DIONEX

Application Update 142

Improved Determination of Trace Anions in High Purity Waters by High-Volume Direct Injection with the EG40

INTRODUCTION

This document describes the use of the EG40 potassium hydroxide (KOH) eluent generator with the IonPac® AS15-5µm (3 x 150 mm) column for trace anion analysis. The EG40 generates high-purity and carbonate-free hydroxide eluents on-line to improve the method performance for determination of target analytes at trace levels.[1,2] The high-volume direct injection technique is used to achieve sensitive detection at low to sub-µg/L levels without the use of a concentrator column, sample loading pump, and additional valve.[3-5] The AS15-5µm column is used to separate common inorganic anions and low molecular weight organic acids in less than 20 minutes. This column provides improved peak efficiencies, which results in lower detection limits than the AS15-9µm column (4 x 250 mm). The analytes are detected by suppressed conductivity with a 2-mm ASRS®-ULTRA operated in the Gas-Assisted Recycle mode. This Application Update expands on work presented in Dionex Technical Note 48, “Determination of Trace Anions in High-Purity Water by High-Volume Direct Injection with the EG40”.[6]

EQUIPMENT

Dionex DX-600 ion chromatography system consisting of:

- GS50 Gradient Pump
- CD25 Conductivity Detector
- LC30 Chromatography Enclosure equipped with Rheodyne Model 9126 injector PEEK, rear loading (P/N 052291)

Columns: IonPac AS15-5µm analytical, 3 x 150 mm (P/N 057594)
IonPac AG15-5µm guard, 3 x 30 mm (P/N 057597)

Trap Columns: ATC-1 Trap, 9 x 24 mm (P/N 037151) placed after pump
ATC Trap, 4 x 35 mm (P/N 043131) placed between the EG40 degas module and injector

Suppressor: ASRS-ULTRA 2-mm (P/N 053947)

EG40 Eluent Generator with EluGen® EGC-KOH cartridge

Gas-Assisted Regeneration Kit (P/N 056886)

Pressurized sample vessel (P/N 037460) and low-pressure 3-way double stack valve (P/N 045009), optional

300 cm of green 0.75-mm (0.30-in.) PEEK tubing to make a 1000-µL sample loop

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

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

Sodium and potassium salts, ACS reagent grade, 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 instead of sodium hydroxide

Fluoride standard 1000 mg/L, 100 mL (Dionex P/N 037158)

Chloride standard 1000 mg/L, 100 mL (Dionex P/N 037159)

Sulfate standard 1000 mg/L, 100 mL (Dionex P/N 037160)

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

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

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

CONDITIONS

Eluent: Potassium hydroxide (EG40 as the source)

Temperature: 30 °C

EG40 Offset Volume: 0 µL

Pump Program					
Time (min)	**Flow (mL/min)**	**A (%)**	**Valve**	**EG40 Conc (mM)**	**Comments**
Initial	0.70	100	Load	60.0	60 mM KOH
0.00	0.70	100	Load	7.0	Load sample loop, equilibrate with 7.0 mM KOH for 5 min
5.00	0.70	100	Inject	7.0	Inject
10.00	0.70	100	Inject	7.0	
17.00	0.70	100	Inject	60.0	60 mM KOH
21.00	0.70	100	Load	60.0	60 mM KOH

Eluent Flow Rate: 0.7 mL/min

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

ASRS Current Setting: 100 mA

Expected Background Conductivity: 1 µS (60 mM KOH)

Expected System Backpressure: 13.8 MPa (2000 psi)

Sample Volume: 1 mL

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 ion standard solutions from Dionex 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 mass 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.

