



**Thermo Scientific**

**Dionex IonPac AS11-HC-4 $\mu$ m**

**Column Product Manual**

**P/N: 065463-04**

**May 2013**

## **Product Manual**

**for**

### **Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column**

(0.4  $\times$  250 mm, P/N 078031)

### **Dionex IonPac AS11-HC-4 $\mu$ m Analytical Column**

(2  $\times$  250 mm, P/N 078035)

(4  $\times$  250 mm, P/N 082313)

### **Dionex IonPac AG11-HC-4 $\mu$ m Capillary Guard Column**

(0.4  $\times$  50 mm, P/N 078032)

### **Dionex IonPac AG11-HC-4 $\mu$ m Guard Column**

(2  $\times$  50 mm, P/N 078036)

(4  $\times$  50 mm, P/N 078034)

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Revision 04, May, 2013, Corrected typo on page 2. Added emphasis to intended use statement on page 3.

## Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:



**SAFETY**

*Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.*



**WARNING**

*Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.*



**CAUTION**

*Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.*



**NOTE**

*Indicates information of general interest.*

**IMPORTANT**

*Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.*

**Tip**

*Highlights helpful information that can make a task easier.*

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# 1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column is specifically designed to provide high resolution of a large number of inorganic anions and organic acid anions from a single sample injection in one gradient run using hydroxide eluent system.

The Dionex IonPac AS11-HC-4µm column is a high resolution, high capacity anion exchange column with selectivity and capacity similar to the Thermo Scientific Dionex IonPac AS11-HC column. The high resolution provides better peak identification and high capacity allows injection of more concentrated samples without overloading the column. Hydroxide is normally used for gradient elution to minimize background shift. Because of high background conductance, sodium carbonate/bicarbonate eluents are not appropriate for gradient analysis but can be used for isocratic applications. By using a hydroxide gradient, strongly retained trivalent ions, such as phosphate and citrate, are efficiently eluted in the same run that also gives baseline resolution of the weakly retained monovalent anions: fluoride, lactate, acetate, formate, and butyrate.

Another benefit of using the Dionex IonPac AS11-HC-4µm column is the ability to easily change the order of elution of ions with different valencies simply by changing the gradient profile. For example, if citrate is present in high enough concentration to interfere with chromate, the chromate peak can be moved ahead of the citrate peak by using a slightly different gradient.

The Dionex IonPac AS11-HC-4µm column is available in  $0.4 \times 250$  mm,  $2 \times 250$  mm and  $4 \times 250$  mm formats, thus supporting flow rates from 0.015 mL/min to 1.5 mL/min. The Dionex IonPac AS11-HC-4µm column is stable between pH 0 and 14 and is compatible with eluents containing 0-100% organic solvents. The Dionex IonPac AG11-HC-4µm guard column is packed with a microporous resin with a lower capacity. The microporous resin ensures optimum long term performance of the guard column.

The Dionex IonPac AS11-HC-4µm Capillary Column ( $0.4 \times 250$  mm) requires only one-hundredth (1/100) the eluent flow rate of a typical 4 mm application. The capillary format has the advantage of less eluent consumption, providing reduced costs.

## 1.1 Dionex IonPac AS11-HC-4µm/Dionex IonPac AG11-HC-4µm Column Packing Specifications

### Resin Characteristics:

|                         |                                         |
|-------------------------|-----------------------------------------|
| Particle Size:          | 4 µm (Analytical/Capillary column*)     |
| Particle Size:          | 11 µm (Guard/Capillary Guard column**)  |
| Particle Cross-linking: | 55%                                     |
| Ion exchange capacity:  | 290 µeq per $4 \times 250$ mm column    |
|                         | 72.5 µeq per $2 \times 250$ mm column   |
|                         | 2.9 µeq per $0.4 \times 250$ mm column  |
|                         | 7 µeq per $4 \times 50$ mm column       |
|                         | 1.75 µeq per $2 \times 50$ mm column    |
|                         | 0.07 µeq per $0.4 \times 250$ mm column |

### Latex Characteristics:

|                   |                                 |
|-------------------|---------------------------------|
| Functional Group: | Alkanol quaternary ammonium ion |
| Hydrophobicity:   | Medium-Low                      |

\* Capillary Column resin composition: supermacroporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene.

\*\* Guard Column resin composition: microporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene.

**Table 1** Dionex IonPac AS11-HC-4 $\mu$ m/Dionex IonPac AG11-HC-4 $\mu$ m Column Operating Parameters

| Column                                                                  | Typical Back Pressure<br>psi (MPa) | Standard<br>Flow Rate<br>mL/min | Maximum Flow<br>Rate mL/min |
|-------------------------------------------------------------------------|------------------------------------|---------------------------------|-----------------------------|
| Dionex IonPac AS11-HC-4 $\mu$ m 0.4 mm<br>Capillary Column              | $\leq 3800$ (26.20)                | 0.015                           | 0.02                        |
| Dionex IonPac AG11-HC-4 $\mu$ m 0.4 mm<br>Capillary Guard Column        | $\leq 150$ (1.03)                  | 0.015                           | 0.02                        |
| Dionex IonPac AS11-HC-4 $\mu$ m and<br>AG11-HC-4 $\mu$ m 0.4 mm columns | $\leq 3950$ (27.23)                | 0.015                           | 0.02                        |
| Dionex IonPac AS11-HC-4 $\mu$ m 2 mm<br>Analytical Column               | $< 3800$ (26.20)                   | 0.38                            | 0.5                         |
| Dionex IonPac AG11-HC-4 $\mu$ m 2 mm<br>Guard Column                    | $\leq 150$ (1.03)                  | 0.38                            | 0.5                         |
| Dionex IonPac AS11-HC-4 $\mu$ m and<br>AG11-HC-4 $\mu$ m 2 mm columns   | $\leq 3950$ (27.23)                | 0.38                            | 0.5                         |
| Dionex IonPac AS11-HC-4 $\mu$ m 4 mm<br>Analytical Column               | $\leq 3800$ (26.20)                | 1.50                            | 2.0                         |
| Dionex IonPac AG11-HC-4 $\mu$ m 4 mm<br>Guard Column                    | $\leq 150$ (1.03)                  | 1.50                            | 2.0                         |
| Dionex IonPac AS11-HC-4 $\mu$ m and<br>AG11-HC-4 $\mu$ m 4 mm columns   | $\leq 3950$ (27.23)                | 1.50                            | 2.0                         |



**NOTE**

*For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.*

## 2. Ion Chromatography Systems

The Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column can only be operated using a Ion Chromatograph capable of operating at 5000 psi or higher such as the Thermo Scientific Dionex ICS-5000<sup>+</sup> or the Thermo Scientific Dionex ICS-4000 Capillary HPIC™ Systems. These systems are capable of operating up to 5000 psi to support the back pressure generated by the Dionex IonPac AS11-HC-4 $\mu$ m Column under standard operating conditions.

See Appendix B, "System Configuration" for specific recommendations including pumps, eluent flow rate, Thermo Scientific Dionex ASRS™ 300 Anion Self-Regenerating Suppressor™, Thermo Scientific Dionex ACES™ 300 Capillary Electrolytic Suppressor, injection loop, system void volume, and tubing back pressure.



### NOTE

***Do not operate suppressors over 40 °C. It is highly recommended to use Dionex ACES 300 at lower temperature (15°C) for optimum performance. Use of a Thermo Scientific Dionex EGC 500 KOH (P/N 075778) for the analytical set-up and Thermo Scientific Dionex EGC KOH (Capillary) (P/N 072076) cartridge for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.***

## 3. Installation

### 3.1 Column Start-Up

The column is shipped using 100 mM Sodium Borate as the storage solution. Prepare the eluent shown on the Quality Assurance Report (QAR), install the column in the chromatography module and direct the column effluent to waste for 30 minutes, and then connect to the suppressor. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

If peak efficiencies or resolution are poorer than the QAR, see Sections 3.13 Installation of the Capillary Column, Section 6.3.5 Poor Efficiency using Capillary Columns for information regarding proper connections and Section 6.3.1 Loss of Column Efficiency.

#### IMPORTANT

*When making any tubing connections (column installation, replacing tubing etc), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette with the flow off before joining two components together. This will prevent air from entering the system and result in a faster equilibration.*

### 3.2 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Borate for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

### 3.3 System Requirements for 0.4 mm Operation

The Dionex IonPac AS11-HC-4 $\mu$ m Capillary Guard and Capillary Columns are designed to be run on a capillary ion chromatograph system equipped with suppressed conductivity detection with the capability of continuously running at 5000 psi or higher. For best performance, it is recommended to run the capillary column only on the Dionex ICS-5000<sup>+</sup> HPIC system or the Dionex ICS-4000 Capillary HPIC system.

### 3.4 System Requirements for 2 mm and 4 mm Operation

The Dionex IonPac AS11-HC-4 $\mu$ m Guard and Analytical Column are designed to be run on an Ion Chromatograph equipped with suppressed conductivity detection with the capability of continuously running at 5000 psi or higher. For best performance, it is recommended to run the analytical column on a system rated 5000 psi or higher such as Dionex ICS-5000<sup>+</sup> HPIC system.

### 3.5 System Void Volume

The Dionex ICS-5000<sup>+</sup> HPIC system and the Dionex ICS-4000 Capillary HPIC system have preconfigured tubing to minimize the system void volume. The capillary tubing should only be replaced with precut tubing of the same type. It should also be noted that due to system configuration differences, the system void time in the capillary system will typically be longer than that observed with the analytical system at the same linear velocity. Slight modification of the method may be required to ensure equivalent retention time and peak resolution.

### 3.6 The Sample Concentrator

The function of a concentrator column in these applications is to strip ions from a measured volume of a relatively clean aqueous sample matrix. This process “concentrates” the desired analyte species onto the concentrator column, lowering detection limits by 2-5 orders of magnitude. The concentrator column is used in lieu of the sample loop.

The Thermo Scientific Dionex Trace Anion Concentrator Low Pressure Column (Dionex TAC-LP1, P/N 046026), the Dionex Trace Anion Concentrator Ultra Low Pressure Column (Dionex TAC-ULP1, P/N 061400), the Dionex Ultra Trace Anion Concentrator Low Pressure Column (Dionex UTAC-LP1, P/N 063079) or (Dionex UTACL2, P/N 079917), the Dionex Ultra Trace Anion Concentrator Ultra Low Pressure Column (Dionex UTAC-ULP1, P/N 063475) or (Dionex UTAC-ULP2, P/N 079918), the Dionex Ultra Trace Anion Concentrator Extremely Low Pressure Column (Dionex UTAC-XLP1, P/N 063459) or (Dionex UTAC-XLP2, P/N 072781), or the Dionex IonPac AG11-HC-4 $\mu$ m Guard Column can be used for trace anion concentration work with the 2 mm and 4 mm Dionex IonPac AS11-HC-4 $\mu$ m columns. For trace anion concentration work with the 0.4 mm Dionex IonPac AS11-HC-4 $\mu$ m column, use the Thermo Scientific Dionex IonSwift MAC-100 Concentrator Column.

Pump the sample onto the concentrator column in the OPPOSITE direction of the eluent flow. When using concentration techniques, do not overload the concentrator column by concentrating an excessive amount of sample. Concentrating an excessive amount of sample can result in inaccurate results being obtained. It is possible during the concentration step for the polyvalent anions such as phosphate and sulfate to elute the weakly retained anions such as fluoride and acetate off the concentrator column. For more detailed information on sample concentration techniques for high sensitivity work and a detailed discussion of anion concentration techniques refer to:

- Section 3, “Operation,” of the Thermo Scientific Dionex Trace Anion Concentrator Low Pressure (Dionex TAC-LP1) and Dionex Ultra Low Pressure (Dionex TAC-ULP1) Column Product Manual (Document No. 034972).
- Section 3, “Operation,” of the Thermo Scientific Dionex Ultra Trace Anion Concentrator Low Pressure (Dionex UTAC-LP1), Dionex Ultra Low Pressure (Dionex UTAC-ULP1), and Dionex Extremely Low Pressure (Dionex UTAC-XLP1) Column Product Manual (Document No. 065091.)
- Section 4, “Operation,” of the Thermo Scientific Dionex Ultra Trace Anion Concentrator 2 Low Pressure (Dionex UTAC-LP2), Dionex Ultra Low Pressure (Dionex UTAC-ULP2), and Dionex Extremely Low Pressure (Dionex UTAC-XLP2) Column Product Manual (Document No. 065376.)



#### CAUTION

***Thermo Scientific Dionex IonPac Trace Anion Concentrator Column, Dionex TAC-2 (P/N 043101), is not optimized for use with hydroxide eluents and should not be used for concentrator work with the Dionex IonPac AS11-HC-4 $\mu$ m column. Instead, Concentrators (Dionex TAC-LP1, TAC-ULP1, UTAC 1, UTAC 2 or Dionex IonSwift MAC-100) or Guards (Dionex IonPac AG11-HC 4 mm or Dionex IonPac AG11-HC 2 mm) should be used.***

## 3.7 The Injection Loop

### 3.7.1 The 0.4 mm System Injection Loop, 0.4 $\mu$ L Internal Loop

For most applications on a 0.4 mm capillary system, a 0.4  $\mu$ L injection loop is sufficient. Generally, do not inject more than 0.5 nanomoles of any one analyte into a 0.4 mm capillary column. Injecting larger numbers of moles of a sample can result in overloading the column, which can affect the detection linearity. For samples containing low concentrations of analytes, larger injection loops can be used to increase sensitivity.

### 3.7.2 The 2 mm System Injection Loop, 2 - 15 $\mu$ L

For most applications on a 2 mm analytical system, a 2 - 15  $\mu$ L injection loop is sufficient. Generally, you should not inject more than 10 nanomoles of any one analyte onto a 2 mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. As a general rule, install an injection loop one-fourth or less ( $<15 \mu$ L) of the loop volume used with a 4 mm analytical system.

### 3.7.3 The 4 mm System Injection Loop, 10 - 50 $\mu$ L

For most applications on a 4 mm analytical system, a 10 - 50  $\mu$ L injection loop is sufficient. Generally, you should not inject more than 40 nanomoles of any one analyte onto the 4 mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity.

## 3.8 The Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard Column

A Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard Column is normally used with the Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column. Retention times will increase by approximately 5% when a guard column is placed in-line prior to the analytical/capillary column under isocratic test conditions. A guard column is placed prior to the analytical/capillary column to prevent sample contaminants from eluting onto the analytical/capillary column. It is easier to clean or replace a guard column than it is an analytical/capillary column. Replacing the Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column.

## 3.9 Installing the Dionex CR-ATC Trap Column for Use with Dionex EGC

For Dionex IonPac AS11-HC-4 $\mu$ m applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC 500 (P/N 075550) for analytical systems and Dionex CR-ATC (Capillary ) (P/N 072078) for the Capillary system should be installed at the Dionex EGC eluent outlet to remove trace level anionic contaminants from the carrier deionized water. See the Dionex CR-TC Product Manual (Document No. 031910) for instructions.

## 3.10 Eluent Storage

Dionex IonPac AS11-HC-4 $\mu$ m columns are designed to be used with hydroxide eluent systems. Deionized water storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

### 3.11 Dionex Anion Self-Regenerating Suppressor Requirements

A Dionex Anion Self-Regenerating Suppressor should be used for applications that require suppressed conductivity detection. It is compatible with solvent containing eluents and aqueous ionic eluents of all concentrations with which the systems and columns are compatible. Aqueous ionic eluents can be used in all Dionex ASRS 300 modes of operation.



**CAUTION**

***Solvent containing eluents should be used in the AutoSuppression External Water Mode.***

For Dionex IonPac AS11-HC-4 $\mu$ m 4 mm Analytical Column, use a Dionex ASRS 300 (4 mm, P/N 061561).

For Dionex IonPac AS11-HC-4 $\mu$ m 2 mm Analytical Column, use a Dionex ASRS 300 (2 mm, P/N 061562).

For Dionex IonPac AS11-HC-4 $\mu$ m 0.4 mm Capillary Column, use a Dionex ACES 300 (2 mm, P/N 072052).

For detailed information on the operation of the Dionex Anion Self-Regenerating Suppressor, see Document No. 031956, the "Product Manual for the Dionex Anion Self-Regenerating Suppressor 300, the Dionex ASRS 300."

For detailed information on the operation of the Dionex Anion Capillary Electrolytic Suppressor 300, see Document No. 065386, the "Product Manual for the Dionex Anion Capillary Electrolytic Suppressor 300, the Dionex ACES 300"

### 3.12 Dionex Anion MicroMembrane Suppressor Requirements

A Thermo Scientific Dionex Anion MicroMembrane Suppressor 300 (Dionex AMMS 300) may be used instead of a Dionex ASRS 300 for applications that require suppressed conductivity detection. Use a Dionex AMMS 300 (4 mm) (P/N 064558) with the Dionex IonPac AS11-HC-4 $\mu$ m 4 mm Analytical Column. For 2 mm operation, use the Dionex AMMS 300 (2 mm) (P/N 064559). They are compatible with all solvents and concentrations with which the systems and columns are compatible.

