

# Determination of trace anions in high purity waters using a large-volume direct injection

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## Goal

To describe the use of a Thermo Scientific™ Dionex™ ICS-6000 system with an eluent generator module equipped with a Thermo Scientific™ Dionex™ EGC 500 potassium hydroxide (KOH) eluent generator cartridge, and a Thermo Scientific™ Dionex™ IonPac™ AS28-Fast-4 $\mu$ m (2 × 150 mm) column for trace-level anion analysis.

## Introduction

Trace anion analysis in high purity water is an important application for the semiconductor, disk drive, electronic components, and nuclear power industries. The measurement of trace levels of ionic impurities throughout the power generation process is critical for the identification and prevention of corrosive conditions in many power plant components. Corrosive ions should be minimized and continuously monitored. Ion chromatography (IC) has become an essential technique for monitoring water quality and for the determination of corrosive ions. Anions at sub- $\mu$ g/L levels can be determined with a large-volume direct-injection approach. The Dionex ICS-6000 eluent



generator module generates high-purity and carbonate-free hydroxide eluents online to improve the method performance of target analytes at trace levels. The large-volume direct injection technique is used to achieve sensitive detection at low to sub- $\mu$ g/L levels without using a concentrator column, sample loading pump, and additional valve. The Dionex IonPac AS28-Fast-4 $\mu$ m column is a high resolution, high capacity anion exchange column with selectivity and capacity similar to the Thermo Scientific™ Dionex™ IonPac™ AS15 column. The high resolution improves peak identification, and the increased ion exchange capacity allows injection of more concentrated samples or larger sample volumes without overloading the column. The Dionex IonPac AS28-Fast-4 $\mu$ m column is designed for the trace analysis of inorganic anions and

monovalent organic acids.<sup>1</sup> Improved peak efficiencies in combination with the smaller column inner diameter result in lower detection limits compared to the Dionex IonPac AS15-5 $\mu$ m (3 × 150 mm) column. The analytes are detected by suppressed conductivity with a 2 mm Thermo Scientific™ Dionex™ ADRS™ 600 suppressor. This application note expands on work presented in Thermo Scientific Application Update 142, “Improved determination of trace anions in high purity waters by high-volume direct injection with the Dionex EG40 eluent generator”.<sup>2</sup>

## Experimental

### Equipment

- Dionex ICS-6000 HPIC system including:
  - Dionex ICS-6000 DP Pump module
  - Dionex ICS-6000 EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-6000 Low Temperature DC Detector/Chromatography module with two injection valves
  - CD Conductivity Detector
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control (P/N 074926)\*, sample syringe, 5 mL (P/N 074308) and buffer line, 8.5 mL (P/N 075520)
- Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm, P/N 088667)
- Thermo Scientific™ Dionex™ IC PEEK Viper Fitting Tubing Assembly Kit (2 mm, P/N 302965)
- Thermo Scientific™ Dionex™ Vial Kit, 10 mL polystyrene with caps and blue septa (P/N 074228)
- Corning™ polystyrene non-treated culture flasks (Fisher Scientific, P/N 08-757-502) for low-level standards and samples

- Gloves, Ansell™ Nitrilite™, nitrile cleanroom Class 5 (Fisher Scientific, P/N 19-014)
- Berkshire™ Gamma Wipe™ 120 cleanroom wipes, polyester (Berkshire, P/N GW120ST15; Fisher Scientific, P/N 18-999-306)
- Thermo Scientific™ Nalgene™ filter flask containers for soaking vials, caps, septa, pipette tips, or connectors (P/N 164-0020)

\*Dionex AS-AP autosampler comes with a pre-installed 250  $\mu$ L sample syringe and a 1.2 mL buffer line. We use large-volume injection for trace anion analysis; thus, we need a 5 mL sample syringe and a 8.5 mL buffer line as replacements.

### Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 M $\Omega$ -cm resistance or better
- Thermo Scientific™ Dionex™ Fluoride standard 1000 mg/L, 100 mL (P/N 037158)
- Thermo Scientific™ Dionex™ Chloride standard 1000 mg/L, 100 mL (P/N 037159)
- Thermo Scientific™ Dionex™ Sulfate standard 1000 mg/L, 100 mL (P/N 037160)
- Thermo Scientific™ Dionex™ Nitrate standard 1000 mg/L, 100 mL (P/N 056497)

### Preparing for successful trace-level (<10 $\mu$ g/L) ion determinations

There are many sources of contamination that can interfere with reliable and consistent trace-level ion determinations. Working in trace analysis, we require a clean environment, methodical practices, and an awareness of laboratory activities and items that pose the risk of contamination. This section discusses and reviews the importance of clean water; an isolated clean, low-particle work environment; the selection and use of laboratory gloves; the impacts of behaviors, social interactions, and personal care products on trace-level anion analysis; and tools suitable for the trace analysis work area (Table 1). For details, please refer to Thermo Scientific Technical Note 112.<sup>3</sup>

**Table 1. Preparing for successful trace level ion determinations**

Item	Recommendation
DI water	Use only freshly generated 18.2 MΩ·cm deionized (DI) water.
Work environment	<ul style="list-style-type: none"> <li>a. Isolate the instrument in a low-traffic area away from heater, air conditioner, and instrument vents, and away from chemical storage and weighing areas.</li> <li>b. Wipe down all surfaces using cleanroom wipes and fresh DI water.</li> <li>c. Minimize group discussions near the work area. People can stir up and emit dust particles and aerosols of ionic contamination.</li> </ul>
Gloves	<ul style="list-style-type: none"> <li>a. Always wear fresh gloves in the trace analysis work area.</li> <li>b. Use powder-free Class 10 or Class 100 undyed nitrile cleanroom gloves (such as Ansell Nitrilite, Fisher Scientific, P/N 19-014-687, 19-014-688, 19-014-689).</li> <li>c. Touch only the glove sleeves when putting on gloves. Never touch the glove tips.</li> <li>d. Select the glove size that results in a comfortable but tight fit. The glove tips should not extend past your fingers more than a few millimeters. Oversize gloves can easily contaminate the sample containers.</li> <li>e. Discard the gloves after touching your face, hair, or extra surfaces, especially any concentrated standards, solid reagents, or paper products.</li> <li>f. Avoid touching the neck or cap of the sample container or standard container.</li> </ul>
Personal	<ul style="list-style-type: none"> <li>a. Avoid personal care products such as deodorant, powders, hair products, makeup, perfume, and lotions.</li> <li>b. Avoid talking, sneezing, laughing, and coughing near the work area or wear a face mask to cover mouth and nose, to prevent the generation of airborne mists of particles containing high concentrations of chloride, sulfate, and organic acids. Note that one grain of salt in a cup of water is equivalent to ~20 mg/L chloride.</li> </ul>
Sample preparation, sample containers, and sample vials	<ul style="list-style-type: none"> <li>a. Use pipette tips only when preparing concentrated standards, such as the intermediate standard, and not for any low-level work.</li> <li>b. Before using pipette tips, soak them in fresh DI water in a pre-cleaned polystyrene flask, and handle them only with stainless steel alloy (SS 416) tweezers to further ensure cleanliness.</li> <li>c. Avoid the use of any cellulose products—including lab wipes, weighing paper, and cotton swabs. These contain high concentrations of chloride and sulfate and therefore are major sources of contamination.</li> <li>d. Use low-particle, low ionic contamination polyester wipes, such as Berkshire Gamma Wipe 120 Class 10 cleanroom wipes.</li> <li>e. Use either cleanroom adhesive tape or labels or use markers to label samples.</li> <li>f. Do not use plastic weighing dishes to handle anything related to standards and samples; instead, tare final containers and transfer materials directly from their source to the final containers.</li> <li>g. Use only polystyrene or FEP (fluorinated ethylene propylene polymer) for containers and only polystyrene for vials. Use only autosampler vial septa that have been verified to be contaminant-free, such as blue septa or (preferably) single-use PTFE septa.</li> <li>h. Use only autosampler vial septa that have been verified to be contaminant-free, such as blue septa or (preferably) single-use PTFE septa.</li> </ul>

### Preparation of solutions and reagents

For successful trace ion determinations, it is crucial to minimize contamination to the sample and standard containers.

### Preparing containers

Preparing samples and standards for trace analysis requires containers with low extractable ionic contamination and special handling techniques. Two types of containers are suited for trace analysis: non-treated polystyrene culture flasks and FEP containers. Non-treated polystyrene (Corning) culture flasks are recommended for storing samples and standards. Never use caps with liners or filters because the liners and filters typically contain mg/L concentrations of chloride and sulfate.

To prepare (clean) the flasks, turn on the 18.2 MΩ·cm DI water and allow it to run for a minute before rinsing the flask. Rinse the flask five times with 18.2 MΩ·cm DI water,

fill the flask to the top (leaving as little air as possible), close it with a cap that has been pre-rinsed five times, and let soak overnight. Repeat the same rinsing and soaking process daily for at least three days.