Table 1 Amounts of Compounds Used to Prepare 1 L of 1000-mg/L Ion Standards

Anion	Compound	Mass (g)
F^-	Sodium fluoride (NaF)	2.210
$C_2H_3O_3^-$	Glycolic acid ($C_2H_4O_3$)	1.000
CH_3COO^-	Sodium acetate ($CH_3COONa \cdot 3H_2O$)	2.305
$HCOO^-$	Sodium formate (HCOONa)	1.511
Cl^-	Sodium chloride (NaCl)	1.648
NO_2^-	Sodium nitrite ($NaNO_2$)	1.499
SO_4^{2-}	Sodium sulfate (Na_2SO_4)	1.479
$C_2O_4^{2-}$	Sodium oxalate ($Na_2C_2O_4$)	1.522
NO_3^-	Sodium nitrate ($NaNO_3$)	1.371
PO_4^{3-}	Potassium phosphate, monobasic (KH_2PO_4)	1.433

Composite Standard Solution

Composite standards of the target analyte at lower 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-µg/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-µg/L (ppb) range should be made fresh daily.

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 17.8 MΩ-cm or greater) to a final weight of 1080 g in an eluent bottle. Avoid the introduction of carbon dioxide from air. *Note: 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 SET-UP

This section describes the procedures for the initial installation and start-up of the ASRS-ULTRA, ATCs, and EGC-KOH EluGen cartridge. Prepare the ASRS according to the *Quickstart Instructions for the ASRS-ULTRA* (Document No. 031368). Prepare the ATCs for use by rinsing with 200 mL of 2 M KOH or 2 M NaOH at 2.0 mL/min. This can be done off-line without the

GS50 by pressurizing an eluent bottle with helium at 34.5 kPa (5 psi). Rinse the two ATCs with deionized water at 2.0 mL/min for 20 min.

Install the EGC-OH EluGen cartridge according to the instructions in the *Operator's Manual for the EG40 Eluent Generator System* (Document No. 031373). Place the 4-mm ATC-1 column between the GS50 outlet and the EGC-KOH cartridge inlet and the 2-mm ATC column between the EG40 degas unit outlet and the injection valve inlet, as shown in Figure 9, "EG40 Flow Path Diagram", in the *EG40 Eluent Generator System Manual*.

Program the EG40 to generate the highest concentration of KOH (60 mM) that will be used by the method and rinse the second ATC column and the separation column for 30 minutes at the method flow rate. For the Gas-Assisted Recycle mode, 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-µL sample loop by cutting a 220 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 2 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 tubing varies by as much as 20% (for example, 0.010 ± 0.002 in.).

Table 2 Volume per Unit Length for Various Tubing Internal Diameters

Material	Color	Internal Diameter		Estimated Volume
		(inches)	(millimeters)	(µL/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

Connect the columns and suppressor in the IC system by using the red 0.005-in. (0.125-mm) PEEK tubing. Keep the lengths of connecting tubing as short as possible to minimize system void volume. This will ensure efficient 3-mm column operation. Carefully use a razor blade or 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.

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 portion of yellow PEEK 0.003-in. (0.075 mm) tubing should be added between the outlet of the degas assembly in the EG40 and the inlet of the injection valve. For optimal EG40 performance, maintain a system backpressure of 15.2 to 16.6 MPa (2200 to 2400 psi). Confirm that there are no leaks anywhere in the chromatographic pathway. For more information see the *Operator's Manual for the EG40 Eluent Generator System* (Document No. 031373).

After stable eluent flow has been established, apply 10–15 psi gas pressure to the Regen In port of the ASRS. A mixture of gas and liquid will be observed exiting the Regen Out port. Turn the power on to supply current to the ASRS; the gas flow rate across the regenerant chamber will be about 100 mL/min at a set pressure of 10–12 psi.

Turn on the EG40 using PeakNet to deliver the highest eluent concentration required by the method. Allow the LC30 oven to stabilize at the 30 °C operating temperature. Assess the quality of the blank by measuring the short-term noise. In a representative 1-minute level portion of the chromatogram, a "peak-to-peak" measurement should be less than 10 nS. It will take at least 4 hours for the system to equilibrate to a stable background conductivity for trace analysis. 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 a 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 placed at the waste port and used to pull sample into the loop. Pulling too hard will introduce unwanted bubbles into the sample loop.