For detailed information on the operation of the Dionex Anion MicroMembrane Suppressor, see Document No. 031727, the "Product Manual for the Dionex Anion MicroMembrane Suppressor 300, the Dionex AMMS 300".

### 3.13 Using Displacement Chemical Regeneration (DCR) with the Chemical Suppression Mode

The Dionex Displacement Chemical Regeneration (Dionex DCR) Mode is recommended for chemical suppression using sulfuric acid and the Dionex Anion MicroMembrane Suppressor (Dionex AMMS 300). See the DCR kit manual, Document P/N 031664, for details.



**SAFETY**

*Use proper safety precautions in handling acids and bases.*

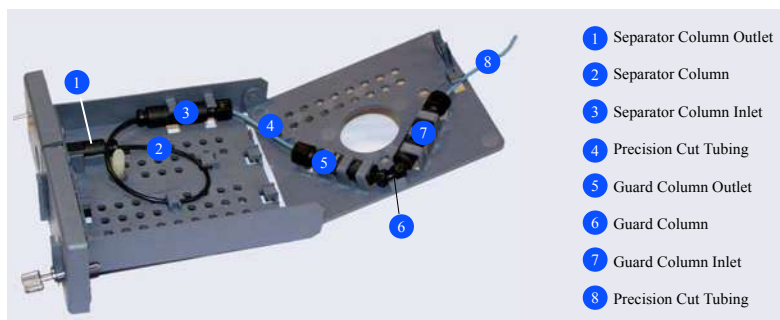
### 3.14 Dionex EGC-KOH Cartridge with Dionex IonPac AS11-HC-4 $\mu$ m Column

The Dionex IonPac AS11-HC-4 $\mu$ m column is recommended for use with ion chromatographs equipped with a Thermo Scientific Dionex Eluent Generator Cartridge (Dionex EGC 500 KOH Cartridge for analytical systems (P/N 075778) or Dionex EGC KOH (Capillary) Cartridge (P/N 072076) for capillary systems. The Dionex Eluent Generator is used to automatically produce potassium hydroxide gradients from deionized water. For detailed information on the operation of the Dionex EGC Cartridges, see Document No. 065018, the "Product Manual for the Dionex Eluent Generator Cartridges".

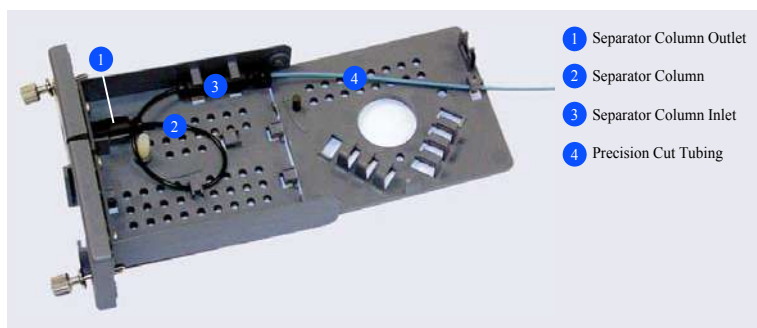
### 3.15 Installation of the Capillary Column

1. Before installing the new separator column, cut off the column label and slide it into the holder on the front of the cartridge (see Figure 6).
2. For reference, Figure 1 shows the column cartridge after installation of both a capillary guard column and a capillary separator column. Figure 2 shows the column cartridge after installation of only a capillary separator column.

**Figure 1** Separator and Guard Columns Installed in Column Cartridge



**Figure 2** Separator Column Only Installed in Column Cartridge



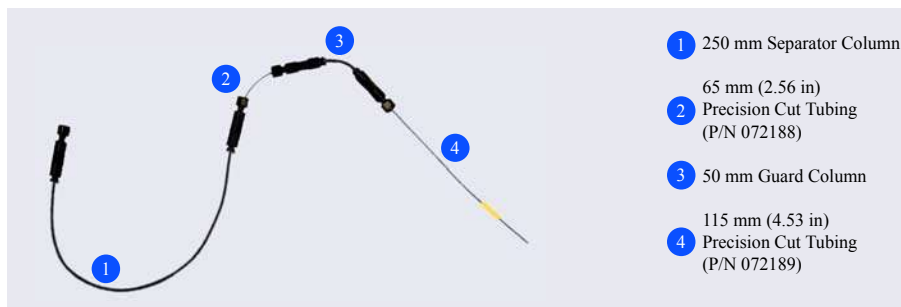
3. Locate the Dionex IC Cube Tubing Kit (P/N 072186) that is shipped with the Dionex IC Cube. The tubing kit includes the following items:

**Table 2** Contents of the Dionex IC Cube Tubing Kit (P/N 072186)

| Part                                                                          | Length / Quantity | Part Number | Used To Connect                                                              |
|-------------------------------------------------------------------------------|-------------------|-------------|------------------------------------------------------------------------------|
| Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue                       | 65 mm (2.56 in)   | 072188      | 50 mm guard column outlet to 250 mm separator column inlet                   |
| Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue, labeled VALVE PORT 3 | 115 mm (4.53 in)  | 072189      | Guard column inlet to injection valve                                        |
| Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue                       | 75 mm (2.93 in)   | 074603      | 35 mm guard column outlet to 150 mm separator column inlet                   |
| Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue, labeled VALVE PORT 3 | 210 mm (8.27 in)  | 072187      | Separator column inlet to injection valve (if a guard column is not present) |
| 0.25 mm (0.010-in) ID PEEK tubing, black                                      | 610 mm (24 in)    | 042690      | EG degas cartridge REGEN OUT to waste (if an EG is not present)              |
| Fitting bolt, 10-32 hex double-cone (smaller), black                          | 3                 | 072949      | Connect precision cut 0.062 mm (0.0025-in) ID PEEK tubing                    |
| Fitting bolt, 10-32 double-cone (larger), black                               | 1                 | 043275      | Connect 0.25 mm (0.010-in) ID PEEK tubing (black)                            |
| Ferrule fitting, 10-32 double-cone, tan                                       | 4                 | 043276      | Use with both sizes of fitting bolts                                         |

4. Refer to the following figures for the precision cut tubing required for your configuration:

**Figure 3**     Tubing Connections for 250 mm Separator Column and 50 mm Guard Column



**Figure 4**     Tubing Connections for Separator Column Only



5. Lift up the lid of the column cartridge to open it.
6. Remove the fitting plug from the outlet fitting on the separator column. Orient the fitting with a flat side up (see Figure 5) and push the fitting into the opening at the front of the column cartridge until it stops.

**Figure 5** Column Outlet Fitting Installed in Column Cartridge



7. Coil the separator column tubing inside the cartridge as shown in Figure 1 or Figure 2. Secure the column tubing and the inlet fitting in the clips on the column cartridge.
8. Secure the inlet and outlet fittings on the guard column (if used) in the column clips on the lid of the column cartridge.
9. Route the guard column inlet tubing (if used) or the separator column inlet tubing through the clip on the top edge of the column cartridge lid.
10. Close the lid (you should hear a click) and route the tubing into the slot on the front of the column cartridge (see Figure 6).



**NOTE**

*If the columns are installed correctly, the cartridge lid snaps closed easily. If the lid does not close easily, do not force it. Open the lid and verify that the columns and tubing are installed correctly and secured in the clips.*

**Figure 6** Column Cartridge Closed

- 1 Separator Column Outlet
- 2 Column Inlet Tubing



## 4. Operation

### 4.1 General Operating Conditions

|                                         |                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample Volume:                          | 0.4 mm: 0.4 µL Loop<br>2 mm: 2.5 µL Loop + 0.8 µL Injection valve dead volume<br>4 mm: 10 µL Loop + 0.8 µL Injection valve dead volume                                                                                                                                                                                                      |
| Column:                                 | 0.4 mm: Dionex IonPac AS11-HC-4µm 0.4 mm Capillary Column +<br>Dionex IonPacAG11-HC-4µm 0.4 mm Capillary Guard Column<br>2 mm: Dionex IonPac AS11-HC-4µm 2 mm Analytical Column +<br>Dionex IonPac AG11-HC-4µm 2 mm Guard Column<br>4 mm: Dionex IonPac AS11-HC-4µm 4 mm Analytical Column +<br>Dionex IonPac AG11-HC-4µm 4 mm Guard Column |
| Eluent:                                 | 30 mM KOH (for Quality Assurance Report)                                                                                                                                                                                                                                                                                                    |
| Eluent Source:                          | 0.4 mm: Dionex EGC -KOH (Capillary) cartridge<br>2 mm and 4 mm: Dionex EGC 500 KOH cartridge                                                                                                                                                                                                                                                |
| Eluent Flow Rate:                       | 0.4 mm: 0.015 mL/min<br>2 mm: 0.38 mL/min<br>4 mm: 1.5 mL/min                                                                                                                                                                                                                                                                               |
| SRS Suppressor:                         | Dionex Anion Self-Regenerating Suppressor, Dionex ASRS 300 (2 or 4 mm)<br>Dionex Anion Capillary Electrolytic Suppression, Dionex ACES 300 (0.4 mm)<br>AutoSuppression Recycle Mode                                                                                                                                                         |
| Expected Background Conductivity:       | < 2 µS                                                                                                                                                                                                                                                                                                                                      |
| Long-term Storage Solution (> 1 week):  | 100 mM Sodium Borate                                                                                                                                                                                                                                                                                                                        |
| Short-term Storage Solution (< 1 week): | Eluent                                                                                                                                                                                                                                                                                                                                      |

### 4.2 Dionex IonPac AS11-HC-4µm Column Operating Precautions

**Table 3** Operating Precautions

| Filter and Degas Eluents and Samples if Necessary |                      |
|---------------------------------------------------|----------------------|
| Eluent pH                                         | Between 0 and 14     |
| Sample pH                                         | Between 0 and 14     |
| Maximum Flow Rate for 0.4 mm Columns              | 0.02 mL/min          |
| Maximum Flow Rate for 2 mm Columns                | 0.5 mL/min           |
| Maximum Flow Rate for 4 mm Columns                | 2.0 mL/min           |
| Maximum Operating Pressure                        | 5,000 psi (34.47MPa) |

### 4.3 Chemical Purity Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic impurities. Chemicals, solvents and deionized water used to prepare eluents must be of the highest purity available. Low trace impurities and low particle levels in eluents also help to protect your ion exchange columns and system components. Thermo Fisher Scientific cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

#### 4.3.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label.

#### 4.3.2 Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The deionized water should be free of ionic impurities, organics, microorganisms and particulate matter larger than 0.2  $\mu\text{m}$ . Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.

#### 4.3.3 Solvents

Solvents can be added to the ionic eluents used with Dionex IonPac AS11-HC-4 $\mu\text{m}$  column to modify the ion exchange process or improve sample solubility. The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at Thermo Fisher Scientific, we have obtained consistent results using Optima® LC/MS Grade Solvents by Fisher Scientific.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the Dionex IonPac AS11-HC-4 $\mu\text{m}$  column is 5,000 psi (34.47MPa).

The Dionex IonPac AS11-HC-4 $\mu\text{m}$  column can withstand common HPLC solvents in a concentration range of 0 - 100%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.



NOTE

*Adding solvent to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use solvent in the eluent when needed for improved resolution of analytes of interest.*

**Table 4** HPLC Solvents for Use with Dionex IonPac AS11-HC-4 $\mu$ m Column

| Solvent         | Maximum Operating Concentration |
|-----------------|---------------------------------|
| Acetonitrile    | 100%                            |
| Methanol        | 100%                            |
| 2-Propanol      | 100%                            |
| Tetrahydrofuran | 20%*                            |

\*Higher concentrations may only be used for limited duration applications such as column clean-up at pressures < 4000 psi.



*The Dionex ASRS 300 and Dionex ACES 300 suppressors must be operated in the AutoSuppression External Water Mode when using eluents containing solvents. Do not use > 40% solvent with the Dionex ASRS 300 and Dionex ACES 300 suppressors in the electrolytic mode (power on).*

## 4.4 Making Eluents that Contain Solvents

When mixing solvents with water, remember to mix solvent with water on a volume to volume basis. For example, if a procedure requires an eluent of 90% acetonitrile, prepare the eluent by adding 900 mL of acetonitrile to an eluent reservoir. Then add 100 mL of deionized water or eluent concentrate to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume eluent is obtained. Premixing water with solvent will minimize the possibility of outgassing.



*When purging or degassing eluents containing solvents, do not purge or degas the eluent excessively since it is possible that a volatile solvent can be “boiled” off from the solution.*



*Always degas and store all eluents in glass or plastic eluent bottles pressurized with helium. Only helium can be used to purge and degas ionic eluents containing solvents, since nitrogen is soluble in solvent containing eluents.*



*Acetonitrile (ACN) hydrolyzes to ammonia and acetate when left exposed to basic solutions. To prevent eluent contamination from acetonitrile hydrolysis, always add acetonitrile to basic aqueous eluents by proportioning the acetonitrile into the basic eluent with the gradient pump. Keep the acetonitrile in a separate eluent bottle containing only acetonitrile and water.*



*Never add the acetonitrile directly to the basic carbonate or hydroxide eluent solutions.*

## 4.5 Eluent Preparation

The Dionex Eluent Generator Cartridge (Dionex EGC 500 KOH cartridge or Dionex EGC (Capillary) cartridge) is used to automatically produce potassium hydroxide gradients from deionized water. Please refer to the Dionex ICS-5000<sup>+</sup> HPIC system (Document No. 065446), or the Dionex ICS-4000 Capillary HPIC system (Document No. 065468) manual for information on the operation of the Eluent Generator.

For detailed information on the operation of the Dionex EGC Cartridges, see Document No. 065018, the "Product Manual for the Dionex Eluent Generator Cartridges".

## 5. Example Applications

### 5.1 Recommendations for Optimum System Performance

The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components and laboratory temperatures.

The Dionex IonPac AS11-HC-4 $\mu$ m column is designed to perform analyses of large numbers of anions of varying valencies through gradient elution. In any type of gradient elution system it is important to use eluents that produce a minimum shift in baseline conductivity during the run, as well as a fast equilibration time from one run to the next. Because potassium hydroxide is converted to water in the suppressor, it is the best choice for an eluent. As long as the capacity of the suppressor is not exceeded, the eluent hydroxide concentration has little effect on background conductivity. For example, a gradient run could begin at a few mM KOH and end at 100 mM KOH, with only a resulting 1 to 2  $\mu$ S total baseline change.

Ensure that your system is properly configured. Fluctuations in operating temperature can affect the retention time and resolution of analytes and should be controlled.

Ensure that adequate equilibration time is allowed between runs. If downward shift in baseline is observed during the isocratic section of the chromatogram, increase the equilibration time.

The addition of chromate to the sample will help stabilize organic acids. If your sample or standard contains organic acids, adding chromate (about 10 mg/L) will help stabilize them from bacterial degradation at room temperature. See the sample chromatogram in Section 5.3, “Gradient Analysis of a Large Number of Anions Using Aqueous KOH Eluent”.

Use a guard/capillary guard column to protect the analytical/capillary column. If column performance deteriorates and it is determined that the guard/capillary guard and analytical/capillary columns have been fouled, refer to the column cleanup protocols in Appendix A, “Column Care.”

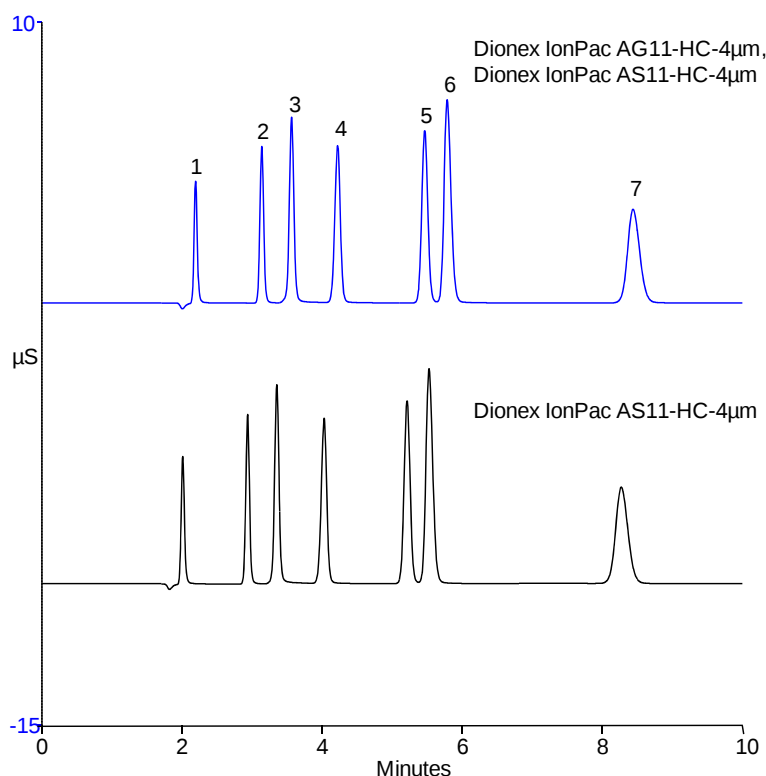
The sensitivity of the IC system can be increased by using sample concentration techniques (see Section 3.6, “The Sample Concentrator”).

