

# Determination of trace anions in high-purity waters by ion chromatography with the Dionex IonPac AS17 column using high-volume direct injection

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## Introduction

This application note describes an ion chromatographic method using the microbore Thermo Scientific™ Dionex™ IonPac™ anion exchange AS17 column to determine trace concentrations of common inorganic anions, low-molecular-weight organic acids, as well as acrylate, methacrylate, benzoate, and phthalate. These contaminants can come from some of the following sources: cleaning agents, adhesives, oils, mold release agents, and solder fluxes.<sup>1,2</sup> Anionic contamination is known to cause corrosion in microelectronic circuitry.<sup>3</sup> The analytes are detected by suppressed conductivity with a 2 mm Thermo Scientific™ Dionex™ ASRS™ ULTRA Self-Regenerating Suppressor operated in the “Gas-Assisted Recycle” mode. Two different gradient methods are described: a 27-min separation and 41-min separation. The Thermo Scientific™ Dionex™ EG40 Eluent Generator generates high-purity and carbonate-free hydroxide eluents online to improve the method performance for determination of target analytes at trace levels.<sup>4,5</sup> The high-volume, direct-injection technique is used to achieve sensitive detection at low- to sub- $\mu\text{g/L}$  levels without the use of a concentrator column or loading pump and valve.<sup>6-8</sup> This application note expands on work presented in Thermo Scientific Technical Note 48, *Determination of Trace Anions in High-Purity Water by High-Volume Direct Injection with the EG40*.<sup>9</sup>

## Experimental

### Equipment

- Thermo Scientific™ Dionex™ DX-600 ion chromatography system\* consisting of:
  - Dionex GS50 Gradient Pump
  - Dionex CD25 Conductivity Detector
  - Dionex LC30 Chromatography Enclosure equipped with a six-port valve

\* Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system or the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

- Columns:
  - Thermo Scientific™ Dionex™ IonPac™ AS17 analytical column, 2 × 250 mm (P/N 055683)
  - Thermo Scientific™ Dionex™ IonPac™ AG17 guard column, 2 × 50 mm (P/N 055685)
  - Thermo Scientific™ Dionex™ IonPac™ ATC-1 Trap column, 9 × 24 mm (Replaced by Thermo Scientific™ Dionex™ IonPac™ ATC-3 Trap column, 9 × 24 mm, P/N 059660) placed after pump
- Dionex ASRS ULTRA suppressor, 2 mm (Replaced by Thermo Scientific™ Dionex™ AERS™ 500 (2 mm), P/N 082541)
- Dionex EG40 Eluent Generator with Thermo Scientific™ Dionex™ EluGen™ EGC-KOH cartridge
- Thermo Scientific™ Dionex™ SRS Gas-Assisted Regeneration Kit (P/N 056886)
- Pressurized sample vessel and low-pressure 4-way valve, 10-32 fittings (P/N 045010), optional
- Thermo Scientific™ Chromeleon™ Chromatography Workstation
- 300 cm of green 0.75 mm (0.030 in.) PEEK™ tubing to make a 1000 µL sample loop

### Reagents and standards

- Deionized water, Type I reagent-grade, 18 MΩ·cm resistance
- ACS reagent-grade materials for preparing anion standards (VWR or other)
- Sodium hydroxide 50% w/w aqueous solution (Fisher Scientific)
- Potassium hydroxide 45% w/w aqueous solution (Fisher Scientific) (optional in place of sodium hydroxide)
- 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)
- 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)

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### Conditions

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Eluent: Potassium hydroxide (Dionex EG40 Eluent Generator as the source)

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Temperature: 30 °C

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Dionex EG40

Offset Volume: 0 µL

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Eluent Flow

Rate: 0.5 mL/min

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Detection: Suppressed conductivity, Dionex ASRS ULTRA Gas-Assisted Recycle mode

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Dionex ASRS

Current Setting: 100 mA

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Expected

Background

Conductivity: 1 µS (40 mM KOH)

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Expected

System

Backpressure: 15.2–16.6 MPa (2200–2400 psi)

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Sample Volume: 1 mL

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**Table 1. Pump program Method 1.**

