provides increased suppressor efficiency, resulting in improved chromatography. In Figure 5, the same standard is compared under the same analytical conditions run on both resin formats of the Fast (4 \times 150 mm), IonPac AS18 chemistry columns: Chromatogram A—Dionex IonPac AS18-Fast column composed of 7.5 μ m resin particles versus Chromatogram B—Dionex IonPac AS18-4 μ m column composed of 4 μ m resin particles. This figure shows the improved peak efficiencies with the 4 μ m column, as exhibited by the smaller peak widths and higher peak response, as well as the higher resolution of the carbonate-bromide and sulfate-nitrate critical pairs.

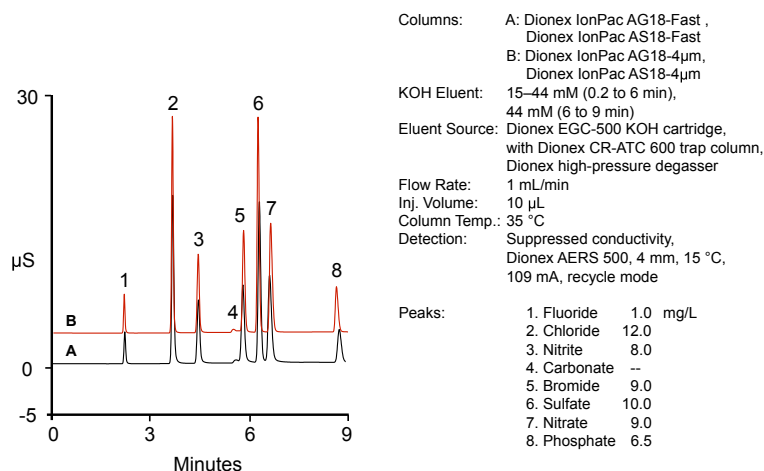


Figure 5. Comparison of Dionex IonPac AS18-Fast columns.

Method Qualification

To evaluate the method, the retention time and peak area precisions were determined by running seven replicate injections of a 50 mg/L mixed anion standard. The results, summarized in Table 3, show excellent precision, < 0.1 and 0.1–0.2 RSDs for retention time and peak area, respectively.

Table 3. Method reproducibilities using a 50 mg/L standard.

	Retention Time		Peak Area	
	(min)	(RSD)	(μ S-min)	(RSD)
Fluoride	2.307 \pm 0.002	0.09	9.237 \pm 0.012	0.13
Chloride	3.714 \pm 0.001	0.04	6.056 \pm 0.011	0.19
Nitrite (NO₂-N)	4.506 \pm 0.006	0.06	4.206 \pm 0.006	0.13
Bromide	5.838 \pm 0.002	0.03	2.370 \pm 0.003	0.14
Sulfate	6.341 \pm 0.002	0.04	4.105 \pm 0.006	0.14
Nitrate (NO₃-N)	6.630 \pm 0.002	0.03	3.300 \pm 0.005	0.14
Phosphate (PO₄-P)	8.714 \pm 0.007	0.08	2.171 \pm 0.004	0.19

n = 7

Replicate injections of seven combined anion standards were used to determine the linear concentration ranges based on peak area. The MDLs were determined using a 20–50x dilution of the lowest calibration standard ($3.14 \times \sigma$ (standard deviation)). The results, summarized in Table 4, show linear responses within the calibration range and MDLs < 2 μ g/L for all anions except bromide.

Table 4. Linearity and MDL results.

	Calibration Range (mg/L)	Coefficient of Determination	MDL Standard (μ g/L)	MDL (μ g/L)
Fluoride	0.04–100	0.99995	1	0.3
Chloride	0.50–200	0.99999	9	0.6
Nitrite (NO₂-N)	0.97–100	0.99961	5	0.1 (0.4 as NO ₂)
Bromide	0.7–100	0.99993	14	2.6
Sulfate	0.8–200	1.00000	16	1.4
Nitrate (NO₃-N)	0.16–100	0.99999	8	0.9 (4.1 as NO ₃)
Phosphate (PO₄-P)	0.17–100	0.99997	9	0.8 (2.6 as PO ₄)

Sample Analysis

This method was applied to municipal drinking and wastewater, surface water, well water, softened well water, inland sea, artificial lake, and pool water samples. Chromatograms of municipal wastewater and surface water samples are shown in Figures 6 and 7. To determine accuracy, recoveries were calculated after adding concentrated anion standards (0.5 to 2x the concentration present) to selected samples: surface, pool, municipal waste, and drinking water. The results, summarized in Table 5, show good recoveries, 89–105%, demonstrating acceptable accuracy.