## 5.2 Dionex IonPac AS11-HC-4 $\mu$ m Column With and Without Guard Column

Isocratic elution of common anions using the Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column has been optimized utilizing a hydroxide eluent. By using this eluent, common inorganic anions can be used to test the performance of the Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column. The Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column should always be used with the Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard Column, see Figure 7, Figure 8, and Figure 9. An operating temperature of 30°C is used to ensure reproducible resolution and retention of analytes. Note that the Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard column is packed with a microporous resin of proportionally lower capacity and contributes approximately 5% increase in retention times when used in-line prior to the Analytical/Capillary column under isocratic test conditions.

**Figure 7** Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column (0.4  $\times$  250 mm) With and Without Capillary Guard Column

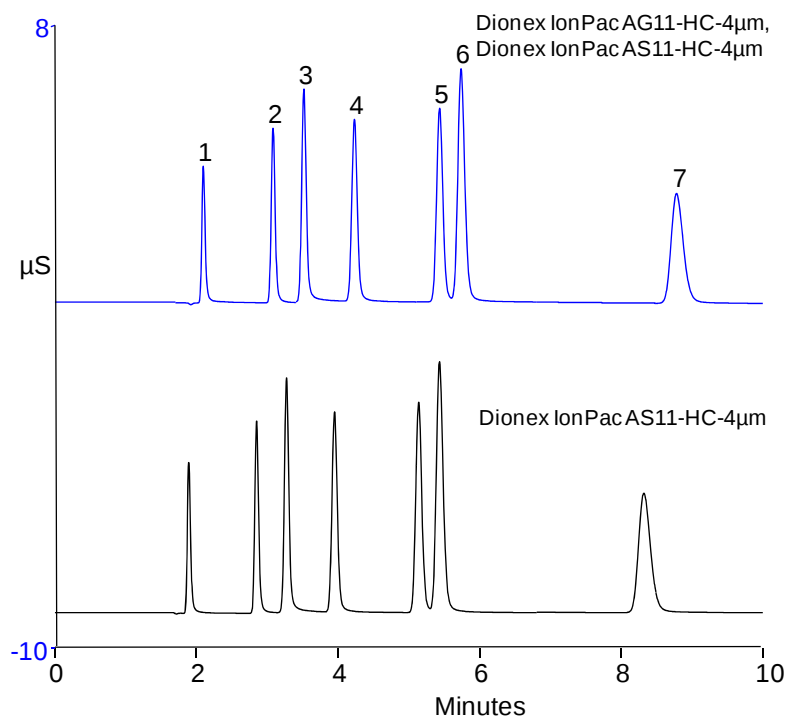
Column: See Chromatogram  
 Eluent : 30 mM KOH  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4  $\mu$ L  
 Temperature: 30 °C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, recycle mode



| Peaks:       | mg/L |
|--------------|------|
| 1. Fluoride  | 0.5  |
| 2. Chloride  | 1.0  |
| 3. Nitrite   | 2.0  |
| 4. Sulfate   | 2.0  |
| 5. Bromide   | 4.0  |
| 6. Nitrate   | 4.0  |
| 7. Phosphate | 6.0  |

**Figure 8** Dionex IonPac AS11-HC-4 $\mu$ m Column (2  $\times$  250 mm) With and Without Guard Column

Column: See Chromatograms  
 Eluent : 30 mM KOH  
 Eluent Source: Dionex EGC 500 KOH cartridge  
 Flow Rate: 0.38 mL/min  
 Inj. Volume: 2.5  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity,  
 Dionex ASRS 300, 2 mm  
 AutoSuppression, recycle mode

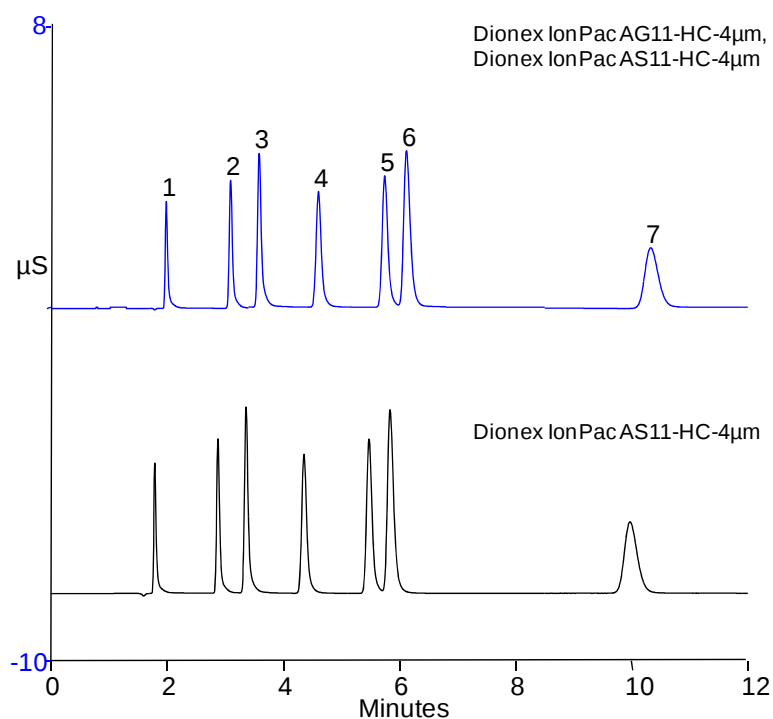


| Peaks:       | mg/L |
|--------------|------|
| 1. Fluoride  | 2.0  |
| 2. Chloride  | 5.0  |
| 3. Nitrite   | 10.0 |
| 4. Sulfate   | 10.0 |
| 5. Bromide   | 20.0 |
| 6. Nitrate   | 20.0 |
| 7. Phosphate | 30.0 |

## 5 – Example Applications

**Figure 9** Dionex IonPac AS11-HC-4 $\mu$ m Column (4  $\times$  250 mm) With and Without Guard Column

Column: See Chromatograms  
 Eluent : 30 mM KOH  
 Eluent Source: Dionex EGC 500 KOH cartridge  
 Flow Rate: 1.5 mL/min  
 Inj. Volume: 10  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity,  
 Dionex ASRS 300, 4 mm  
 AutoSuppression, recycle mode

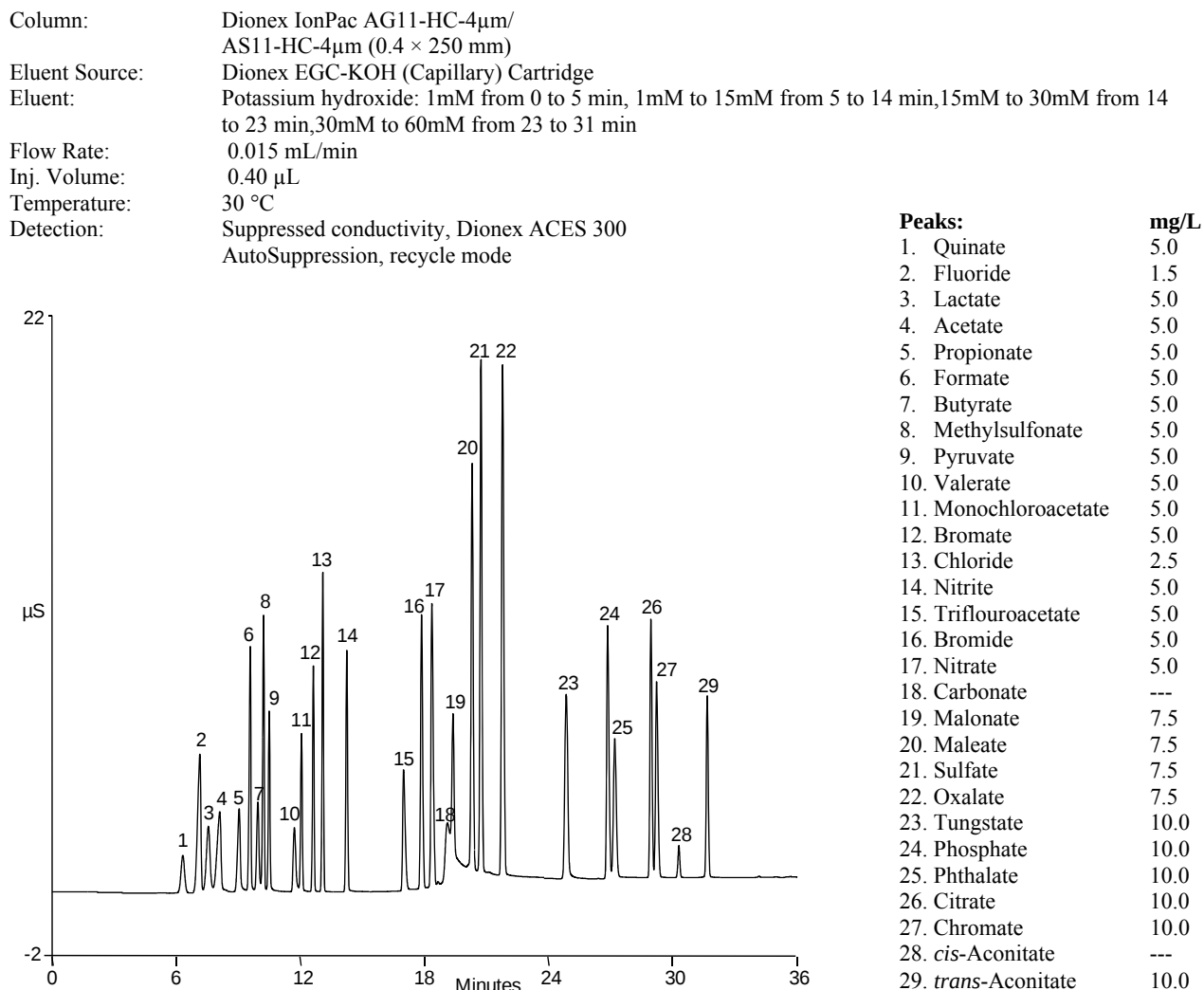


| Peaks:       | mg/L |
|--------------|------|
| 1. Fluoride  | 2.0  |
| 2. Chloride  | 5.0  |
| 3. Nitrite   | 10.0 |
| 4. Sulfate   | 10.0 |
| 5. Bromide   | 20.0 |
| 6. Nitrate   | 20.0 |
| 7. Phosphate | 30.0 |

### 5.3 Gradient Analysis of a Large Number of Anions using Aqueous KOH Eluent

Figure 10 illustrates the separation of a large number of inorganic anions and organic acids using the Dionex IonPac AS11-HC-4 $\mu$ m capillary column with a potassium hydroxide gradient. Note excellent separation of the early eluting peaks such as quinate, fluoride and lactate due to the high efficiency of this column while analysis time is similar to the Dionex IonPac AS11-HC capillary column.

**Figure 10** Gradient Analysis of a Large Number of Anions using Aqueous KOH Eluent

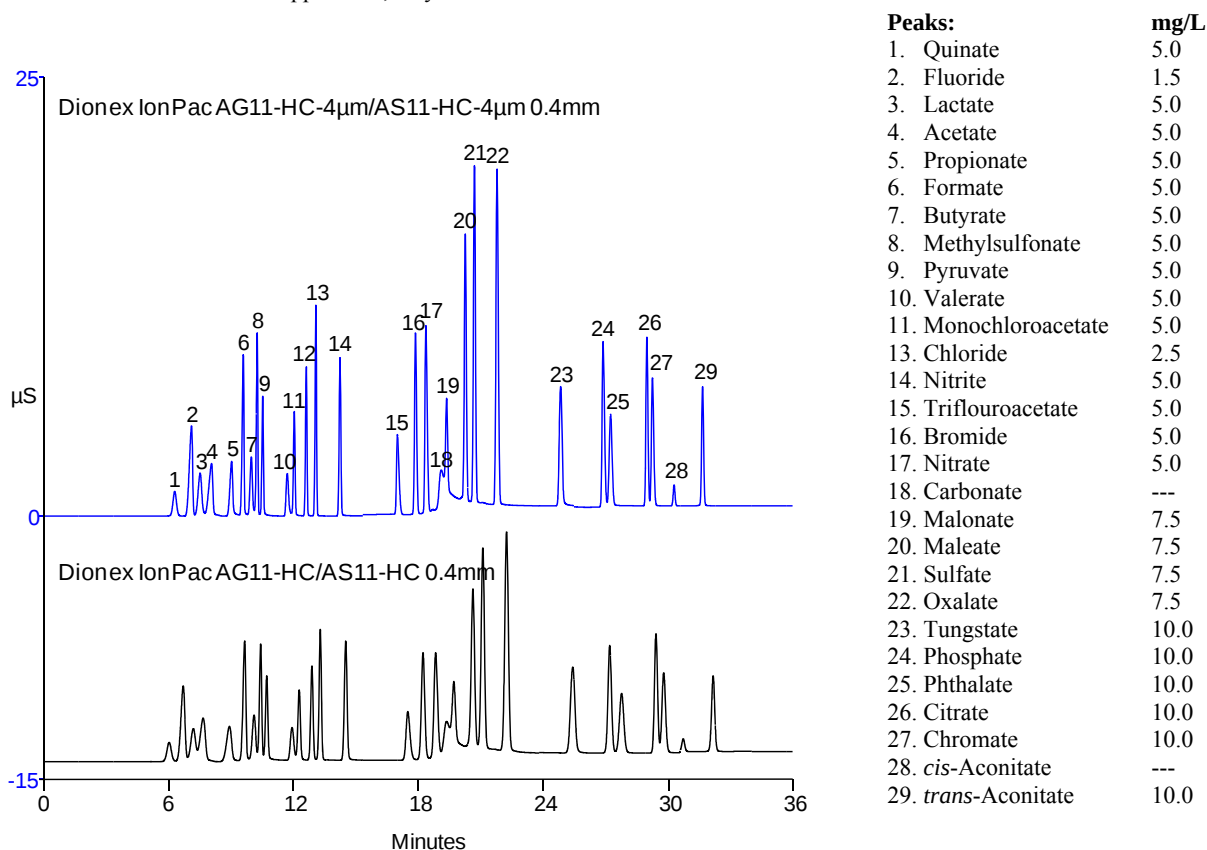


## 5.4 Comparison of Dionex IonPac AS11-HC and Dionex IonPac AS11-HC-4 $\mu$ m Columns

The new Dionex IonPac AS11-HC-4 $\mu$ m column is packed with smaller resin particles than the current Dionex IonPac AS11-HC column. The smaller resin particles produce more efficient peaks as shown in Figure 11A and 11B below. Note the improved resolution of the early eluting peaks when using the Dionex IonPac AS11-HC-4 $\mu$ m column. In general, all the peaks are sharper and taller, providing better resolution and sensitivity when using the Dionex IonPac AS11-HC-4 $\mu$ m column. Some variation in the 0.4 mm and 4 mm chromatogram profile (see Figure 11A and 11B) can be noticed due to slightly different delay volume for the two systems. In order to address the variations in the system delay volume, isocratic part of the method (see Figure 11A) has been modified for the 0.4 mm as compared to 4 mm method shown in Figure 11B.