Time (min)	Flow (mL/ min)	A (%)	Valve	Dionex EG40 Conc. (mM)	Comments
Initial	0.50	100	Load	20.0	20 mM KOH
0.00	0.50	100	Load	1.0	Load sample loop, equilibrate 1.0 mM KOH for 5 min
5.00	0.50	100	Inject	1.0	Inject
15.00	0.50	100	Inject	1.0	1 mM KOH
19.00	0.50	100	Load	12.0	12 mM KOH
25.00	0.50	100	Load	20.0	20 mM KOH
27.00	0.50	100	Load	20.0	20 mM KOH

**Table 2. Pump program Method 2.**

Time (min)	Flow (mL/ min)	A (%)	Valve	Dionex EG40 Conc. (mM)	Comments
Initial	0.50	100	Load	40.0	40 mM KOH
0.00	0.50	100	Load	0.3	Load sample loop, equilibrate 0.3 mM KOH for 5 min
5.00	0.50	100	Inject	0.3	Inject
11.00	0.50	100	Inject	0.3	0.3 mM KOH
13.00	0.50	100	Inject	1.0	1 mM KOH
24.00	0.50	100	Load	10.0	10 mM KOH
41.00	0.50	100	Load	40.0	40 mM KOH

## Preparation of solutions and reagents

### Standard solutions

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

Several of the analytes of interest are available as 1000 mg/L anion standard solutions from Thermo Fisher Scientific or other commercial sources. In cases where commercial standards are not available, 1000 mg/L standards can be prepared by dissolving the appropriate amounts of the corresponding mass for the target analytes in 1000 mL of deionized water according to Table 1. 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.

### Composite standard solution

Composite standards at lower analyte concentrations are prepared from the 1000 mg/L standards above. Select a range similar to the expected analyte concentrations in the samples. Take aliquots from this dilute standard to make working standards at the low- $\mu\text{g/L}$  (ppb) down to the high ng/L (ppt) range. Dilute stock standards at the low-mg/L (ppm) levels should be prepared fresh weekly. Working standards at the low- $\mu\text{g/L}$  (ppb) range should be made fresh daily.

**Table 3. Amounts of compounds used to prepare 1 L of 1000 mg/L ion standards.**

Anion	Compound	Mass (g)
Fluoride	Sodium fluoride (NaF)	2.210
Acetate	Sodium acetate (CH <sub>3</sub> COONa•3H <sub>2</sub> O)	2.305
Formate	Sodium formate (HCOONa)	1.511
Acrylate	Sodium acrylate (H <sub>2</sub> C=CHCO <sub>2</sub> Na)	1.324
Methacrylate	Sodium methacrylate (H <sub>2</sub> C=C(CH <sub>3</sub> )CO <sub>2</sub> Na)	1.270
Chloride	Sodium chloride (NaCl)	1.648
Nitrite	Sodium nitrite (NaNO <sub>2</sub> )	1.499
Bromide	Sodium bromide (NaBr)	1.288
Nitrate	Sodium nitrate (NaNO <sub>3</sub> )	1.371
Benzoate	Sodium benzoate (C <sub>6</sub> H <sub>5</sub> CO <sub>2</sub> Na)	1.190
Sulfate	Sodium sulfate (Na <sub>2</sub> SO <sub>4</sub> )	1.479
Oxalate	Sodium oxalate (Na <sub>2</sub> C <sub>2</sub> O <sub>4</sub> )	1.522
Phthalate	Phthalic acid (C <sub>6</sub> H <sub>4</sub> -1,2-(CO <sub>2</sub> H) <sub>2</sub> )	1.000
Phosphate	Potassium phosphate, monobasic (KH <sub>2</sub> PO <sub>4</sub> )	1.433

## ATC regeneration solution

### 2 M Sodium hydroxide

Dilute 160 g of 50% (w/w) sodium hydroxide with degassed, deionized water (with a specific resistance of 18 M $\Omega$ -cm) to a final weight of 1080 g in an eluent bottle. Avoid the introduction of carbon dioxide from the air. Note that 2 M potassium hydroxide can be used instead of 2 M sodium hydroxide. Preparation is the same as above, except that 249 g of 45% potassium hydroxide is used for a final weight of 1090 g.