### Standard solutions

#### Stock anion standard solution (1000 mg/L)

Several of the analytes of interest are available as 1000 mg/L ion standard solutions from Thermo Fisher Scientific or other commercial sources. In cases where standards are not available, 1000 mg/L standards can be prepared by dissolving the appropriate amounts of the corresponding dried salt in 100 mL of DI water according to Table 2. We recommend making a 100 mL final volume of 1000 mg/L stock standards in 125 mL high-density polyethylene (HDPE) containers. Concentrated standards are stable for at least one month when stored at 4 °C.

**Table 2. Amounts of compounds used to prepare 100 mL of 1000 mg/L anion standards**

Anion	Compound	M (g/mol)	Mass (g)
F <sup>-</sup>	Sodium fluoride	41.99	0.221
C <sub>2</sub> H <sub>3</sub> O <sub>3</sub> <sup>-</sup>	Glycolic acid	76.05	0.100
CH <sub>3</sub> COO <sup>-</sup>	Sodium acetate	82.04	0.139
HCOO <sup>-</sup>	Sodium formate	68.01	0.151
Cl <sup>-</sup>	Sodium chloride	58.44	0.165
NO <sub>2</sub> <sup>-</sup>	Sodium nitrite	69.00	0.150
SO <sub>4</sub> <sup>2-</sup>	Sodium sulfate	142.04	0.148
C <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	sodium oxalate	134.04	0.152
NO <sub>3</sub> <sup>-</sup>	Sodium nitrate	84.99	0.137
PO <sub>4</sub> <sup>3-</sup>	Potassium phosphate	136.09	0.143
Br <sup>-</sup>	Potassium bromide	119.01	0.149

### Mixed standard solution

To prepare the mixed intermediate anion standard, turn on and leave on the DI water faucet until the standard is prepared. Follow the same rinsing process on the pre-cleaned flask by rinsing it five times with DI water. Shake out the excess water and tare the flask on a top loading balance. Fill the flask directly from the DI water faucet with DI water to ~250 ± 1 g. If the water weight is <249 g, quickly put the flask under the faucet. If the flask contains >251 g, gently flick the excess water into the sink. (Turn off the DI water.) Record the weight. Using a micropipette with a disposable pipette tip, transfer 25 µL each of the certified 1,000 mg/L fluoride, acetate, glycolate, formate, chloride, nitrite, sulfate, oxalate, bromide, nitrate, and phosphate standards into the flask containing the previously weighed DI water. Record the final weight, cap, gently mix, and recalculate the actual concentrations based on the volume of standard in the total weight. The density of water of 0.99707 g/mL is used for calculations. The approximate concentrations for each anion are ~100 µg/L. This flask should only be used for this standard because it can introduce contamination in lower concentration samples and standards. Therefore, this flask should never be used to store samples or working standards unless the container is re-cleaned and verified clean by testing to <10 ng/L ion concentrations.

### Preparing the working standards

To prepare the working standards, for example, a 1 µg/L mixed anion standard, turn on and leave on the DI water until the preparation of the standard is complete. Rinse the pre-cleaned flask five times, shake out the excess water, tare the flask on the top-loading balance, and fill the flask directly with the running water to ~247.5 ± 1 g. Do not use a transfer container because this is another potential contamination source. If the water weight is <247 g, quickly put the flask under the faucet. If the flask contains >248 g, gently flick the excess water from the flask into the sink. Record the weight. Pour ~ 2.5 ± 0.5 g of the 100 µg/L intermediate anion standard into the flask carefully and slowly. Record the actual weight, cap, gently shake, and recalculate the concentrations. (Turn off the DI water.) Using the same technique, prepare a 100 ng/L working standard by pouring 25 ± 0.5 g of the 1 µg/L solution into a pre-cleaned flask containing 225 ± 1 g of DI water.

### System preparation and setup

Install the Dionex EGC 500 KOH cartridge and Dionex CR-ATC 600 continuously regenerated anion trap column. Condition the devices according to instructions in the product manuals<sup>4,5</sup> and the Dionex ICS-6000 System Operator's manual.<sup>6</sup> Prepare the Dionex ADRS 600 suppressor for use by hydrating the internal membrane. Refer to the product manual for step-by-step instructions on the hydration of the 2 mm Dionex ADRS 600 suppressor.<sup>7</sup> To minimize damage to the suppressor during hydration, do not add backpressure tubing coils after the suppressor. To complete the hydration step, wait an additional 20 min without eluent flow before installing the suppressor in the recycle mode. Connect Regen-Out on the suppressor to Regen-In on the Dionex CR-ATC 600 anion trap column. Install and condition the guard and separation columns for 40 min using the initial eluent conditions.