**Figure 11A** Comparison of Dionex IonPac AS11-HC and Dionex IonPac AS11-HC-4 $\mu$ m Capillary Columns (0.4  $\times$  250 mm)

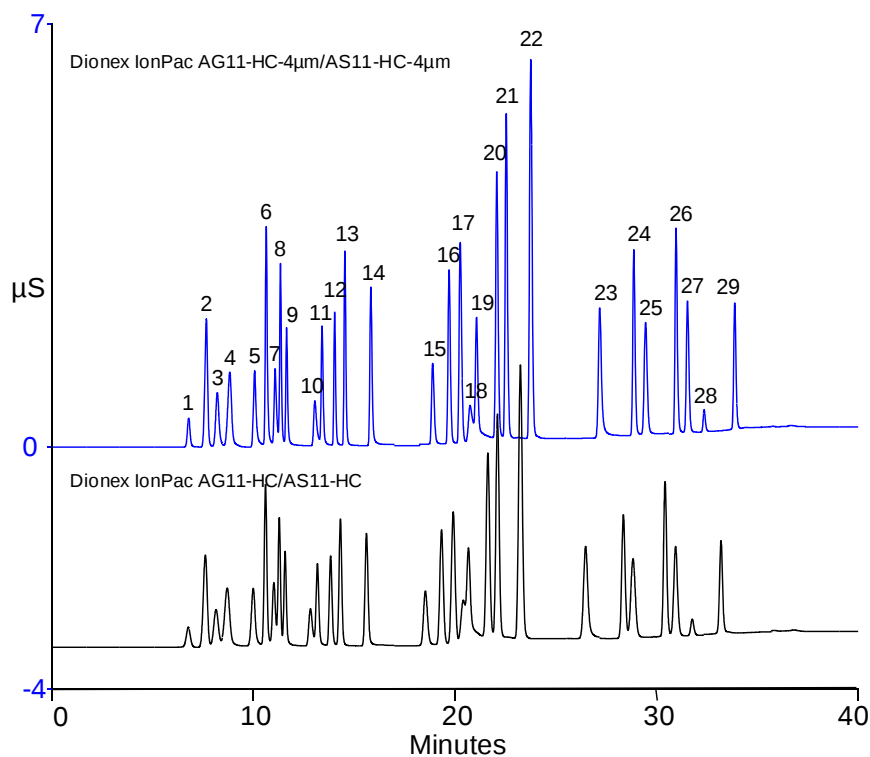
Column: See Chromatogram  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Eluent: Potassium hydroxide: 1mM from 0 to 5 min, 1mM to 15mM from 5 to 14 min, 15mM to 30mM from 14 to 23 min, 30mM to 60mM from 23 to 31 min  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.40  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed conductivity, Dionex ACES 300  
 AutoSuppression, recycle mode



**Figure 11B** Comparison of Dionex IonPac AS11-HC and Dionex IonPac AS11-HC-4 $\mu$ m Analytical Columns (4  $\times$  250 mm)

Column: See Chromatograms  
 Eluent Source: Dionex EGC 500 KOH cartridge  
 Eluent : 1mM KOH from 0 to 7 min,  
 1-15mM KOH from 7 to 16 min;  
 15 to 30mM KOH from 16 to 25 min  
 30 to 60mM KOH from 25 to 33 min  
 Flow Rate: 1.5 mL/min  
 Inj. Volume: 10  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity,  
 Dionex ASRS 300, 4 mm,  
 AutoSuppression, recycle mode

| Peaks:                      | mg/L |
|-----------------------------|------|
| 1. Quinate                  | 5.0  |
| 2. Fluoride                 | 1.5  |
| 3. Lactate                  | 5.0  |
| 4. Acetate                  | 5.0  |
| 5. Propionate               | 5.0  |
| 6. Formate                  | 5.0  |
| 7. Butyrate                 | 5.0  |
| 8. Methylsulfonate          | 5.0  |
| 9. Pyruvate                 | 5.0  |
| 10. Valerate                | 5.0  |
| 11. Monochloroacetate       | 5.0  |
| 13. Chloride                | 2.5  |
| 14. Nitrite                 | 5.0  |
| 15. Trifluoroacetate        | 5.0  |
| 16. Bromide                 | 5.0  |
| 17. Nitrate                 | 5.0  |
| 18. Carbonate               | ---  |
| 19. Malonate                | 7.5  |
| 20. Maleate                 | 7.5  |
| 21. Sulfate                 | 7.5  |
| 22. Oxalate                 | 7.5  |
| 23. Tungstate               | 10.0 |
| 24. Phosphate               | 10.0 |
| 25. Phthalate               | 10.0 |
| 26. Citrate                 | 10.0 |
| 27. Chromate                | 10.0 |
| 28. <i>cis</i> -Aconitate   | ---  |
| 29. <i>trans</i> -Aconitate | 10.0 |



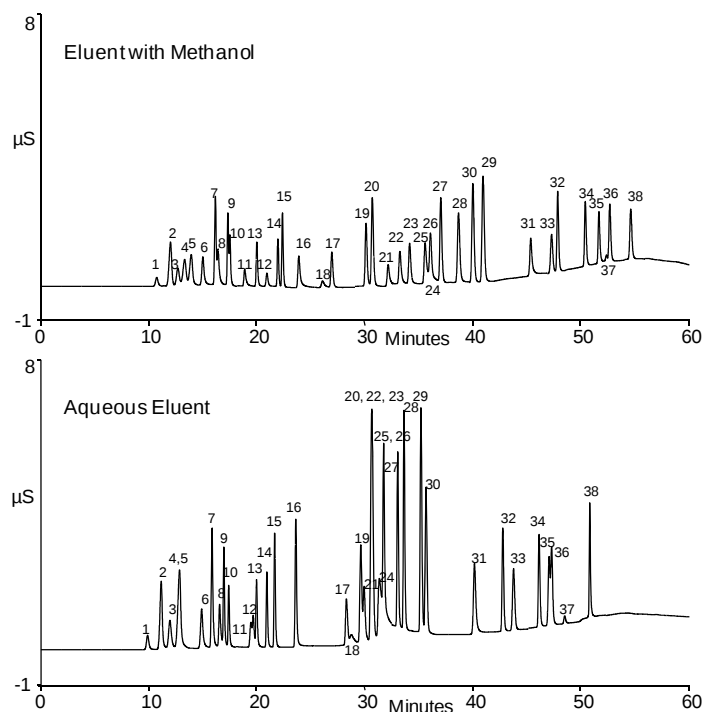
## 5.5 Separation of a Large Number of Inorganic Anions and Organic Acid Anions Using a KOH Gradient without and with Solvent

Figure 12 uses an optimized potassium hydroxide gradient as well as a potassium hydroxide gradient with methanol for the separation of a large number of inorganic anions and organic acid anions. Note that, early eluting peaks such as formate and butyrate are better separated with aqueous eluent, whereas later eluting peaks such as succinate and malate co-elute. However, by adding organic solvent (methanol) to the eluent, succinate and malate peaks are nearly baseline resolved. Note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode. Also note that adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when improved resolution of analytes of interest is necessary.

**Figure 12** Separation of a Large Number of Inorganic Anions and Organic Acid Anions Using a KOH Gradient without and with Solvent

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (4  $\times$  250 mm)  
Eluent with Methanol : 1mM KOH with 10% methanol from 0 to 10.7min,  
1-15mM KOH with 10% to 20% methanol from 10.7 to 24min,  
15 to 30mM KOH with 20% methanol from 24 to 37.3 min,  
30 to 60mM KOH with 20 to 10% methanol from 37.3 to 50.6 min  
Aqueous Eluent : 1mM KOH from 0 to 10.7min,  
1-15mM KOH from 10.7 to 24min,  
15 to 30mM KOH from 24 to 37.3 min  
30 to 60mM KOH from 37.3 to 50.6 min  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 1.0 mL/min  
Inj. Volume: 10  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 4 mm,  
AutoSuppression, \*external water mode

| Peaks:                      | mg/L |
|-----------------------------|------|
| 1. Quinate                  | 10   |
| 2. Fluoride                 | 3    |
| 3. Lactate                  | 10   |
| 4. Acetate                  | 10   |
| 5. Glycolate                | 10   |
| 6. Propionate               | 10   |
| 7. Formate                  | 10   |
| 8. Butyrate                 | 10   |
| 9. Methylsulfonate          | 10   |
| 10. Pyruvate                | 10   |
| 11. Valerate                | 10   |
| 12. Galacturonate           | 10   |
| 13. Monochloroacetate       | 10   |
| 14. Bromate                 | 10   |
| 15. Chloride                | 5    |
| 16. Nitrite                 | 10   |
| 17. Trifluoroacetate        | 10   |
| 18. Sorbate                 | 10   |
| 19. Bromide                 | 10   |
| 20. Nitrate                 | 10   |
| 21. Glutarate               | 10   |
| 22. Succinate               | 15   |
| 23. Malate                  | 15   |
| 24. Carbonate               | 15   |
| 25. Malonate                | 15   |
| 26. Tartrate                | 15   |
| 27. Maleate                 | 15   |
| 28. Sulfate                 | 15   |
| 29. Oxalate                 | 15   |
| 30. Fumarate                | 15   |
| 31. Tungstate               | 20   |
| 32. Phosphate               | 20   |
| 33. Phthalate               | 20   |
| 34. Citrate                 | 20   |
| 35. Chromate                | 20   |
| 36. Isocitrate              | 20   |
| 37. <i>cis</i> -Aconitate   | --   |
| 38. <i>trans</i> -Aconitate | 20   |



\*External water mode at 10 mL/min

## 5.6 Analysis of Beer Samples Using Dionex IonPac AS11-HC-4µm Capillary Column

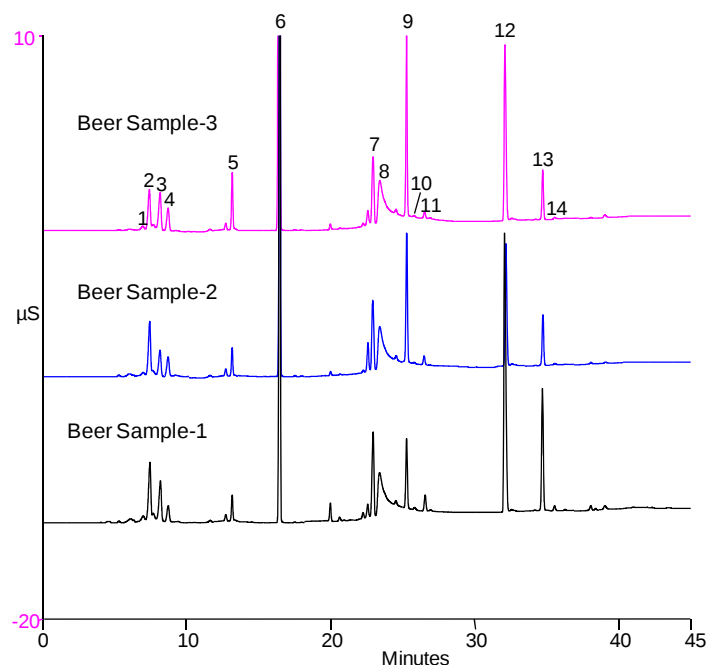
This section shows an optimized potassium hydroxide gradient (Figure 13A) as well as a potassium hydroxide gradient with methanol (Figure 13B) for analysis of beer samples using Dionex IonPac AS11-HC-4µm Capillary column. The beer samples were diluted 1:25 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge as described below. Note that, under aqueous eluent conditions, succinate and malate co-elute. However, by adding 10% methanol to the eluent (10% methanol was added to the deionized water reservoir by volume) these peaks are nearly baseline resolved. Also, note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode (0.12 mL/min using a pump). Adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when needed for improved resolution of analytes of interest.

### Dionex OnGuard RP Sample Pretreatment Procedure

1. Wash Dionex OnGuard RP with 10 mL methanol
2. Next wash with 10 mL deionized water
3. Discard 3-4 mL diluted sample then collect next 6-7 mL sample.

**Figure 13A** Analysis of Beer Samples Using an Aqueous Gradient with the Dionex IonPac AS11-HC-4µm Capillary Column

Column: Dionex IonPac AG11-HC-4µm/AS11-HC-4µm (0.4 × 250 mm)  
 Eluent: 1mM KOH from 0 to 8min, 1-15mM KOH from 8 to 18 min; 15 to 30mM KOH from 18 to 28 min  
 30 to 60mM KOH from 28 to 38 min  
 Eluent Source: Dionex EGC-KOH (Capillary) cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4 µL  
 Temperature: 30 °C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, recycle mode  
 Sample: Beer with 1:25 sample dilution



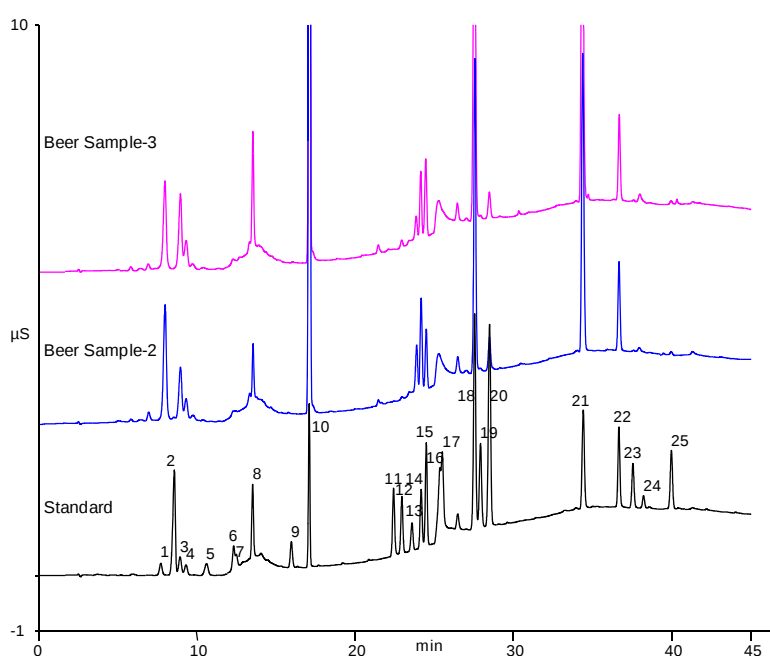
### Peaks:

1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Pyruvate
6. Chloride
7. Succinate+Malate\*
8. Carbonate
9. Sulfate
10. Oxalate
11. Fumarate
12. Phosphate
13. Citrate
14. Isocitrate

\*Succinate and Malate co-elute under aqueous conditions

**Figure 13B** Analysis of Beer Samples Using an Aqueous Gradient with Solvent and the Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column

Column: Dionex IonPac AG11-HC-4 $\mu$ m/AS11-HC-4 $\mu$ m (0.4  $\times$  250 mm)  
 Eluent + Methanol\*: 1mM KOH from 0 to 8min, 1-15mM KOH from 8 to 18 min; 15 to 30mM KOH from 18 to 28 min; 30 to 60mM KOH from 28 to 38 min  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, external water mode  
 Sample: Beer with 1:25 sample dilution

**Peaks (standard):**

| Peaks (standard):           | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 1     |
| 2. Fluoride                 | 0.6   |
| 3. Lactate                  | 1     |
| 4. Acetate                  | 1     |
| 5. Propionate               | 1     |
| 6. Formate                  | 1     |
| 7. Butyrate                 | 1     |
| 8. Pyruvate                 | 2     |
| 9. Galacturonate            | 2     |
| 10. Chloride                | 1     |
| 11. Bromide                 | 1     |
| 12. Nitrate                 | 1     |
| 13. Glutarate               | 2     |
| 14. Succinate               | 2     |
| 15. Malate                  | 2     |
| 16. Carbonate               | --    |
| 17. Tartrate                | 2     |
| 18. Sulfate                 | 2     |
| 19. Fumarate                | 2     |
| 20. Oxalate                 | 2     |
| 21. Phosphate               | 3     |
| 22. Citrate                 | 3     |
| 23. Isocitrate              | 3     |
| 24. <i>cis</i> -Aconitate   | --    |
| 25. <i>trans</i> -Aconitate | 3     |

\* 10% methanol was added to the deionized water reservoir by volume

## 5.7 Analysis of Beer Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Analytical Column

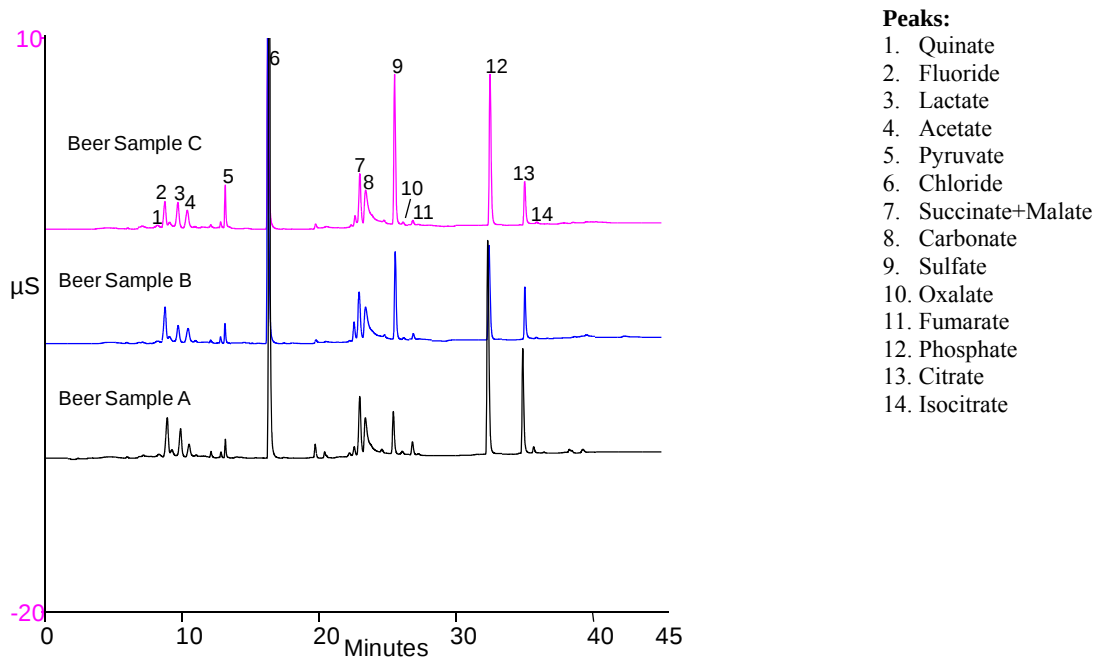
This section shows an optimized potassium hydroxide gradient (Figure 14A) as well as a potassium hydroxide gradient with methanol (Figure 14B) for analysis of beer samples using Dionex IonPac AS11-HC-4 $\mu$ m Analytical column. The beer samples were diluted 1:5 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge. Note that, under aqueous eluent conditions, succinate and malate co-elute. However, by adding organic solvent (methanol) to the eluent, these peaks are nearly baseline resolved. Note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode. Also, note that by adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when improved resolution of analytes of interest is necessary. The Figure 14B demonstrates the excellent separation of formate and butyrate by spiking the beer sample with 10 ppm of butyrate. Beer samples are analyzed for the presence of butyrate which indicates the presence of contaminant bacteria during the beer production process.