## System preparation and setup

This section describes the procedures for the initial installation and start-up of the Dionex ASRS ULTRA suppressor, Dionex IonPac ATC-1 trap column, and Dionex EGC-KOH EluGen cartridge. Prepare the Dionex ASRS ULTRA suppressor according to the *Quickstart Instructions for the Dionex ASRS ULTRA* (Document No. 031368). Prepare the Dionex IonPac ATC-1 trap column for use by rinsing it with 200 mL of 2 M KOH or 2 M NaOH at 2.0 mL/min. This preparation can be done offline without the Dionex GS50 gradient pump by pressurizing an eluent bottle with helium at 34.5 kPa (5 psi). Rinse the Dionex IonPac ATC-1 trap column with deionized water at 2.0 mL/min for 20 min and then stop.

Install the Dionex EGC-OH EluGen cartridge according to the instructions in the *Operator's Manual for the EG40 Eluent Generator System* (Document Number 031373). Place the 4 mm Dionex ATC-1 column between the Dionex GS50 gradient pump outlet and the Dionex EGC-KOH cartridge inlet, as shown in Figure 9, "EG40 Flow Path Diagram" in the *EG40 Eluent Generator system manual*.

Program the Dionex EG40 eluent generator to generate the highest concentration of KOH that will be used by the method and equilibrate the separation column for 30 min at the method flow rate. To operate the Dionex ASRS ULTRA suppressor for the "Gas-Assisted Recycle" mode, use the Dionex Gas-Assisted Regeneration Kit (P/N 056886). Connect the "Regen-In" line (shorter tubing from the tee) to the SRS "Regen-In" port. Connect the line from the "Cell-Out" on the conductivity cell to the "From-Cell" line (longer tubing from the tee). Connect a gas line to the regulator. Compressed air can be used in place of helium or nitrogen if it is purified with suitable traps to remove moisture, hydrocarbons, carbon dioxide, and other particulate matter. For more information, see the *SRS Gas-Assisted Regeneration Kit Installation and Use Instructions* (Document No. 031665-02).

Make a 1000  $\mu$ L sample loop by cutting a 220 cm portion of the green 0.030 in. (0.75 mm) i.d. PEEK tubing. If a loop or tubing with different internal diameter is desired, refer to Table 4 to calculate the length needed. The volume of a loop can be verified by measuring the weight difference between the sample loop filled with deionized water and the empty loop. The inside diameter of the PEEK tubing can vary by as much as 20% (for example, 0.010  $\pm$  0.002 in.). Connect the columns and suppressor in the IC system by using the red 0.005 in. (0.125 mm) or yellow 0.003 in. (0.075 mm) PEEK tubing. Keep the lengths of connecting tubing as short as possible to minimize the system void volume. Minimizing ensures efficient 2 mm column operation. Carefully use a plastic tubing cutter to ensure that the surfaces of the tubing cuts have straight, smooth surfaces. Irregularity on the surface of a tubing end can result in unwanted additional dead volume.

**Table 4. Volume per unit length for various tubing internal diameters.**

Material	Color	Internal Diameter (Inches)	Internal Diameter (mm)	Est. Vol. ( $\mu$ L/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

Turn on the gradient pump to begin the flow of eluent through the system. If the system backpressure is below 14 MPa (2000 psi), a length of yellow PEEK 0.003 in. (0.075 mm) tubing should be added between the outlet of the degas assembly in the Dionex EG40 eluent generator and inlet of the injection valve. For optimal Dionex EG40 eluent generator performance, maintain a system backpressure of 15.2–16.6 MPa (2200–2400 psi). Confirm that no leaks exist anywhere in the chromatographic pathway. For more information, see the *Operator's Manual for the EG40 Eluent Generator System* (Document Number 031373).

After stable eluent flow has been established, apply 10–15 psi gas pressure to the "Regen-In" port of the Dionex ASRS ULTRA suppressor. A mixture of gas and liquid will be observed exiting the "Regen-Out" port.

Turn the power on to supply current to the Dionex ASRS ULTRA suppressor; the gas flow rate across the regenerant chamber will be about 100 mL/min at a set gas pressure of 10–12 psi.