Install and configure the Dionex AS-AP Autosampler in Push Mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361) to calibrate the sample transfer line to ensure accurate and precise sample injections. Due to the large sample injection volumes in this application, a sample syringe size of 5 mL must be installed. A buffer line assembly of 8.5 mL size is required.

Make a 500  $\mu\text{L}$  sample loop by cutting a 110 cm portion of the green 0.030 in. (0.75 mm) i.d. PEEK tubing. If a different loop or tubing with a different internal diameter is desired, refer to Table 3 to calculate the length needed. The volume of a loop can be verified by measuring the weight difference between the sample loop filled with DI water and the empty loop. The inside diameter of tubing varies by as much as 20% (for example, 0.010  $\pm$  0.002 in.)

**Table 3. Volume per unit length for various tubing internal diameters**

Material	Color	Internal diameter (in)	Internal diameter (mm)	Estimated vol. ( $\mu\text{L}/\text{cm}$ )
PEEK	Red	0.005	0.125	0.126
PEEK	Black	0.010	0.250	0.506
PEEK	Orange	0.020	0.500	2.022
PEEK	Green	0.030	0.750	4.550

### System operation

To start the system, turn on the pump and immediately turn on the Dionex EGC 500 eluent generator cartridge. Turn on the Dionex CR-ATC 600 trap and Dionex ADRS 600 suppressor when liquid is flowing through the devices. Confirm that there are no leaks in the chromatographic pathway. Allow the system to equilibrate for 30 min and run a system blank. If properly operating, the system should display the background conductance and noise listed under Conditions (Table 4). Assess the quality of the blank by measuring the short-term noise. In a representative one-minute level portion of the chromatogram, a “peak-to-peak” measurement should be less than 5 nS/cm. It will take at least 4 h for the system to equilibrate to a stable background conductivity for trace analysis. At times there will be brief increases in conductivity that will minimize upon further operation of the system. The elements of the chromatographic instrumentation’s flow path (eluent containers, injector, pump, valves, tubing, columns, suppressor, and conductivity cell) are all potential sources of contamination. Use caution when switching from a system setup that has previously seen significant concentrations of anions. Rinse with high-purity water to reduce residual contamination. It is therefore a good practice to run a system overnight to equilibrate prior to using it the following day.

## Results and discussion

### Separation

The Dionex IonPac AS28-Fast-4 $\mu\text{m}$  (2 x 150 mm) column is well suited for trace-level analysis of common inorganic anions and low molecular weight organic acids. For trace

**Table 4. Chromatography conditions**

Parameter	Value																	
System	Dionex ICS-6000																	
Columns	Dionex IonPac AS28-Fast-4 $\mu\text{m}$ , analytical, 2 x 150 mm (P/N 088749) Dionex IonPac AG28-Fast-4 $\mu\text{m}$ , guard, 2 x 30 mm (P/N 088750)																	
Eluent source	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600 trap column																	
Gradient program	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>KOH (mM)</th> <th>Equilibration stage</th> </tr> </thead> <tbody> <tr> <td>-5</td> <td>7</td> <td rowspan="5"></td> </tr> <tr> <td>0</td> <td>7</td> </tr> <tr> <td>6</td> <td>7</td> </tr> <tr> <td>8</td> <td>20</td> </tr> <tr> <td>13</td> <td>72</td> </tr> <tr> <td>25</td> <td>72</td> <td></td> </tr> </tbody> </table>	Time (min)	KOH (mM)	Equilibration stage	-5	7		0	7	6	7	8	20	13	72	25	72	
Time (min)	KOH (mM)	Equilibration stage																
-5	7																	
0	7																	
6	7																	
8	20																	
13	72																	
25	72																	
Flow rate	0.3 mL/min																	
Column temp.	30 °C																	
Autosampler temp.	10 °C																	
Injection volume	500 $\mu\text{L}$																	
Detection	Suppressed conductivity																	
Suppressor	Dionex ADRS 600 Suppressor (2 mm) (P/N 088667), recycle mode, 4.0 V, (for constant voltage mode), 54 mA (for constant current mode)																	
Run time	30 min																	
Background conductance	~0.4–0.6 $\mu\text{S}/\text{cm}$																	
Noise	<1 nS/cm																	
System backpressure	~2700 psi (100 psi = 0.689 MPa)																	

analysis, a large-loop injection combined with gradient elution was used. The column provides improved peak efficiencies compared to the Dionex IonPac AS15-5 $\mu\text{m}$  (3 x 150 mm) column, which results in lower detection limits. The Dionex ADRS 600 Suppressor delivers low background and noise for sensitivity at trace levels.