### Dionex OnGuard RP Sample Pretreatment Procedure

1. Wash Dionex OnGuard RP with 10 mL methanol
2. Next wash with 10 mL deionized water
3. Discard 3–4 mL diluted sample then collect next 6–7 mL sample.

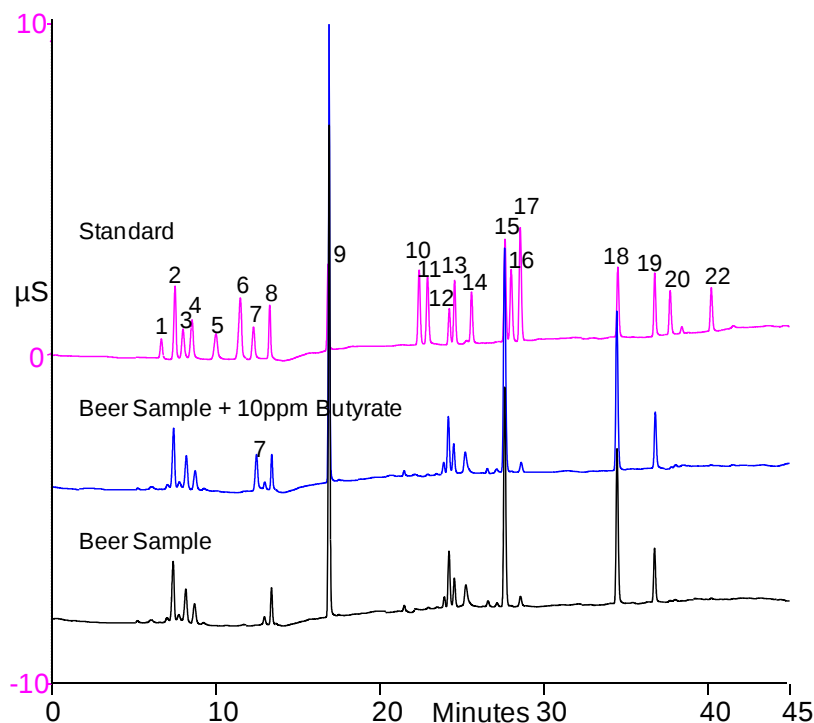
**Figure 14A** Analysis of Beer Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Column (4  $\times$  250 mm)

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (4  $\times$  250 mm)  
Eluent: 1mM KOH from 0 to 8min,  
1-15mM KOH from 8 to 18 min;  
15 to 30mM KOH from 18 to 28 min  
30 to 60mM KOH from 28 to 38 min  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 1.5 mL/min  
Inj. Volume: 10  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 4 mm,  
AutoSuppression, recycle mode  
Sample: Beer sample with 1:5 dilution



**Figure 14B** Analysis of Beer Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Column (2  $\times$  250 mm)

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (2  $\times$  250 mm)  
 Eluent with Methanol: 1mM KOH from 0 to 8min with 2% Methanol,  
2% to 10% Methanol at 8.1min.  
1-15mM KOH from 8.1 to 18 min +10% Methanol;  
15 to 30mM KOH from 18 to 28 min +10% Methanol;  
30 to 60mM KOH from 28 to 38 min +10% Methanol  
 Eluent Source: Dionex EGC 500 KOH cartridge  
 Flow Rate: 0.38 mL/min  
 Inj. Volume: 2.5  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity,  
Dionex ASRS 300, 2 mm,  
AutoSuppression external water mode  
 Sample: Beer sample with 1:5 dilution



| Peaks (standard):           | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 5     |
| 2. Fluoride                 | 3     |
| 3. Lactate                  | 5     |
| 4. Acetate                  | 5     |
| 5. Propionate               | 5     |
| 6. Formate                  | 5     |
| 7. Butyrate                 | 5     |
| 8. Pyruvate                 | 10    |
| 9. Chloride                 | 5     |
| 10. Bromide                 | 5     |
| 11. Nitrate                 | 5     |
| 12. Succinate               | 10    |
| 13. Malate                  | 10    |
| 14. Tartrate                | 10    |
| 15. Sulfate                 | 10    |
| 16. Fumarate                | 10    |
| 17. Oxalate                 | 10    |
| 18. Phosphate               | 15    |
| 19. Citrate                 | 15    |
| 20. Isocitrate              | 15    |
| 21. <i>cis</i> -Aconitate   | --    |
| 22. <i>trans</i> -Aconitate | 15    |

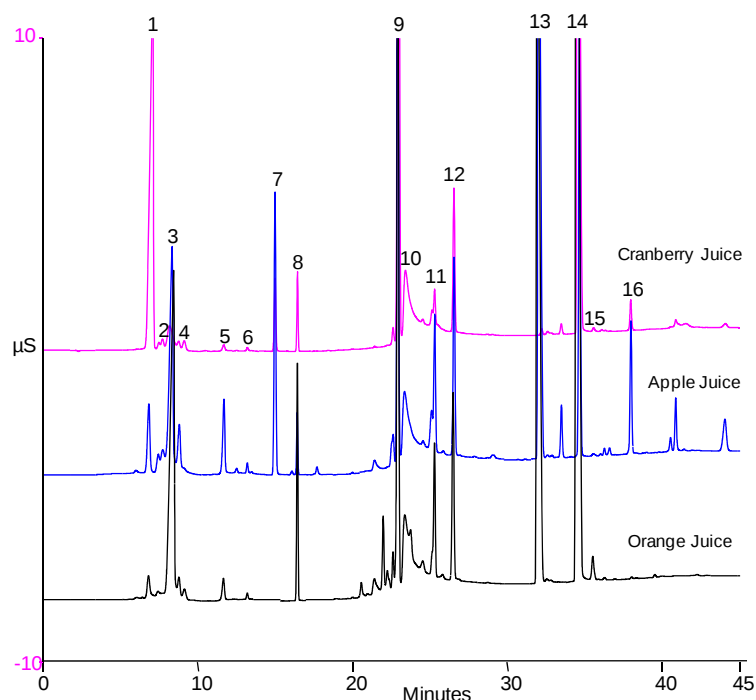
## 5.8 Analysis of Fruit Juice Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column

This section shows an optimized potassium hydroxide gradient (Figure 15A) as well as a potassium hydroxide gradient with methanol (Figure 15B) for analysis of various juice samples. The juice samples were diluted 1:50 with deionized water and filtered through a 0.45  $\mu$ m syringe filter. Note that, under aqueous eluent conditions, succinate and malate co-elute. However, by adding 10% methanol to the eluent (10% methanol was added to the deionized water reservoir by volume) both of these peaks are nearly baseline resolved. Also, note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode (0.12 mL/min using a pump). Adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when needed for improved resolution of analytes of interest.

If a low level of succinate is present in the juice sample and if succinate and malate separation is not required, it is recommended to use potassium hydroxide aqueous gradient for this separation to take advantage of operating in the recycle mode.

**Figure 15A** Analysis of Juice Samples Using an Aqueous Gradient with the Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column

Column: Dionex IonPac AG11-HC-4 $\mu$ m/AS11-HC-4 $\mu$ m (0.4  $\times$  250 mm)  
 Eluent: 1mM KOH from 0 to 8min, 1-15mM KOH from 8 to 18 min; 15 to 30mM KOH from 18 to 28 min  
 30 to 60mM KOH from 28 to 38 min  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, recycle mode  
 Sample: Juice sample diluted 1:50



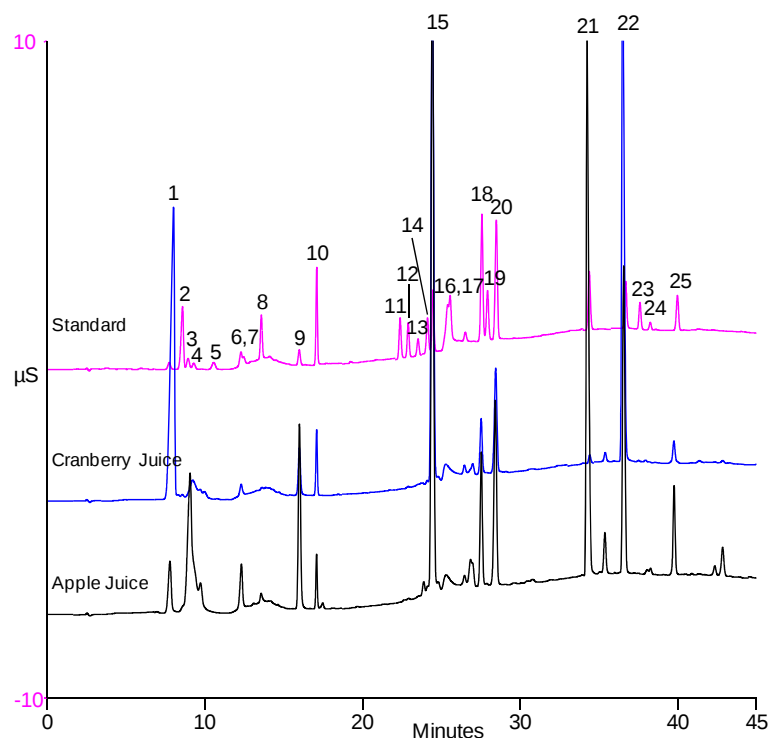
### Peaks:

1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Formate
6. Pyruvate
7. Galacturonate
8. Chloride
9. Succinate+Malate\*
10. Carbonate
11. Sulfate
12. Oxalate
13. Phosphate
14. Citrate
15. Isocitrate
16. *trans*-Aconitate

\*Malate and Succinate co-elute under aqueous conditions

**Figure 15B** Analysis of Juice Samples Using an Aqueous Gradient with Solvent and the Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column

Column: Dionex IonPac AG11-HC-4 $\mu$ m/AS11-HC-4 $\mu$ m (0.4  $\times$  250 mm)  
 Eluent + Methanol\*: 1mM KOH from 0 to 8min, 1-15mM KOH from 8 to 18 min; 15 to 30mM KOH from 18 to 28 min; 30 to 60mM KOH from 28 to 38 min  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, recycle mode  
 Sample: Juice sample diluted 1:50



| Peaks (standard):           | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 1     |
| 2. Fluoride                 | 0.6   |
| 3. Lactate                  | 1     |
| 4. Acetate                  | 1     |
| 5. Propionate               | 1     |
| 6. Formate                  | 1     |
| 7. Butyrate                 | 1     |
| 8. Pyruvate                 | 2     |
| 9. Galacturonate            | 2     |
| 10. Chloride                | 1     |
| 11. Bromide                 | 1     |
| 12. Nitrate                 | 1     |
| 13. Glutarate               | 2     |
| 14. Succinate               | 2     |
| 15. Malate                  | 2     |
| 16. Carbonate               | --    |
| 17. Tartrate                | 2     |
| 18. Sulfate                 | 2     |
| 19. Fumarate                | 2     |
| 20. Oxalate                 | 2     |
| 21. Phosphate               | 3     |
| 22. Citrate                 | 3     |
| 23. Isocitrate              | 3     |
| 24. <i>cis</i> -Aconitate   | --    |
| 25. <i>trans</i> -Aconitate | 3     |

\* 10% methanol was added to the deionized water reservoir by volume

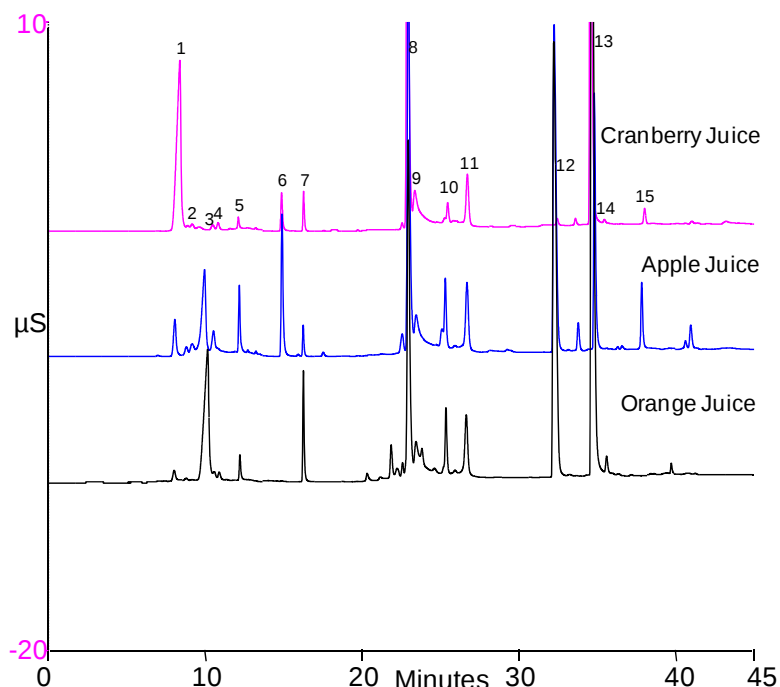
## 5.9 Analysis of Fruit Juice Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Analytical Column

This section shows an optimized potassium hydroxide gradient (Figure 16A, 16B) as well as a potassium hydroxide gradient with methanol (Figure 16C) for analysis of various juice samples. The juice samples were diluted 1:10 with deionized water and filtered through a 0.45  $\mu$ m syringe filter. Note that, under aqueous eluent conditions, succinate and malate co-elute. However, by adding organic solvent (methanol) to the eluent, both of these peaks are nearly baseline resolved. Also, note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode and methanol can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when needed for improved resolution of analytes of interest.

Figure 16C demonstrates that this batch of apple juice did not have any significant amount of succinate. So therefore it is recommended that if fruit juice sample contains no succinate and/or if succinate and malate separation is not required, use potassium hydroxide aqueous gradient for this separation to take advantage of operating in the recycle mode.