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 Dionex Technical Note 48, “Determination of Trace Anions in High-Purity Water by High-Volume Direct Injection with the EG40”. An AS40 or 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.

DISCUSSION AND RESULTS

The EG40 Eluent Generator electrolytically produces high-purity KOH eluents using deionized water as the carrier stream.[1] The EG40 can generate hydroxide eluents that are free of carbonate contamination. For gradient separations, the use of the EG40 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 method performance at trace levels.[2]

The IonPac AS15-5µm (3 x 150 mm) column is well suited for trace-level analysis of common inorganic anions and low molecular weight organic acids. For trace analysis a large loop injection was used with a weak beginning eluent concentration. The column provides improved peak efficiencies, which results in lower detection limits than the AS15-9µm (4 x 250 mm) column. The use of a shorter column provides the advantage of a faster run time. The ASRS-ULTRA delivers low background and noise for sensitivity at trace levels.

The Gas-Assisted Recycle mode was chosen to supply the regenerant solution to the ASRS. It provides the benefit of low noise without the need for external water, significantly reducing water usage and waste. Gas-Assisted Recycle mode reduces peak-to-peak noise from an ASRS by a factor of 4 to 10 when using the eluent as the source for regenerant. A DS-3 Detection Stabilizer minimizes the effects of cell drift due to temperature fluctuations.

The separation begins with an eluent concentration of 7 mM KOH to elute the weakly retained ions such as fluoride, glycolate, acetate, and formate. A gradient from 7 mM to 60 mM KOH is used to separate the more strongly retained ions such as sulfate and phosphate. The chromatographic baseline shift is typically less than 200 nS. The separation is performed at 30 °C to provide consistent retention times during trace analysis. Figure 1 shows a typical chromatogram for a standard 11 anions at trace levels.

Column:	IonPac® AG15, AS15, 3 mm
Eluent:	7 mM Potassium hydroxide for 5 min, 5-12 min gradient to 60 mM
Eluent Source:	EG40
Flow Rate:	0.7 mL/min
Injection:	1 mL
Temperature:	30 °C
Detection:	Suppressed conductivity, ASRS-ULTRA, Gas-Assisted Recycle mode

Peaks:				
	1. Fluoride	0.32 µg/L (ppb)	7. Carbonate	—
	2. Glycolate	0.84	8. Sulfate	0.83
	3. Acetate	1.1	9. Oxalate	0.82
	4. Formate	1.2	10. Bromide	2.9
	5. Chloride	0.34	11. Nitrate	0.87
	6. Nitrite	0.35	12. Phosphate	2.9

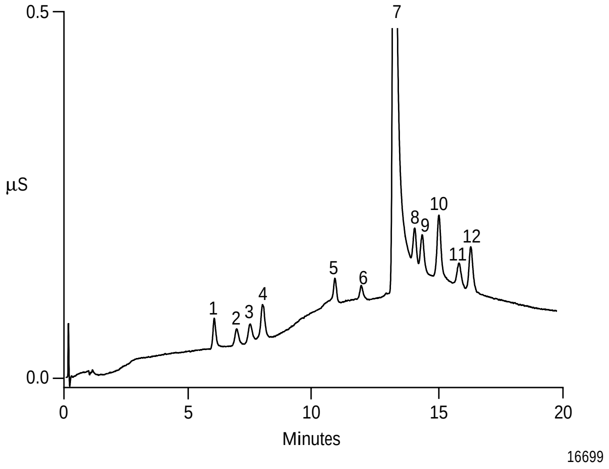

Figure 1. Trace anion determination using the IonPac AS15-5µm (3 x 150 mm).

A representative blank for this method is shown in Figure 2. Trace levels of fluoride, acetate, and formate originated from the point-of-use deionized water system. Determining a blank establishes a starting point above which anion determinations can be made.