Turn on the Dionex EG40 eluent generator to deliver the highest eluent concentration required by the method using the Chromeleon software's run program. Allow the Dionex LC30 oven to stabilize at 30 °C. Determine the quality of the blank by measuring the short-term noise. In a representative 1 min level portion of the chromatogram, a peak-to-peak measurement should be less than 10 nS. For trace analysis, it may take 12 h or more for the system to equilibrate to a stable low background conductivity. It is a good practice to run a system overnight to equilibrate for use the following day.

The sample is loaded with either a syringe or pressurized reservoir. When using a syringe, take care not to introduce contamination by contact of the sample with the syringe. The black rubber plunger in disposable plastic syringes can be a source of significant contamination. To avoid contamination, the syringe should be used to pull sample into the loop when placed at the waste port. Take care not to introduce bubbles into the loop by pulling too hard.

When loading sample with a pressurized reservoir, a low-pressure, double-stack valve at the waste port regulates when the sample is loaded into the loop. More details about sample loading strategies can be found in Thermo Scientific Technical Note 48, *Determination of Trace Anions in High-Purity Water by High-Volume Direct Injection with the EG40*. A Dionex AS40 or Dionex AS50 autosampler is not suitable for the determination of anions at concentrations below 10 µg/L (ppb) because the pathway of an autosampler contributes unwanted anionic contamination.

## Results and discussion

The Dionex EG40 eluent generator electrolytically produces high-purity KOH eluents using deionized water as the carrier stream.<sup>4</sup> It can generate eluents that are free of carbonate contamination. For gradient separations, it provides negligible baseline shifts, greater retention time reproducibility, and better method precision. Background conductivity is lower, providing the best signal-to-noise ratio. These features enhance the

ion chromatographic performance for the determination of anions with the Dionex EG40 eluent generator at trace levels.<sup>5</sup>

The use of the Thermo Scientific™ Dionex™ IonPac™ AS15-5µm column was evaluated for the determination of anions at trace levels in a previous study.<sup>10</sup> Table 5 summarizes the retention time results for the analytes of interest using a gradient from 7 to 60 mM KOH. Notice that several analytes are not well resolved: acrylate (10.1 min), chloride 10.8 min), carbonate (13.0 min), benzoate (13.3 min), and methacrylate (13.4 min). However, this column has very good resolution of fluoride and the weakly retained organic acids such as acetate, glycolate, and formate. For more information about this separation, see Application Update 142, *Improved Determination of Trace Anions in High-Purity Waters by High-Volume Direct Injection with the EG40*.<sup>10</sup>

**Table 5. Anion retention times by high-volume direct-injection ion chromatography with the Dionex EG40 eluent generator and the Dionex IonPac AS15-5µm column.**

Anion	Retention Time (min)
Fluoride	6.2
Glycolate	7.0
Acetate	7.7
Formate	8.1
Acrylate	10.1
Chloride	10.8
Nitrite	12.0
Carbonate	13.0
Benzoate	13.3
Methacrylate	13.4
Sulfate	14.2
Oxalate	14.5
Bromide	15.2
Nitrate	16.0
Phosphate	16.5
Phthalate	21.2

Conditions (see Application Update 142, *Improved Determination of Trace Anions in High Purity Waters by High-Volume Direct Injection with the EG40*).