**Figure 16A** Analysis of Juice Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Column (4  $\times$  250 mm)

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (4  $\times$  250 mm)  
Eluent: 1mM KOH from 0 to 8min,  
1-15mM KOH from 8 to 18 min;  
15 to 30mM KOH from 18 to 28 min  
30 to 60mM KOH from 28 to 38 min  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 1.5 mL/min  
Inj. Volume: 10  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 4 mm,  
AutoSuppression, recycle mode  
Sample: Juice sample with 1:10 dilution



### Peaks:

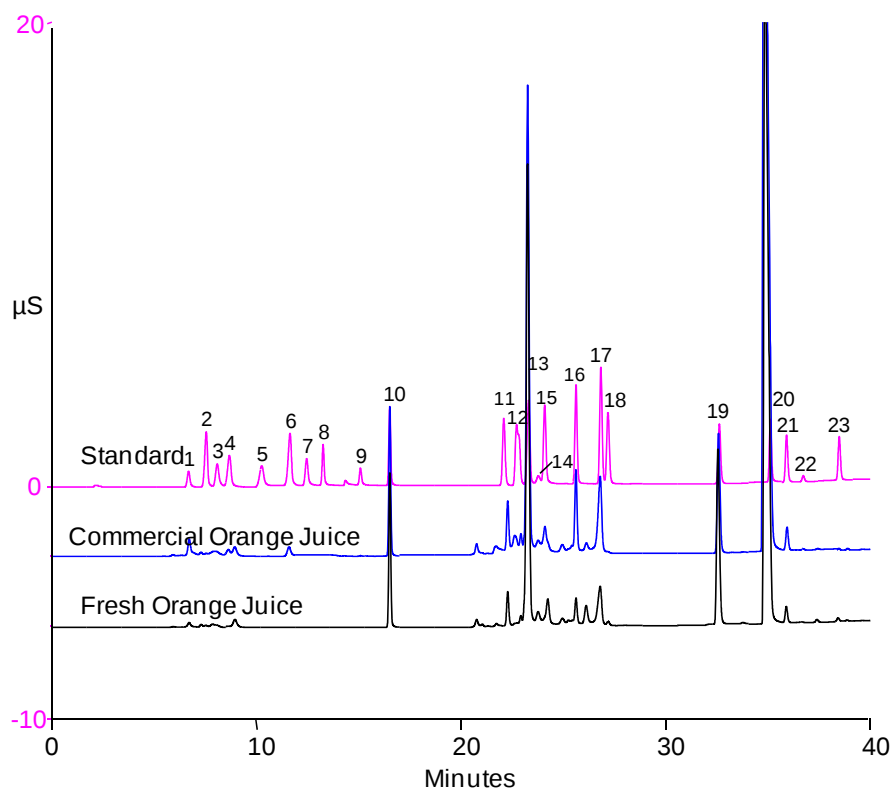
1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Formate
6. Galacturonate
7. Chloride
8. Succinate+Malate
9. Carbonate
10. Sulfate
11. Oxalate
12. Phosphate
13. Citrate
14. Isocitrate
15. *trans*-Aconitate



## 5 – Example Applications

**Figure 16B** Analysis of Orange Juice Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Column (2  $\times$  250 mm)

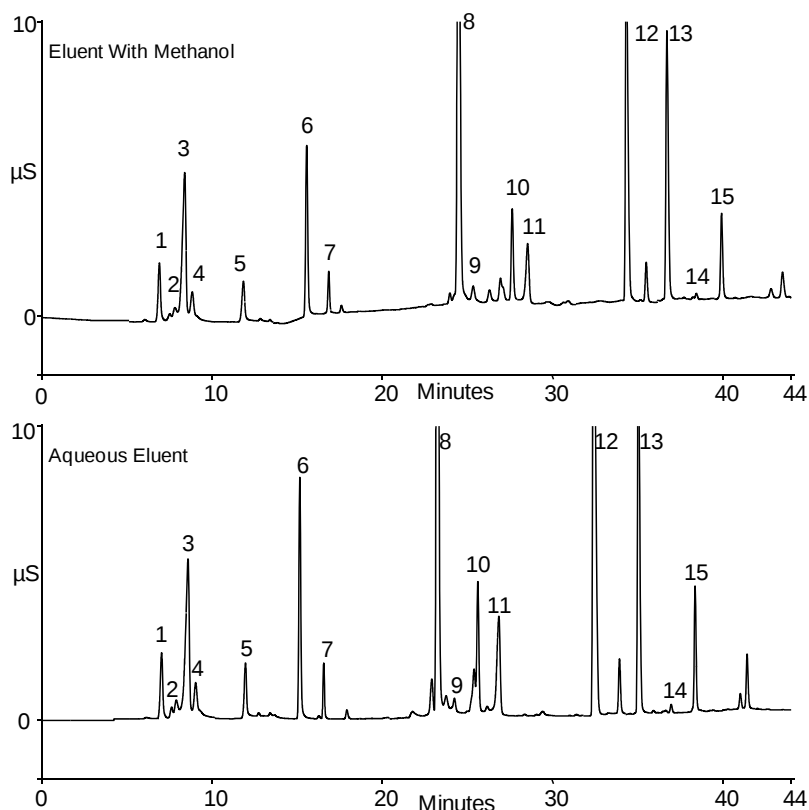
Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (2  $\times$  250 mm)  
Eluent : 1mM KOH from 0 to 8min,  
1-15mM KOH from 8 to 18 min;  
15 to 30mM KOH from 18 to 28 min  
30 to 60mM KOH from 28 to 38 min  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 0.38 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 2 mm,  
AutoSuppression, recycle mode  
Sample: Orange Juice with 1:10 sample dilution



| Peaks (standard):           | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 5     |
| 2. Fluoride                 | 3     |
| 3. Lactate                  | 5     |
| 4. Acetate                  | 5     |
| 5. Propionate               | 5     |
| 6. Formate                  | 5     |
| 7. Butyrate                 | 5     |
| 8. Pyruvate                 | 5     |
| 9. Galacturonate            | 5     |
| 10. Chloride                | 5     |
| 11. Bromide                 | 5     |
| 12. Nitrate+Glutarate       | 5+5   |
| 13. Malate+Succinate        | 10+10 |
| 14. Carbonate               | 10    |
| 15. Tartrate+Malonate       | 10+10 |
| 16. Sulfate                 | 10    |
| 17. Oxalate                 | 10    |
| 18. Fumarate                | 10    |
| 19. Phosphate               | 15    |
| 20. Citrate                 | 15    |
| 21. Isocitrate              | 15    |
| 22. <i>cis</i> -Aconitate   | --    |
| 23. <i>trans</i> -Aconitate | 15    |

**Figure 16C** Analysis of an Apple Juice Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Column With and Without Methanol

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (2  $\times$  250 mm)  
Aqueous Eluent : 1mM KOH from 0 to 8min,  
1-15mM KOH from 8 to 18 min;  
15 to 30mM KOH from 18 to 28 min  
30 to 60mM KOH from 28 to 38 min  
Eluent with Methanol : 1mM KOH from 0 to 8min with 2% Methanol,  
2% to 10% Methanol at 8.1min.  
1-15mM KOH from 8.1 to 18 min +10% Methanol;  
15 to 30mM KOH from 18 to 28 min +10% Methanol;  
30 to 60mM KOH from 28 to 38 min +10% Methanol  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 0.38 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 2 mm,  
AutoSuppression, external water mode  
Sample: Apple Juice with 1:10 sample dilution



**Peaks:**

1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Formate
6. Galacturonate
7. Chloride
8. Malate
9. Carbonate
10. Sulfate
11. Oxalate
12. Phosphate
13. Citrate
14. *cis*-Aconitate
15. *trans*-Aconitate

\*No Succinate found in this Apple Juice Sample

\*Regenerant flow rate: 5 mL/min

## 5.10 Analysis of a Wine Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column

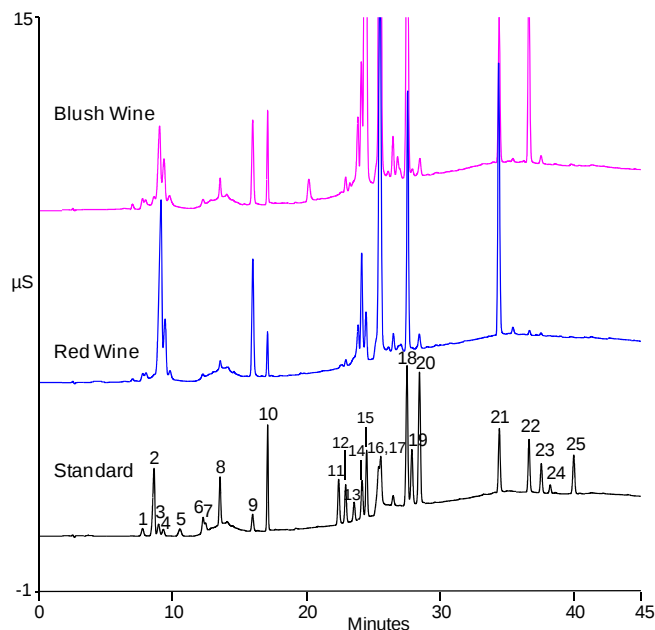
Figure 17 shows an optimized potassium hydroxide gradient with methanol for analysis of a wine sample. The wine sample was diluted 1:100 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge. Under aqueous eluent conditions, succinate and malate co-elute. However, by adding 10% methanol to the eluent (10% methanol was added to the deionized water reservoir by volume) these peaks are nearly baseline resolved. Also, note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode (0.12 mL/min using a pump). Note that adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when needed for improved resolution of analytes of interest.

### Dionex OnGuard RP Sample Pretreatment Procedure

1. Wash Dionex OnGuard RP with 10 mL methanol
2. Next wash with 10 mL deionized water
3. Discard 3-4 mL diluted sample then collect next 6-7 mL sample.

**Figure 17** Analysis of Wine Samples Using the Dionex IonPac AS11-HC-4 $\mu$ m Capillary Column and Gradient Chromatography

Column: Dionex IonPac AG11-HC-4 $\mu$ m/AS11-HC-4 $\mu$ m (0.4  $\times$  250 mm)  
 Eluent + Methanol\*: 1mM KOH from 0 to 8min, 1-15mM KOH from 8 to 18 min; 15 to 30mM KOH from 18 to 28 min  
 30 to 60mM KOH from 28 to 38 min  
 Eluent Source: Dionex EGC-KOH (Capillary) Cartridge  
 Flow Rate: 0.015 mL/min  
 Inj. Volume: 0.4  $\mu$ L  
 Temperature: 30  $^{\circ}$ C  
 Detection: Suppressed Conductivity, Dionex ACES 300  
 AutoSuppression, external water mode  
 Sample: Wine sample diluted 1:100



| Peaks:                      | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 1     |
| 2. Fluoride                 | 0.6   |
| 3. Lactate                  | 1     |
| 4. Acetate                  | 1     |
| 5. Propionate               | 1     |
| 6. Formate                  | 1     |
| 7. Butyrate                 | 1     |
| 8. Pyruvate                 | 2     |
| 9. Galacturonate            | 2     |
| 10. Chloride                | 1     |
| 11. Bromide                 | 1     |
| 12. Nitrate                 | 1     |
| 13. Glutarate               | 2     |
| 14. Succinate               | 2     |
| 15. Malate                  | 2     |
| 16. Carbonate               | --    |
| 17. Tartrate                | 2     |
| 18. Sulfate                 | 2     |
| 19. Fumarate                | 2     |
| 20. Oxalate                 | 2     |
| 21. Phosphate               | 3     |
| 22. Citrate                 | 3     |
| 23. Isocitrate              | 3     |
| 24. <i>cis</i> -Aconitate   | --    |
| 25. <i>trans</i> -Aconitate | 3     |

\* 10% methanol was added to the deionized water reservoir by volume

## 5.11 Analysis of a Wine Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Analytical Column

Figures 18A and 18B show an optimized potassium hydroxide gradient with methanol for analysis of a wine sample. The wine sample was diluted 1:20 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge. Under aqueous eluent conditions, succinate and malate co-elute. However, by adding organic solvent (methanol) to the eluent, these peaks are nearly baseline resolved. In order to improve the separation of malate and succinate further, it is recommended to increase the methanol level to 20% and reduced the flow rate 1 mL/min to avoid any over pressure issues with the system.

Note that when adding methanol to the eluent stream, the suppressor must be operated in the external water mode. Also, note that adding methanol to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use methanol when needed improved resolution of analytes of interest.

### Dionex OnGuard RP Sample Pretreatment Procedure

1. Wash Dionex OnGuard RP with 10 mL methanol
2. Next wash with 10 mL deionized water
3. Discard 3-4 mL diluted sample then collect next 6-7 mL sample.

**Figure 18A** Analysis of Wine Samples Using Dionex IonPac AS11-HC-4 $\mu$ m Column With and Without Methanol

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (4  $\times$  250 mm)

Eluent : 1mM KOH from 0 to 8min,  
1-15mM KOH from 8 to 18 min;  
15 to 30mM KOH from 18 to 28 min  
30 to 60mM KOH from 28 to 38 min

Eluent with Methanol : 1mM KOH from 0 to 8min with 2% Methanol,  
2% to 10% Methanol at 11.1min.  
1-15mM KOH from 8 to 18 min +10% Methanol;  
15 to 30mM KOH from 18 to 28 min +10% Methanol;  
30 to 60mM KOH from 28 to 38 min +10% Methanol

Eluent Source: Dionex EGC 500 KOH cartridge

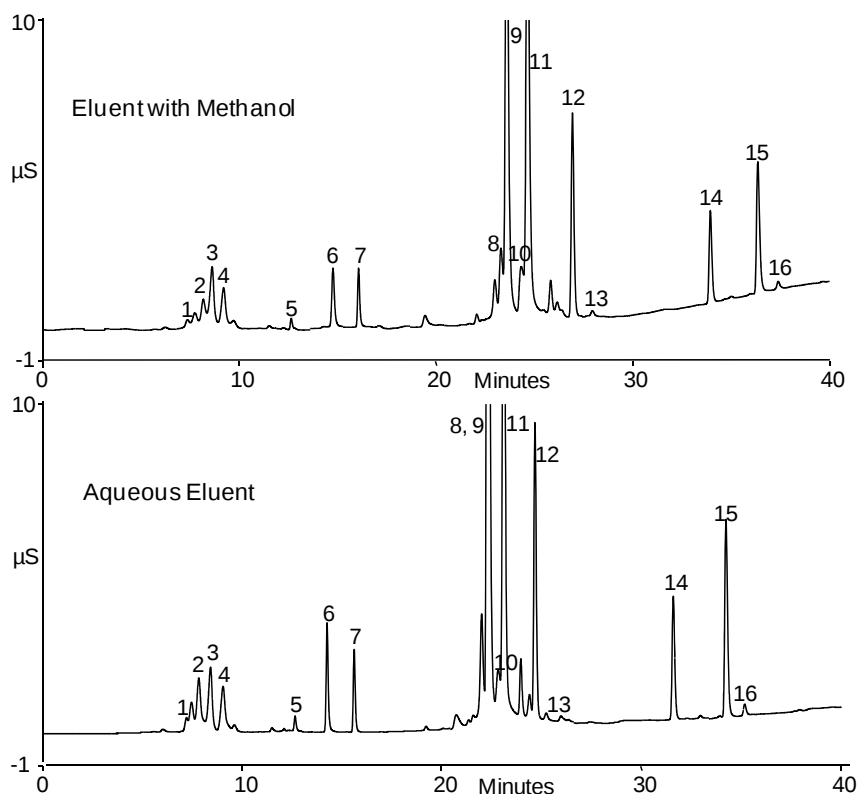
Flow Rate: 1.5 mL/min

Inj. Volume: 10  $\mu$ L

Temperature: 30  $^{\circ}$ C

Detection: Suppressed Conductivity,  
Dionex ASRS 300, 4 mm,  
AutoSuppression, External water mode

Sample: Blush Wine sample with 1:20 dilution

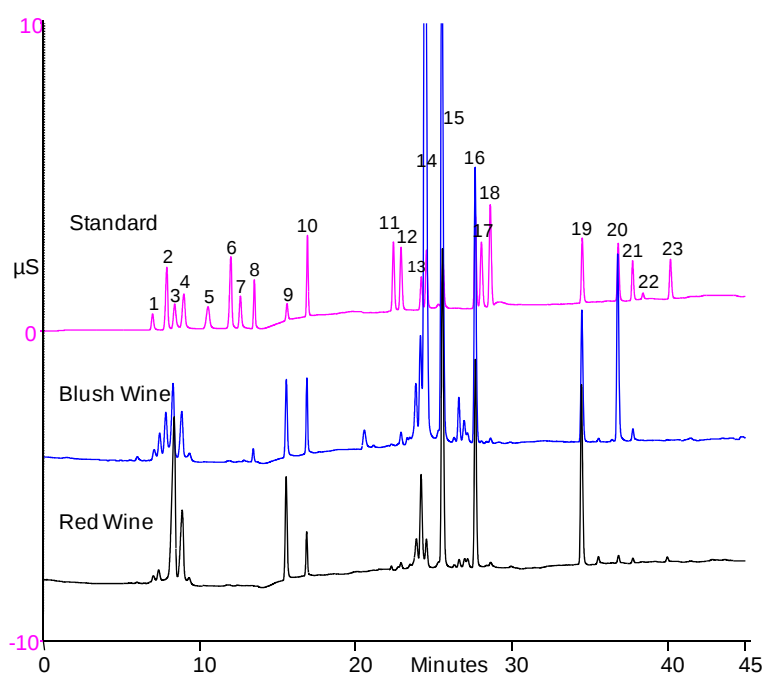


**Peaks:**

1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Pyruvate
6. Galacturonate
7. Chloride
8. Succinate
9. Malate
10. Carbonate
11. Tartrate
12. Sulfate
13. Fumarate
14. Phosphate
15. Citrate
16. Isocitrate

**Figure 18B** Analysis of Wine Sample Using Dionex IonPac AS11-HC-4 $\mu$ m Column (2  $\times$  250 mm)

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
Dionex IonPac AS11-HC-4 $\mu$ m (2  $\times$  250 mm)  
Eluent with Methanol : 1mM KOH from 0 to 8min with 2% Methanol,  
2% to 10% Methanol at 8.1min.  
1-15mM KOH from 8 to 18 min +10% Methanol;  
15 to 30mM KOH from 18 to 28 min +10% Methanol;  
30 to 60mM KOH from 28 to 38 min +10% Methanol  
Eluent Source: Dionex EGC 500 KOH cartridge  
Flow Rate: 0.38 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Temperature: 30  $^{\circ}$ C  
Detection: Suppressed Conductivity,  
Dionex ASRS 300, 2 mm,  
AutoSuppression external water mode  
Sample: Wine with 1:20 sample dilution



| Peaks (standard):           | mg/mL |
|-----------------------------|-------|
| 1. Quinate                  | 5     |
| 2. Fluoride                 | 3     |
| 3. Lactate                  | 5     |
| 4. Acetate                  | 5     |
| 5. Propionate               | 5     |
| 6. Formate                  | 5     |
| 7. Butyrate                 | 5     |
| 8. Pyruvate                 | 10    |
| 9. Galacturonate            | 10    |
| 10. Chloride                | 5     |
| 11. Bromide                 | 5     |
| 12. Nitrate                 | 5     |
| 13. Succinate               | 10    |
| 14. Malate                  | 10    |
| 15. Tartrate                | 10    |
| 16. Sulfate                 | 10    |
| 17. Fumarate                | 10    |
| 18. Oxalate                 | 10    |
| 19. Phosphate               | 15    |
| 20. Citrate                 | 15    |
| 21. Isocitrate              | 15    |
| 22. <i>cis</i> -Aconitate   | --    |
| 23. <i>trans</i> -Aconitate | 15    |

### 5.12 Separations of Heat Stable Salts in Methyldiethanolamine Using a Shallow Gradient and High Pressure IC

Crude natural gas (methane) must be chemically processed to remove impurities before the gas can be sold as a pure product. Some natural gas wells may contain high concentrations of carbon dioxide (CO<sub>2</sub>) and hydrogen sulfide (H<sub>2</sub>S), the later known as “sour gas” because of the mercaptan odor.<sup>1,2</sup> The high sulfide content and acidity from dissolved carbon dioxide cause corrosion of metal pipes and acid rain making sour gas undesirable as a commercial product. Therefore, sour gas is typically treated with amine (amine rich) solutions (e.g., ethanolamine, methanolamine, and methyldiethanolamine) to absorb hydrogen sulfide and neutralize carbon dioxide.<sup>1,2</sup> The resulting pure natural gas, defined as containing < 5.7 mg/m<sup>3</sup> hydrogen sulfide, is called “sweet” gas by the industry.<sup>1</sup> The amine processing solutions (amine lean) are stripped and regenerated to remove the sulfur compounds as elemental sulfur. However, the amine solutions also extract other contaminants which form salts of organic acids and sulfur species, such as oxalate, propionate, formate, acetate, thiosulfate, and thiocyanate.<sup>2</sup> These anions are collectively termed “heat stable amine salts” because the salts are not removed by the amine stripping process. Furthermore, the high salt content in the amine solution increases the solution viscosity, reduces acid-absorbing capacity and increases corrosion-induced maintenance costs. These amine waste solutions must also be characterized to comply with discharge permits. Figure 19 below shows the separation of heat stable amine salts in water and up to 30% Methyldiethanolamine using the Dionex IonPac AS11-HC-4μm column.