The separation begins with an eluent concentration of 7 mM KOH to elute the weakly retained ions, such as fluoride, glycolate, acetate, and formate, followed by a gradient from 7 mM to 20 mM KOH to separate chloride and nitrate, and then 20 to 72 mM KOH to elute the more strongly retained ions, such as sulfate and phosphate. Figure 1 shows a typical chromatogram for a standard 11 anions at trace levels. The large peak at ~17 min is carbonate, the size of which will vary with the amount of dissolved carbon dioxide in the sample. The chromatographic baseline shift is approximately 200–300 nS/cm. The separation is performed at 30 °C to provide consistent retention times during analysis.

1. Fluoride	0.125 µg/L (ppb)	7. Carbonate	-
2. Acetate	0.313	8. Sulfate	0.625
3. Glycolate	0.313	9. Oxalate	0.313
4. Formate	0.313	10. Bromide	0.625
5. Chloride	0.313	11. Nitrate	0.625
6. Nitrite	0.625	12. Phosphate	1.250

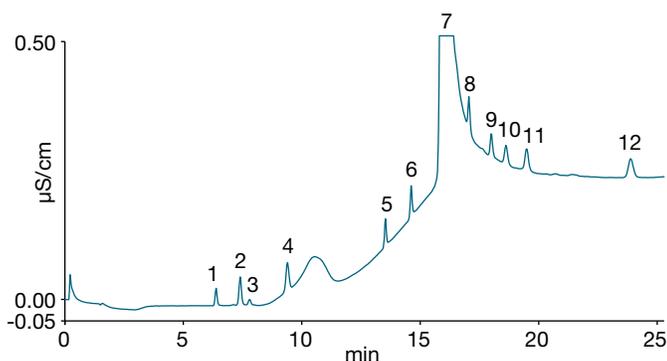


Figure 1. Trace anion determination using a Dionex IonPac AS28-Fast-4µm column

### System and water blanks

In all trace ion applications, the system and water blanks can often compromise detection limits of the analytical method and therefore are an important parameter when assessing the method qualification. The system blank is a chromatographic run with no injection being made by the autosampler, but the system is operated for the whole run with the method conditions. To set a system blank injection in Thermo Scientific™ Chromeleon™ software, put a “blank” in the “type of sample” column in the sequence. A representative system blank and DI water blank for this method are shown in Figure 2. Trace levels of fluoride, acetate, and formate originated primarily from the point-of-use DI water system. Chloride and sulfate were also detected at a low level, sub-µg/L, in the DI water injection blank, thereby limiting the detection limit to three times the average contamination. As with most trace ion applications, lower ionic contamination can be achieved by continuously running the system. The DI water blank establishes the concentrations above which anion determinations can be made.

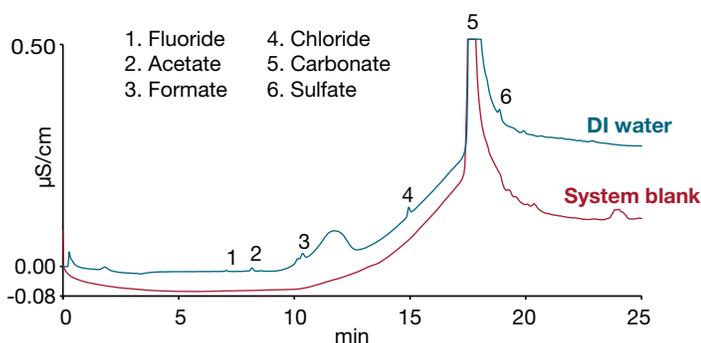


Figure 2. Chromatograms of system blank (no injection) and DI water injection blank

### Calibration

Calibration curves were obtained with standards prepared in DI water using the concentrations listed in Table 5. Figure 3 displays the chromatograms of four calibration standards. Calibration curves for the anions of interest were linear and had coefficients of determination ( $r^2$ ) greater than 0.99, except for acetate, which was 0.98 (Table 6).