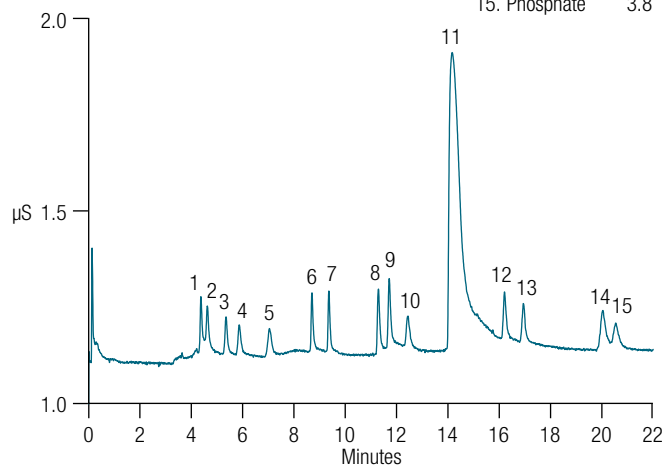
The Dionex IonPac AS17 column is chosen for this method because it provides the best selectivity for the analytes of interest to the electronics industry: common inorganic anions, low-molecular-weight organic acids, acrylate, methacrylate, benzoate, and phthalate. For trace analysis, the large-loop injection technique is used with a weak starting eluent concentration. The Dionex ASRS ULTRA suppressor delivers low background and noise for sensitivity at trace levels. The “Gas-Assisted Recycle” mode is chosen to supply the regenerant solution to it. This mode provides the benefit of low noise without the need for external water, significantly reducing water usage and waste. The “Gas-Assisted Recycle” mode reduces peak-to-peak noise from a Dionex ASRS ULTRA suppressor by a factor of 4 to 10 while using the eluent as the source for regenerant. The use of the Thermo Scientific™ Dionex™ DS-3 conductivity cell minimizes the effects of cell drift and temperature fluctuations.

Two gradient methods were developed in this study: Method 1 has a separation time of 27 min and Method 2 takes 41 min. Method 1 is best suited for well-characterized samples where fast sample throughput is important. Method 2 provides better separation of analytes and is suited for samples containing target analytes at disparate concentrations.

Both methods begin with a 5 min equilibration at the starting eluent concentration. This dilute eluent is used to elute the weakly retained ions such as fluoride, acetate, and formate. A gradient to a higher KOH concentration is used to separate the more strongly retained ions, such as sulfate and phosphate. The chromatographic baseline shift during the gradient is typically less than 200 nS when using the Dionex EG40 eluent generator. The separations are performed at 30 °C to provide consistent retention times during trace analysis. Typical chromatograms for low-level standards for Method 1 and Method 2 are shown in Figures 1 and 2, respectively.

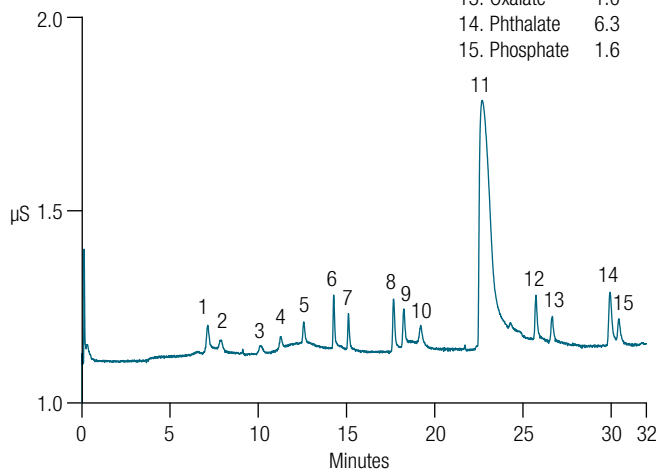
A representative deionized water blank for both methods is shown in Figures 3 and 4, respectively. Determining a blank establishes a starting point above which trace-level anion determinations can be made. No significant contaminants were detected except an unidentified peak and carbonate. The use of a high quality deionized water purification system minimizes the amount of carbonate.

Column:	Dionex IonPac AG17, AS17, 2 mm	Peaks:	
Eluent:	1.0 mM potassium hydroxide for 3 min 1 to 12 mM from 3–10 min 12 to 20 mM from 10–14 min	1. Fluoride	0.61 µg/L
Eluent Source:	Dionex EG40	2. Acetate	7.0
Flow Rate:	0.5 mL/min	3. Formate	1.5
Injection:	1 mL	4. Acrylate	3.7
Temperature:	30 °C	5. Methacrylate	4.2
Detection:	Suppressed conductivity, Dionex ASRS ULTRA Gas-assisted recycle mode	6. Chloride	1.2
		7. Nitrite	2.0
		8. Bromide	3.1
		9. Nitrate	3.8
		10. Benzoate	7.0
		11. Carbonate	–
		12. Sulfate	2.1
		13. Oxalate	1.6
		14. Phthalate	10.8
		15. Phosphate	3.8