1. Natural Gas.org, [http://www.naturalgas.org/naturalgas/processing\\_ng.asp](http://www.naturalgas.org/naturalgas/processing_ng.asp)

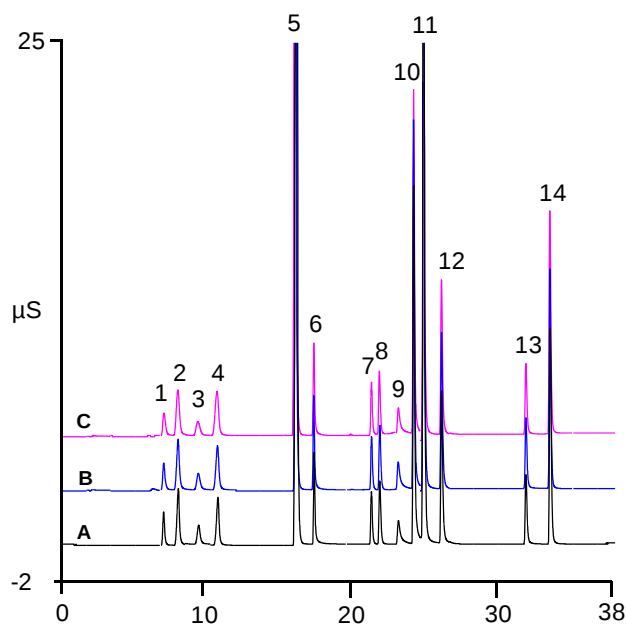
2. P.C. Rooney, T.R. Bacon and M.S DuPart *Hydrocarbon Processing* **1996**, March, 95-103 and **1997**, April, 65-71

## 5 – Example Applications

**Figure 19** Separations of Heat Stable Salts in Methyl-diethanolamine Matrix Using the Dionex IonPac AS11-HC-4 $\mu$ m Column (2  $\times$  250 mm)

Column: Dionex IonPac AG11-HC-4 $\mu$ m,  
AS11-HC-4 $\mu$ m (2  $\times$  250 mm)  
Eluent Source: Dionex, EGC 500 KOH  
Gradient: Potassium hydroxide  
1 mM from 0 to 8 min,  
1–30 mM from 8 to 28 min  
3–72 mM from 28 to 38 min  
Flow Rate: 0.38 mL/min  
Inj. Volume: 25  $\mu$ L  
Column Temp.: 30  $^{\circ}$ C  
Detection: Suppressed conductivity  
Dionex ASRS 300, 2 mm, recycle  
Samples: Heat stable salts in  
A: Water; B: 10% Methyl-diethanolamine  
C: 30% Methyl-diethanolamine

| Peaks (standard): | mg/mL |
|-------------------|-------|
| 1. Fluoride       | 2     |
| 2. Acetate        | 10    |
| 3. Propionate     | 10    |
| 4. Formate        | 10    |
| 5. Chloride       | 100   |
| 6. Nitrite        | 10    |
| 7. Bromide        | 10    |
| 8. Nitrate        | 100   |
| 8. Nitrate        | 100   |
| 9. Carbonate      | 50    |
| 10. Sulfite       | 100   |
| 11. Sulfate       | 100   |
| 12. Oxalate       | 20    |
| 13. Phosphate     | 20    |
| 14. Thiosulfate   | 10    |



## 6. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex IonPac AS11-HC-4 $\mu$ m columns. For more information on problems that originate with the Ion Chromatograph (IC) or the suppressor, refer to the Troubleshooting Guide in the appropriate operator's manual. **For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.**

**Table 5**     Dionex IonPac AS11-HC-4 $\mu$ m/Dionex IonPac AG11-HC-4 $\mu$ m Troubleshooting Summary

| Observation                         | Cause                                | Action                                                   | Reference Section         |
|-------------------------------------|--------------------------------------|----------------------------------------------------------|---------------------------|
| <b>High Back Pressure</b>           | Unknown                              | Isolate Blocked Component                                | 6.1.1                     |
|                                     | Plugged Column Bed Supports          | Replace Bed Supports, Filter Eluents, and Filter Samples | 6.1.2                     |
|                                     | Other System Components              | Unplug, Replace                                          | Component Manual          |
| <b>High Background Conductivity</b> | Contaminated Eluents                 | Remake Eluents                                           | 6.2, 6.2.1                |
|                                     | Contaminated Trap Column             | Clean Trap Column                                        | 6.2.2                     |
|                                     | Contaminated Guard or Analyte Column | Clean Guard and Analytical/Capillary Column              | 6.2.3                     |
|                                     | Contaminated Suppressor              | Clean Suppressor                                         | 6.2.5, Component Manual   |
|                                     | Contaminated Hardware                | Clean Component                                          | 6.2.4 Component Manual    |
| <b>Poor Resolution</b>              | Method Not Optimized                 | Optimize Method                                          | 6.3.A, B                  |
| <b>Poor Efficiency</b>              | Large System Void Volumes            | Replumb System                                           | 6.3.1.B, Component Manual |
|                                     | Column Headspace                     | Replace Column                                           | 6.3.1.A                   |
|                                     | Improper Connections                 | Remake Connections                                       | 6.3.1.C                   |
| <b>Short Retention Times</b>        | Flow Rate Too fast                   | Recalibrate Pump                                         | 6.3.2.A                   |
|                                     | Conc. Incorrect Eluents              | Remake Eluents                                           | 6.3.2.B                   |
|                                     | Column Contamination                 | Clean Column                                             | 6.3.2.C, 6.3.2.D          |
| <b>Poor Front End Resolution</b>    | Conc. Incorrect Eluents              | Remake Eluents                                           | 6.3.3.A                   |
|                                     | Column Overloading                   | Reduce Sample Size                                       | 6.3.3.B, 3.3.1, 3.3.2     |
|                                     | Sluggish Injection Valve             | Service Valve                                            | 6.3.3.C, Component Manual |
|                                     | Large System Void Volumes            | Replumb System                                           | 6.3.3.D, Component Manual |
| <b>Spurious Peaks</b>               | Sample Contaminated                  | Pretreat Samples                                         | 6.3.4.A                   |
|                                     | Sluggish Injection Valve             | Service Valve                                            | 6.3.3.B, Component Manual |

## 6.1 High Back Pressure

### 6.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex IonPac AG11-HC-4 $\mu$ m Guard/Capillary Guard Column plus the Dionex IonPac AS11-HC-4 $\mu$ m Analytical/Capillary Column when using the test chromatogram conditions should be equal or less than 4500 psi. If the system pressure is higher than 4500 psi, it is advisable to determine the cause of the high system pressure. The system should be operated with a Thermo Scientific Dionex High-Pressure In-Line Filter (P/N 074505) which is positioned between the Gradient Pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

- A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate if necessary with a stop watch and graduated cylinder.
- B. Determine which part of the system is causing the high pressure. High pressure could be due to a plugged tubing or tubing with collapsed or pinched walls, an injection valve with a clogged port, a column with particulates clogging the bed support, a clogged High-Pressure In-Line Filter, the suppressor or the detector cell.

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 500 psi. Continue adding system components (injection valve, column(s), suppressor and detector) one by one, while monitoring the system pressure. The pressure should increase up to a maximum when the Guard/Capillary Guard and Analytical/Capillary columns are connected (see Table 6, “Typical Dionex IonPac AS11-HC-4 $\mu$ m/Dionex IonPac AG11-HC-4 $\mu$ m Operating Back Pressures”).

The suppressor may add up to 100 psi (0.69 MPa). The EGC 500 KOH can add up to 500 psi at 1.5 mL/min. No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

**Table 6** Typical Dionex IonPac AS11-HC-4 $\mu$ m/Dionex IonPac AG11-HC-4 $\mu$ m Operating Back Pressures

| Column                                                                               | Typical Back Pressure<br>psi (MPa) | Standard<br>Flow Rate<br>mL/min | Maximum Flow<br>Rate mL/min |
|--------------------------------------------------------------------------------------|------------------------------------|---------------------------------|-----------------------------|
| Dionex IonPac AS11-HC-4 $\mu$ m 4 mm Analytical Column                               | $\leq 3800$ (26.20)                | 1.50                            | 2.0                         |
| A Dionex IonPac G11-HC-4 $\mu$ m 4 mm Guard Column                                   | $\leq 150$ (1.03)                  | 1.50                            | 2.0                         |
| Dionex IonPac AS11-HC-4 $\mu$ m and AG11-HC-4 $\mu$ m 4 mm columns                   | $\leq 3950$ (27.23)                | 1.50                            | 2.0                         |
| Dionex IonPac AS11-HC-4 $\mu$ m 2 mm Analytical Column                               | $\leq 3800$ (26.20)                | 0.38                            | 0.5                         |
| Dionex IonPac AG11-HC-4 $\mu$ m 2 mm Guard Column                                    | $\leq 150$ (1.03)                  | 0.38                            | 0.5                         |
| Dionex IonPac AS11-HC-4 $\mu$ m and AG11-HC-4 $\mu$ m 2 mm columns                   | $\leq 3950$ (27.23)                | 0.38                            | 0.5                         |
| Dionex IonPac AS11-HC-4 $\mu$ m 0.4 mm Capillary Column                              | $\leq 3800$ (26.20)                | 0.015                           | 0.02                        |
| AG11-HC-4 $\mu$ m 0.4 mm Capillary Guard Column                                      | $\leq 150$ (1.03)                  | 0.015                           | 0.02                        |
| AS11-HC-4 $\mu$ m and AG11-HC-4 $\mu$ m 0.4 mm Capillary and Capillary Guard columns | $\leq 3950$ (27.23)                | 0.015                           | 0.02                        |

### 6.1.2 Replacing Column Bed Support Assemblies for 2 mm and 4 mm columns

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

- A. Disconnect the column from the system.
- B. Carefully unscrew the inlet (top) column fitting. Use two open-end wrenches.
- C. Remove the bed support. Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you do not scratch the walls of the end fitting. Discard the old bed support assembly.
- D. Place a new bed support assembly into the end fitting. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

**Table 7** Product Information

| Product              | Dionex IonPac AS11-HC-4µm<br>4 mm Columns (P/N) | Dionex IonPac AS11-HC-4µm<br>2 mm Columns (P/N) | Dionex IonPac AS11-HC-4µm<br>0.4 mm Columns (P/N) |
|----------------------|-------------------------------------------------|-------------------------------------------------|---------------------------------------------------|
| Analytical Column    | 082313                                          | 078035                                          | 078031                                            |
| Guard Column         | 078034                                          | 078036                                          | 078032                                            |
| Bed Support Assembly | 042955                                          | 044689                                          | N/A                                               |
| End Fitting          | 052809                                          | 043278                                          | N/A                                               |



#### CAUTION

*If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting.*

- E. Screw the end fitting back onto the column. Tighten it finger-tight, then an additional 1/4 turn (25 in × lb). Tighten further only if leaks are observed.
- F. Reconnect the column to the system and resume operation.

### 6.1.3 Filter Eluent

Eluents containing particulate material or bacteria may clog the column inlet bed support. Filter water used for eluents through a 0.45 µm filter.

### 6.1.4 Filter Samples

Samples containing particulate material may clog the column inlet bed support. Filter samples through a 0.45 µm filter prior to injection.

## 6.2 High Background

In a properly working system, the background conductivity level for the standard eluent system is shown below:

**Table 8** Background Conductivity

| Eluent                           | Expected Background Conductivity |
|----------------------------------|----------------------------------|
| 1.0 mM KOH                       | 0.5 - 0.8 µS                     |
| 60 mM KOH                        | 0.6-1.5 µS                       |
| 60 mM KOH/15% CH <sub>3</sub> OH | 1-3 µS                           |

### 6.2.1 Preparation of Eluents

- A. Make sure that the eluents and the regenerant are made correctly.
- B. Make sure that the eluents are made from chemicals with the recommended purity.
- C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

### 6.2.2 Contaminated Dionex CR-ATC Column

- A. Install a Dionex CR-ATC Anion Trap Column (P/N 075550 or 072078) if using a Dionex Eluent Generator with Dionex EGC 500 KOH or Dionex EGC KOH (Capillary) cartridge.
- B. If the Dionex CR-ATC becomes contaminated, please refer to Section 6, Clean-Up, in the Dionex CR-ATC Product Manual (Document No. 031910).

### 6.2.3 A Contaminated Guard/Capillary Guard or Analytical/Capillary Column

- A. Remove the columns from the system.
- B. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent. If the background conductivity decreases, the column(s) is (are) the cause of the high background conductivity.
- C. To eliminate downtime, clean or replace the analytical/capillary column at the first sign of column performance degradation. Clean the column as instructed in, “Column Cleanup” (See Appendix A “Column Care”).

### 6.2.4 Contaminated Hardware

Eliminate the hardware as the source of the high background conductivity.

- A. Bypass the columns and the suppressor.
- B. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.
- C. Pump deionized water with a specific resistance of 18.2 megohm-cm through the system.
- D. The background conductivity should be less than 2  $\mu$ S. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

### 6.2.5 A Contaminated Suppressor

If the above items have been checked and the problem persists, the suppressor is probably causing the problem. For details on Dionex Anion Self-Regenerating Suppressor operation, refer to the Dionex Anion Self-Regenerating Suppressor 300 Product Manual (Document No. 031956). For details on Dionex Anion Membrane Suppressor 300 operation, refer to the Product Manual (Document No. 031727) for assistance. For details on the Dionex Anion Capillary Electrolytic Suppressor 300 (Dionex ACES 300) operation, refer to the product manual (Document No. 065388) for assistance.

## 6.3 Poor Peak Resolution

One of the unique features of the Dionex IonPac AS11-HC-4 $\mu$ m is fast equilibration time in gradient applications from the last eluent (high ionic strength) to the first eluent (low ionic strength). The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration. Typically equilibration times range from 7 to 10 minutes.

If increased separation is needed for the first group of peaks, reduce the initial eluent concentration.

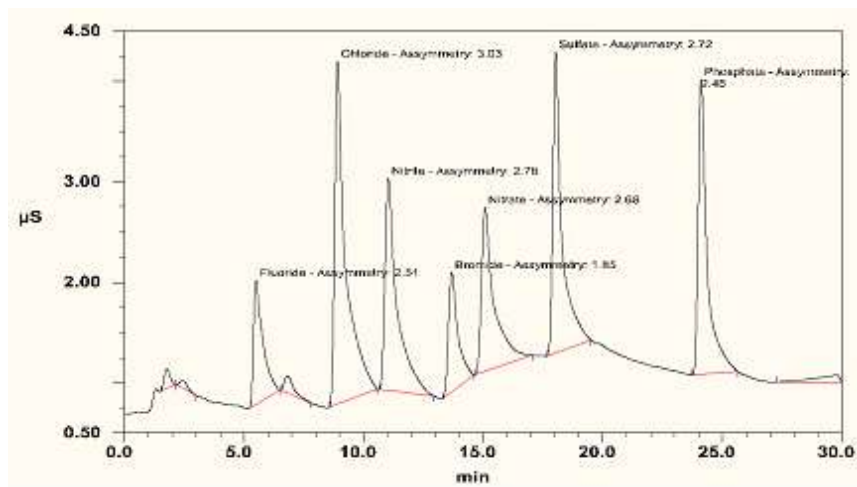
Due to different system configurations, the gradient profile may not match the gradient shown in the example. The gradient conditions can be adjusted to improve resolution or to adjust retention times either by changing the gradient timing or by changing the initial and/or final eluent concentration.