Method detection limits (MDLs) established for the target anions are listed in Table 3. 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.[7] Calibration curves were obtained with standards prepared in deionized water using

Column:	IonPac® AG15, AS15, 3 mm
Eluent:	7 mM Potassium hydroxide for 5 min, 5-12 min gradient to 60 mM
Eluent Source:	EG40
Flow Rate:	0.7 mL/min
Injection:	1 mL
Temperature:	30 °C
Detection:	Suppressed conductivity, ASRS-ULTRA, Gas-Assisted Recycle mode

Peaks:		
	1. Fluoride	0.32 µg/L (ppb)
	2. Acetate	0.15
	3. Formate	0.11
	4. Carbonate	—

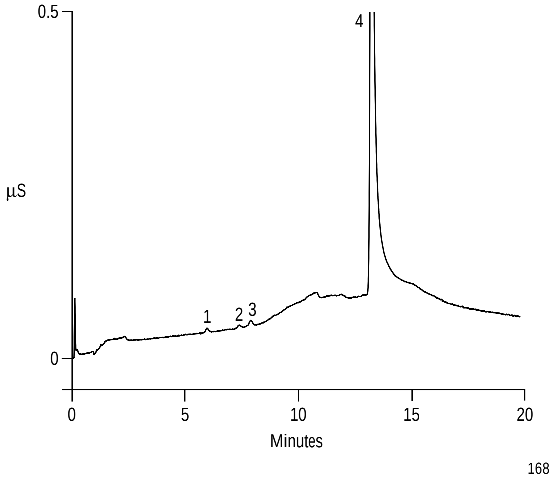

Figure 2. Representative blank for trace analysis using the IonPac AS15-5µm (3 x 150 mm).

Table 3 Method Detection Limits[a] for Anions by High-Volume Direct Injection Ion Chromatography with the EG40 and the IonPac AS15-5µm (3 x 150 mm)

Anion	MDL[a] µg/L (ppb)
Fluoride	0.040
Glycolate	0.065
Acetate	0.49
Formate	0.42
Chloride	0.17
Nitrite	0.13
Sulfate	0.12
Oxalate	0.11
Bromide	0.27
Nitrate	0.25
Phosphate	0.18

[a] MDL = (S.D.) x (t_s) 99%, where (t_s) is for a 99% single sided Student's t-test distribution for n=7.

Table 4 Calibration Curve Concentrations (µg/L) for Anions by High-Volume Direct Injection Ion Chromatography with the EG40 and the IonPac AS15-5µm (3 x 150 mm)

Anion	Levels 1	2	3
Fluoride	0.1	0.3	1.0
Glycolate	0.3	1.0	3.0
Acetate	0.3	1.0	3.0
Formate	0.3	1.0	3.0
Chloride	0.1	0.3	1.0
Nitrite	0.1	0.3	1.0
Sulfate	0.3	1.0	3.0
Oxalate	0.3	1.0	3.0
Bromide	1.0	3.0	10
Nitrate	0.3	1.0	3.0
Phosphate	1.0	3.0	10

the concentrations listed in Table 4. Calibration curves for the anions of interest were linear and had coefficients of determination (r^2) greater than 0.99.

PRECAUTIONS

Special care must be taken to minimize contamination when performing trace analysis. It is very important to use only the highest quality deionized water. When conducting analyses 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 17.8 MΩ-cm or better 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. Take care when switching from a system set-up that had previously seen significant concentrations of anions. Rinse with high purity water to reduce residual contamination.

The ATCs should be periodically regenerated with the procedure described in the System Preparation and

Set-up section. Monitoring the blank for any significant increase in anionic contamination will indicate when regeneration is necessary. Monitoring the baseline shift during the EG40 hydroxide gradient will also indicate when regeneration is necessary. A significant increase beyond 200 nS indicates that the ATC 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 the usage rate of the instrument.

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