**Figure 1. Trace anion determination using the Dionex IonPac AS17 column with Method 1.**

Column:	Dionex IonPac AG17, AS17, 2 mm	Peaks:	
Eluent:	0.3 mM potassium hydroxide for 6 min 0.3 to 1 mM from 6–8 min 1–10 mM from 8–19 min, 10 to 40 mM from 19–36 min	1. Fluoride	0.24 µg/L
Eluent Source:	Dionex EG40	2. Acetate	0.82
Flow Rate:	0.5 mL/min	3. Formate	0.57
Injection:	1 mL	4. Acrylate	0.87
Temperature:	30 °C	5. Methacrylate	1.9
Detection:	Suppressed conductivity, Dionex ASRS ULTRA Gas-assisted recycle mode	6. Chloride	0.72
		7. Nitrite	0.90
		8. Bromide	1.8
		9. Nitrate	1.3
		10. Benzoate	2.5
		11. Carbonate	–
		12. Sulfate	1.2
		13. Oxalate	1.0
		14. Phthalate	6.3
		15. Phosphate	1.6



**Figure 2. Trace anion determination using the Dionex IonPac AS17 column with Method 2.**

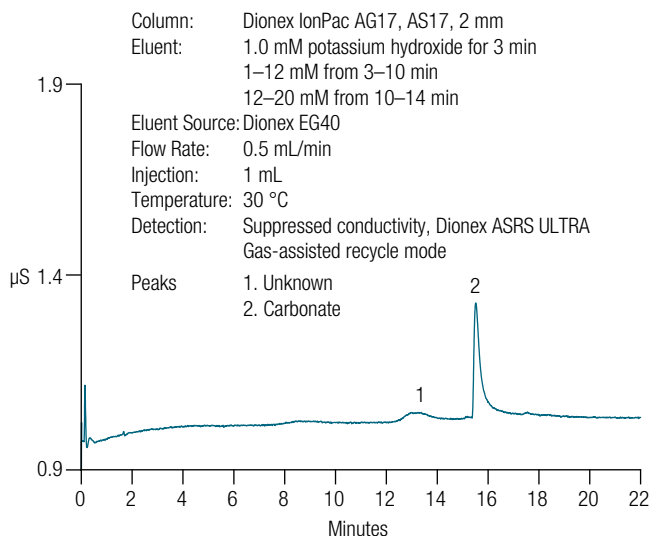


Figure 3. Representative blank for trace analysis with Method 1.

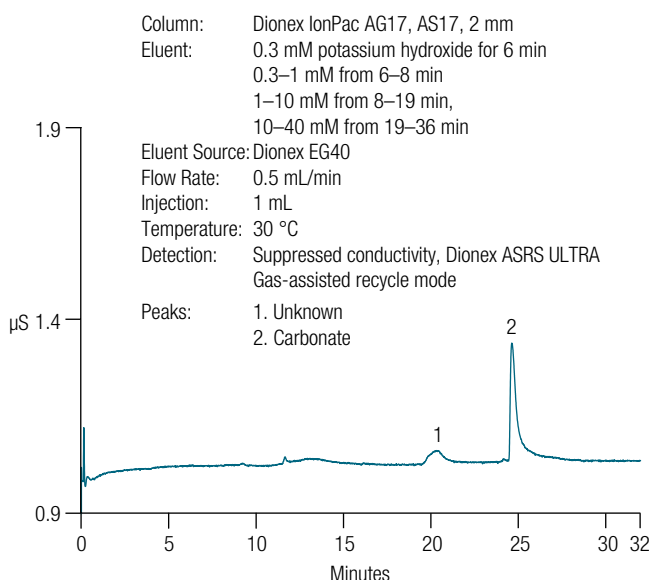


Figure 4. Representative blank for trace analysis with Method 2.