Over the course of the analysis of triplicate injections of four calibration standards, the relative standard deviations (RSDs) of retention times of all eluted peaks ( $n = 12$ ) ranged from 0.03 to 0.17%. (Table 7)

Table 5. Concentrations of calibration standards

	Level 1 (µg/L)	Level 2 (µg/L)	Level 3 (µg/L)	Level 4 (µg/L)
1. Fluoride	0.125	0.250	0.500	1.00
2. Acetate	0.313	0.625	1.25	2.50
3. Glycolate	0.313	0.625	1.25	2.50
4. Formate	0.313	0.625	1.25	2.50
5. Chloride	0.313	0.625	1.25	2.50
6. Nitrite	0.625	1.25	2.50	5.00
7. Sulfate	0.625	1.25	2.50	5.00
8. Oxalate	0.313	0.625	1.25	2.50
9. Bromide	1.25	2.50	5.00	10.0
10. Nitrate	1.25	2.50	5.00	10.0
11. Phosphate	1.25	2.50	5.00	10.0

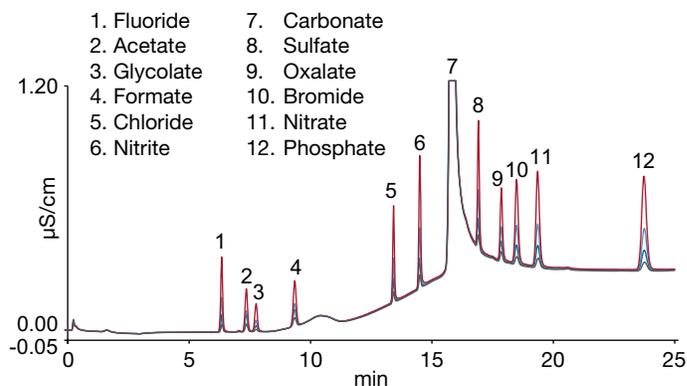


Figure 3. Chromatograms of calibration standards 1 to 4

**Table 6. Calibrations**

Analyte	Levels (µg/L)	Coefficient of determination (r <sup>2</sup> )
Fluoride	0.125–1.00	0.998
Acetate	0.313–2.50	0.980
Glycolate	0.313–2.50	0.997
Formate	0.313–2.50	0.989
Chloride	0.313–2.50	0.997
Nitrite	0.625–5.00	0.999
Sulfate	0.625–5.00	0.999
Oxalate	0.313–2.50	0.996
Bromide	1.25–10.0	0.999
Nitrate	1.25–10.0	0.998
Phosphate	1.25–10.0	0.997

**Table 7. Retention time reproducibilities**

Analyte	RT reproducibility RSD (n=12)
Fluoride	0.17
Acetate	0.14
Glycolate	0.12
Formate	0.07
Chloride	0.06
Nitrite	0.06
Sulfate	0.05
Oxalate	0.05
Bromide	0.05
Nitrate	0.04
Phosphate	0.03

**Method detection limit**

Method detection limits (MDLs) were calculated based on three times the signal-to-noise (S/N) ratio. Baseline noise (N) was determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the analyte peak. The signal (S) was determined from the average peak height of

three injections of the lowest level standard (3–5 times the estimated MDL). Table 8 summarizes the method detection limits (MDLs) for the target analytes. In our experiments, a standard MDL calculation method cannot be used for five anions—fluoride, acetate, formate, chloride, and sulfate—because they were found as contaminants in the DI water blanks (Figure 2). In these cases, and if the contamination amount is more than 3× S/N, the amount of contamination can be considered the MDL. In other cases, the MDL is what is determined with the low concentration standard. MDLs will vary from laboratory to laboratory depending on water quality and laboratory environment, and ultimately each laboratory must decide how they handle MDLs in case of low-level contamination.

**Table 8. Method detection limits**

Analyte	MDL (µg/L)
Fluoride*	0.030
Acetate*	0.271
Glycolate	0.127
Formate*	0.303
Chloride*	0.156
Nitrite	0.015
Sulfate*	0.313
Oxalate	0.156
Bromide	0.066
Nitrate	0.044
Phosphate	0.085

\*Found as contaminants in the DI water blanks

**Conclusion**

Using a high capacity Dionex IonPac AS28-Fast-4µm (2 × 150 mm) column with an electrolytically generated, high purity KOH eluent enables the determination of trace anions in high-purity water. It is possible to achieve sub-µg/L detection limits for anions in high purity water by direct injection. This approach is easier to operate than methods that use a concentrator column and loading pump. This method demonstrated good linearity, sub-µg/L detection limits, and good precision for the target anions.

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