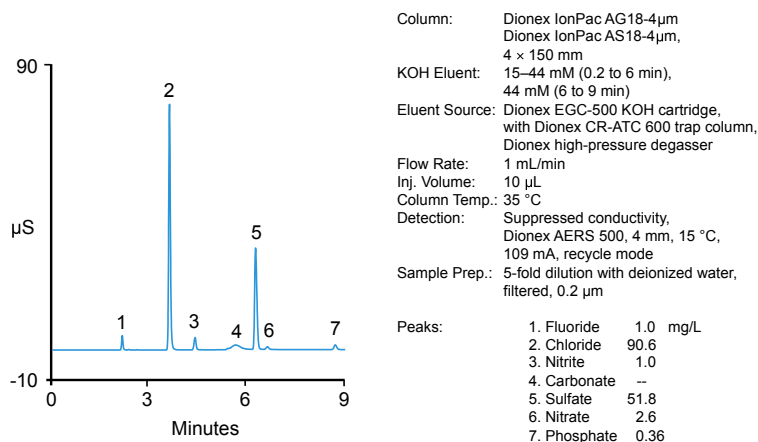


Figure 6. Determination of anions in a diluted municipal wastewater sample.

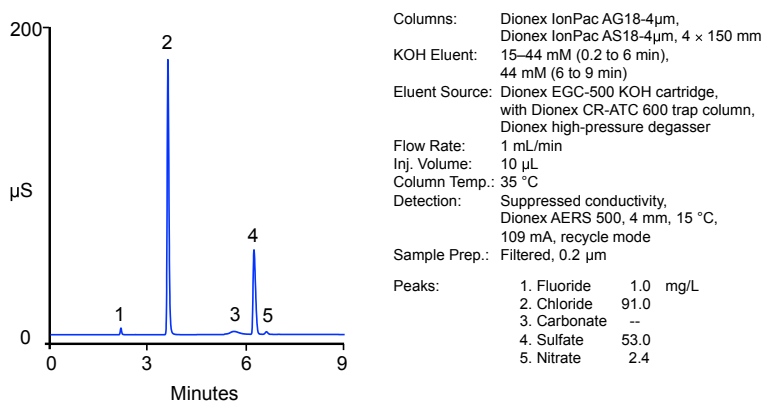


Figure 7. Determination of anions in an undiluted surface water sample.

Table 5. Recovery results.

Municipal Wastewater	Added (mg/L)	Recovery (%)	Surface water	Added (mg/L)	Recovery (%)
Fluoride	1	102	Fluoride	1.0	100
Chloride	20	103	Chloride	80	103
Nitrite- <i>N</i>	2	103	Nitrite- <i>N</i>	1	94.9
Bromide	2	89.5	Bromide	1	90.2
Sulfate	10	95.3	Sulfate	35	104
Nitrate- <i>N</i>	1	102	Nitrate- <i>N</i>	5	95.1
Phosphate- <i>P</i>	2	98.7	Phosphate- <i>P</i>	1	97.0

Pool water	Added (mg/L)	Recovery (%)	Municipal Drinking water	Added (mg/L)	Recovery (%)
Fluoride	1	104	Fluoride	1.0	101
Chlorite	detected	--			
Chloride	100	96.7	Chloride	20	99.6
Nitrite- <i>N</i>	1	98.8	Nitrite- <i>N</i>	1	98.0
Bromide	1	90.5	Bromide	1	88.7
Sulfate	3	91.4	Sulfate	10	104
Nitrate- <i>N</i>	5	99.6	Nitrate- <i>N</i>	2	91.2
Phosphate- <i>P</i>	1	105	Phosphate- <i>P</i>	1	96.0

Conclusion

Fast analysis of environmental water samples containing anions at ppm concentrations were demonstrated using the high resolution capabilities of the Dionex IonPac AS18-4 μ m column facilitated by the high-pressure capabilities of the Dionex Integriion HPIC system.

In this update of AN154:

- The method was updated with the latest column, suppressor, and IC instrument technology.
- The method demonstrated reliability and accuracy with standards and samples as shown by the retention time and peak area reproducibilities with a RSD of < 0.1 and < 0.2, respectively, improved chromatography and resolution of critical peak pairs, and 89–105% recoveries.
- The run time was reduced to 12 min, saving four minutes of analysis time for characterizing a sample containing seven anions (including phosphate) per sample.

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