Method detection limits (MDLs) for the target analytes were established using Method 1 (Table 6). Method 2 yielded comparable results. The MDLs for the target analytes were determined by multiplying the standard deviation of seven replicate injections for the lowest-level standard with the Student's *t* value for the 99% confidence level.<sup>11</sup> Calibration curves were obtained using standards prepared in deionized water. The analyte concentrations in the calibration standards are listed in Table 7. Three replicate injections were used at each concentration level. Results for the anions of interest yielded a linear response with coefficients of determination (*r*<sup>2</sup>) greater than 0.99. To accurately determine the area of a peak at trace levels, it may be necessary to manually draw the baselines using tools

in the Chromeleon software. Remember that the lowest quantifiable analyte concentration is generally 3 to 5 times greater than the lowest detectable concentration.<sup>12</sup>

Table 6. Method detection limits for anions by high-volume direct-injection ion chromatography with the Dionex EG40 eluent generator and the Dionex IonPac AS17 2 mm column.

Anion	MDL <sup>a</sup> µg/L (ppb)
Fluoride	0.040
Acetate	1.1
Formate	0.85
Acrylate	0.44
Methacrylate	0.35
Chloride	0.12
Nitrite	0.23
Bromide	0.28
Nitrate	0.12
Benzoate	0.76
Sulfate	0.23
Oxalate	0.21
Phthalate	0.84
Phosphate	0.88

<sup>a</sup>MDL = (S.D.) × (*t*<sub>99%</sub>), where (*t*<sub>99%</sub>) is for a 99% single-sided Student's *t* test distribution for n = 7

Table 7. Calibration curve concentrations (µg/L) for anions by high-volume direct-injection ion chromatography with the Dionex EG40 eluent generator and the Dionex IonPac AS17 2 mm column.

Anion	Labels		
	1	2	3
Fluoride	0.1	0.3	1.0
Acetate	1.0	3.0	10.0
Formate	1.0	3.0	10.0
Acrylate	0.3	1.0	3.0
Methacrylate	1.0	3.0	10.0
Chloride	0.1	0.3	1.0
Nitrite	0.1	0.3	1.0
Bromide	0.3	1.0	3.0
Nitrate	0.3	1.0	3.0
Benzoate	1.0	3.0	10.0
Sulfate	0.3	1.0	3.0
Oxalate	0.3	1.0	3.0
Phthalate	1.0	3.0	10.0
Phosphate	1.0	3.0	10.0

## Precautions

Special care must be taken to minimize contamination when performing trace analysis. The quality of the blank will be an important determining factor to the concentration of ions that can be reliably determined in a sample. It is very important to use only the highest quality deionized water. When conducting analysis at trace levels, the sources of contamination are numerous. To minimize contamination, wear disposable, powder-free PVC gloves. After putting them on, rinse with deionized water and air dry. Do not dry with paper towels. All containers should be dedicated for this analysis and copiously rinsed with 18 M $\Omega$ -cm deionized water before use. Exercise caution when handling anything that could have contact with the blank, unknown, or standards. The flow path of the chromatographic instrumentation (eluent containers, injector, pump, valves, tubing, columns, suppressor, and conductivity cell) are all potential sources of contamination.

For this reason, the flow path should be minimized wherever possible. Take care when switching from a system setup that had previously seen significant concentrations of anions. Rinse with high-purity water to reduce residual contamination. The Dionex IonPac ATC-1 trap column should be periodically regenerated with the procedure described in the “System Preparation and Setup” section. Monitoring the blank for any significant increase in anionic contamination will indicate when regeneration is necessary. Monitoring the baseline shift during the Dionex EG40 eluent generator hydroxide gradient will indicate when regeneration is necessary.

A significant increase beyond 200 nS indicates that the Dionex IonPac ATC-1 trap column has exceeded its capacity to trap ionic contaminants and should be regenerated. The frequency of regeneration depends on the quality of the deionized water and usage rate of the instrument. Since the completion of this work, a new ATC—the Thermo Scientific™ Dionex™ IonPac™ ATC-HC (P/N 059604)—was introduced that extends the time between ATC regeneration.

If unexpected broad peaks appear in the chromatogram, it may be necessary to modify the gradient. These peaks can result from retained species of previous injections. Confirm this possibility by using an eluent concentration higher than the ending concentration of the gradient. It is best to run with this higher concentration for a period of time after all the peaks of interest have eluted. To confirm that the column has been cleaned of retained species, rerun a sample to observe the quality of the separation.

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