- A. Keep the eluent concentrations constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.
- B. Change the initial and/or final eluent concentration and adjust the gradient time. This approach requires more time to develop and more knowledge in methods development work. Its advantage is that it allows a method to be tailored for a particular application, where selectivity, resolution, and total run time are optimized. Be aware poor peak resolution can be due to any or all of the following factors.

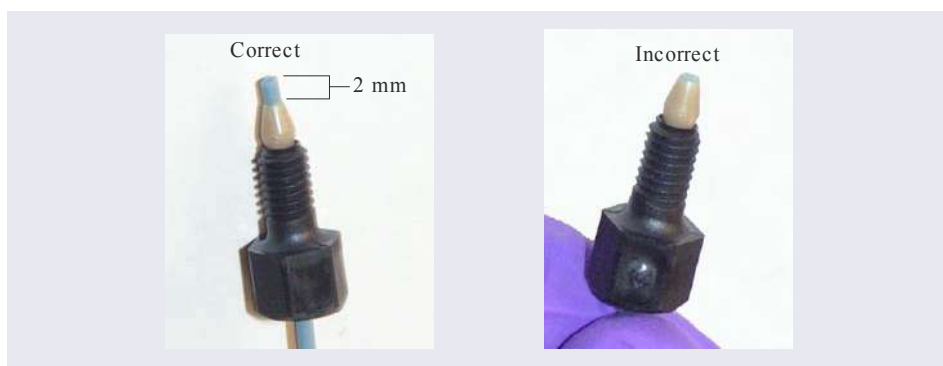
### 6.3.1 Loss of Column Efficiency

- A. Check to see if headspace has developed in the guard or analytical column (this cannot be checked on capillary columns). This is usually due to improper use of the column such as submitting it to high pressures. Remove the column's top end fitting (see Section 6.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. The column must be replaced.
- B. Extra-column effects can result in sample band dispersion, making the peaks' elution less efficient. Make sure you are using PEEK tubing with an ID of no greater than 0.010" for 4 mm systems or no greater than 0.005" for 2 mm systems to make all eluent liquid line connections between the injection valve and the detector cell inlet. Cut the tubing lengths as short as possible. Check for leaks. For capillary systems, only use precut tubing of the same type.
- C. If tubing is not connected properly from the inlet and outlet of the column, it can cause low efficiency. When installing AS11-HC-4 $\mu$ m columns, it is recommended to turn off the pump while connecting the column inlet and the column outlet to the suppressor. This will avoid any slippage of the ferrule under high pressure conditions which can cause low peak efficiencies.

Incorrectly installed fittings on capillary tubing can increase void volumes, causing chromatograms with tailing peaks.

**Figure 20** Tailing Peaks Caused by Incorrectly Installed Capillary Tubing Fittings

When connecting a capillary tube fitting, make sure that the ferrule and fitting bolt are at least 2 mm (0.1 in) from the end of the tubing before you insert the tubing into the port. Do not place the ferrule and fitting bolt flush with the end of the tubing. Figure 21 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing.

**Figure 21** Correct and Incorrect Ferrule and Fitting Bolt Placement for Capillary Tubing Connections

## 6.3.2 Shortened Retention Times



## NOTE

***Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.***

- A. Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder.
- B. Check to see if the eluent compositions and concentrations are correct. An eluent that is too concentrated will cause the peaks to elute faster. Prepare fresh eluent.



## NOTE

***If you are using a gradient pump to proportion the eluent, components from two or three different eluent reservoirs, the resulting eluent composition may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.***

- C. Column contamination can lead to a loss of column capacity. This is because all of the anion exchange sites will no longer be available for the sample ions. For example, polyvalent anions from the sample or metals may concentrate on the column. Refer to Appendix A “Column Care”, for recommended column cleanup procedures.



## NOTE

***Possible sources of column contamination are impurities in chemicals and in the deionized water used for eluents or components of the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of 18.2 megohm-cm.***

- D. Diluting the eluent will improve peak resolution, but will also increase the analytes' retention times. If a 10% dilution of the eluent is not sufficient to obtain the desired peak resolution, or if the resulting increase in retention times is unacceptable, clean the column (see, Appendix A, “Column Care”).

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes directing the column effluent to waste. Then connect the column to the suppressor. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column.



## NOTE

***For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.***

### 6.3.3 Loss of Front End Resolution

If poor resolution or efficiency is observed for the peaks eluting near the system void volume compared to the later eluting peaks, check the following:

- A. Improper eluent concentration may be the problem. If manually prepared eluent is used, remake the eluent as required for your application and ensure that the water and chemicals used are of the required purity. If Dionex eluent generator is used to generate the eluent; check the flow rate, as pump flow rate will affect the eluent concentration.
- B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical/capillary column by either diluting the sample or injecting a smaller volume onto the column.
- C. Sluggish operation of the injection valve may be the problem. Check the air pressure and make sure there are no gas leaks or partially plugged port faces. Refer to the valve manual for instructions.
- D. Improperly swept out volumes anywhere in the system prior to the guard and analytical/capillary columns may be the problem. Swap components, one at a time, in the system prior to the analytical/capillary column and test for front-end resolution after every system change.

### 6.3.4 Spurious Peaks

- A. The columns may be contaminated. If the samples contain an appreciable level of polyvalent ions and the column is used with a weak eluent system, the retention times for the analytes will then decrease and be spurious, inefficient (broad) peaks that can show up at unexpected times. Clean the column as indicated in Appendix A “Column Care”.



**NOTE**

*For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.*

- B. The injection valve may need maintenance. When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset can show up as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

## Appendix A – Column Care

### A.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Dionex IonPac AS11-HC-4 $\mu$ m columns is 5,000 psi (34.47 MPa).

### A.2 Column Start-Up

The column is shipped using 100 mM Sodium Borate as the storage solution. Prepare the eluent shown on the Quality Assurance Report (QAR), install the column in the chromatography module and direct the column effluent to waste for 30 minutes, and then connect to the suppressor. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

If peak efficiencies or resolution are poorer than the QAR, see Sections 3.13 Installation of the Capillary Column, Section 6.3 Poor Peak Res and Section 6.3.1 Loss of Column Efficiency.

#### IMPORTANT

*When making any tubing connections (column installation, replacing tubing etc), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette with the flow off before joining two components together. This will prevent air from entering the system and result in a faster equilibration.*

### A.3 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Borate for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

### A.4 Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble, or organic contaminants. They can be combined into one gradient protocol if desired; however, the following precautions should be observed. When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to < 5% levels and the ionic strength of the eluent to < 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

**WARNING**

- *Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column.*
- *High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column.*
- *High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible, that have eluent components in one eluent that will precipitate out in the other eluent or by using an acid eluent followed by a base eluent which may create a neutralization pressure band.*
- *The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.*

**A.4.1 Choosing the Appropriate Cleanup Solution**

| Contamination                               | Solution                                                                                                                                                                                                                                                           |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hydrophilic Contamination of Low Valence    | Concentrated hydroxide solutions such as a 10X concentrate of the most concentrated eluent used in the application is sufficient to remove hydrophilic contamination of low valence.                                                                               |
| High Valence Hydrophilic Ions Contamination | Concentrated acid solutions such as 1 to 3 M HCl will remove high valence hydrophilic ions by ion suppression and elution by the chloride ion.                                                                                                                     |
| Metal Contamination                         | Metal contamination often results in asymmetric peak shapes and/or variable analyte recoveries. For example, iron or aluminum contamination often results in tailing of sulfate and phosphate. Aluminum contamination can also result in low phosphate recoveries. |
|                                             | Concentrated acid solutions such as 1 to 3 M HCl remove a variety of metals. If after acid treatment, the chromatography still suggests metal contamination, treatment with chelating acids such as 0.2 M oxalic acid is recommended.                              |
| Nonionic and Hydrophobic Contamination      | Organic solvents can be used alone if the contamination is nonionic and hydrophobic. The degree of nonpolar character of the solvent should be increased as the degree of hydrophobicity of the contamination within the range of acceptable solvents.             |
| Ionic and Hydrophobic Contamination         | Concentrated acid solutions such as 1 to 3 M HCl can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic. The acid suppresses ionization and ion exchange interactions of the contamination with the resin.             |
|                                             | A frequently used cleanup solution is 200 mM HCl in 80% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage.                                                     |

### A.4.2 Column Cleanup Procedure

- A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in Section B.4.1, "Choosing the Appropriate Cleanup Solution."
- B. Disconnect the suppressor from the columns and direct the column effluent to waste. If your system is configured with both a guard column and an analytical/capillary column, reverse the order of the guard and analytical/capillary column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels.



#### CAUTION

***When cleaning an analytical/capillary column and a guard column in series, ensure that the guard column is placed after the analytical/capillary column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical/capillary column and irreversibly damage it. If in doubt, clean each column separately.***

- C. Set the pump flow rate to 1.0 mL/min for a 4 mm analytical and/or guard column, 0.25 mL/min for a 2 mm analytical and/or guard column and 0.010 mL/min for 0.4 mm capillary and/or capillary guard column.
- D. Rinse the column for 10 minutes with deionized water before pumping the chosen cleanup solution over the column.
- E. Pump the cleanup solution through the column for at least 60 minutes. If column is contaminated heavily, then clean the column for four hours to overnight.
- F. Rinse the column for 10 minutes with deionized water before pumping eluent over the column.
- G. Equilibrate the column(s) with eluent still directing the column effluent to waste for at least 60 minutes before resuming normal operation.

Reinstall the guard/capillary guard column in line between the injection valve and the analytical/capillary column and reconnect the analytical/capillary column to the suppressor.

## Appendix B – System Configuration

Table B1 Configuration

| CONFIGURATION                                                                                                                                                                                                                                                           | 2 mm                                                                                                                     | 4 mm                                                                                                                     | 0.4 mm                                                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Eluent Flow Rate</b>                                                                                                                                                                                                                                                 | 0.38 mL/min                                                                                                              | 1.5 µL/min                                                                                                               | 0.015 µL/min                                                                                                                                       |
| <b>SRS Suppressor</b>                                                                                                                                                                                                                                                   | Dionex ASRS 300<br>(P/N 061562)                                                                                          | Dionex ASRS 300<br>(P/N 061561)                                                                                          | N/A                                                                                                                                                |
| <b>MMS Suppressor</b>                                                                                                                                                                                                                                                   | Dionex AMMS 300<br>(P/N 056751)                                                                                          | Dionex AMMS 300<br>(P/N 056750)                                                                                          | N/A                                                                                                                                                |
| <b>ACES Suppressor</b>                                                                                                                                                                                                                                                  | N/A                                                                                                                      | N/A                                                                                                                      | Dionex ACES 300<br>(P/N 072052)                                                                                                                    |
| <b>NOTE:</b><br>Do not run suppressors over 40°C. If application requires a higher temperature, place suppressor outside of chromatographic oven.                                                                                                                       |                                                                                                                          |                                                                                                                          |                                                                                                                                                    |
| <b>Injection Loop</b>                                                                                                                                                                                                                                                   | 2 - 15 µL<br>Use the Rheodyne Microinjection Valve, Model No. 9126 P/N 044697) for full loop injections <15 µL.          | 10 - 50 µL                                                                                                               | 0.4 µL<br>(typical)                                                                                                                                |
| <b>System Void Volume</b>                                                                                                                                                                                                                                               | Eliminate switching valves, couplers and use only the 2 mm Dionex GM-4 Mixer (P/N 049135).                               | Eliminate switching valves, couplers and use the Dionex GM-2, GM-3 or recommended gradient mixers.                       | Use only an IC system equipped for capillary analysis where dead volumes are fixed such as the Dionex ICS-5000 <sup>+</sup> HPIC capillary system. |
| <b>Pumps</b>                                                                                                                                                                                                                                                            | IC single or dual pump capable of operating up to 5000 psi or higher such as the Dionex ICS-5000 <sup>+</sup> HPIC pump. | IC single or dual pump capable of operating up to 5000 psi or higher such as the Dionex ICS-5000 <sup>+</sup> HPIC pump. | Use only a pump designed for capillary flow rates such as the Dionex ICS-5000 <sup>+</sup> HPIC capillary pump.                                    |
| <b>NOTE:</b><br>Use of a Dionex EGC 500 KOH cartridge (P/N 075778 or 072076 in conjunction with a Dionex CR-ATC 500 P/N 075550 or 072078) for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients. |                                                                                                                          |                                                                                                                          |                                                                                                                                                    |
| <b>Chromatographic Module</b>                                                                                                                                                                                                                                           | A thermally controlled column oven such as the Dionex ICS-5000 <sup>+</sup> DC                                           | A thermally controlled column oven such as the Dionex ICS-5000 <sup>+</sup> DC                                           | A thermally controlled column compartment such as the Dionex ICS-5000 DC equipped with Dionex IC-Cube.                                             |

Table B2 Tubing Back Pressures

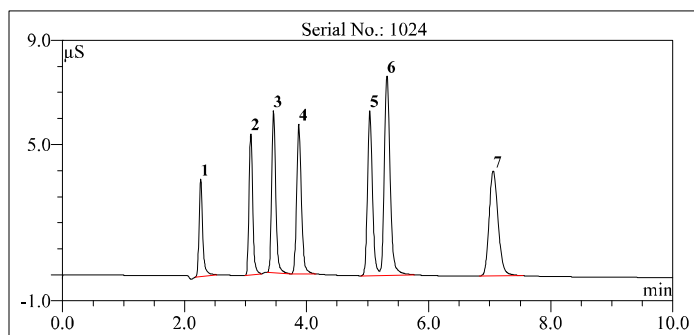
| Color      | Part Number | I.D. inch | I.D. cm | Volume mL/ft | Back Pressure Psi/ft. at 1 mL/min | Back Pressure Psi/ft. at 0.25 mL/min | Back Pressure Psi/cm. at 1 mL/min |
|------------|-------------|-----------|---------|--------------|-----------------------------------|--------------------------------------|-----------------------------------|
| Green      | 044777      | 0.030     | 0.076   | 0.137        | 0.086                             | 0.021                                | 0.003                             |
| Orange     | 042855      | 0.020     | 0.051   | 0.061        | 0.435                             | 0.109                                | 0.015                             |
| Blue       | 049714      | 0.013     | 0.033   | 0.026        | 2.437                             | 0.609                                | 0.081                             |
| Black      | 042690      | 0.010     | 0.025   | 0.015        | 6.960                             | 1.740                                | 0.232                             |
| Red        | 044221      | 0.005     | 0.013   | 0.004        | 111.360                           | 27.840                               | 3.712                             |
| Yellow     | 049715      | 0.003     | 0.008   | 0.001        | 859.259                           | 214.815                              | 28.642                            |
| Light Blue | 071870      | 0.0025    | 0.006   | 0.0009       | 1766.0                            | 441.0                                | 58.0                              |

# Appendix C – Quality Assurance Reports

**Dionex IonPac™ AS11-HC-4µm**  
**Capillary (0.4 x 250 mm)**  
**Product No. 078031**

**Date:** 14-Sep-12 13:17  
**Serial No. :** 001024  
**Lot No. :** 011-33-156A

**Eluent:** 30 mM Potassium Hydroxide (KOH)  
**Eluent Source:** Dionex EGC-KOH (Capillary)  
**Flow Rate:** 0.015 mL/min  
**Temperature:** 30 °C  
**Detection:** Suppressed Conductivity  
**Suppressor:** Dionex Anion Capillary Electrolytic Suppressor (Dionex ACES 300)  
 AutoSuppression™ Recycle Mode  
**Applied Current:** 10 mA  
**Injection Volume:** 0.40 µL (Internal Loop)  
**Storage Solution:** 100 mM Sodium Tetraborate



| No. | Peak Name | Ret.Time<br>(min) | Asymmetry<br>(AIA) | Resolution<br>(EP) | Efficiency<br>(EP) | Concentration<br>(mg/L) |
|-----|-----------|-------------------|--------------------|--------------------|--------------------|-------------------------|
| 1   | Fluoride  | 2.26              | 1.4                | 7.99               | 7840               | 0.5                     |
| 2   | Chloride  | 3.08              | 1.3                | 3.39               | 14154              | 1.3                     |
| 3   | Nitrite   | 3.46              | 1.3                | 3.27               | 13971              | 2.5                     |
| 4   | Sulfate   | 3.87              | 1.2                | 8.19               | 12513              | 2.5                     |
| 5   | Bromide   | 5.04              | 1.2                | 1.81               | 18929              | 5.0                     |
| 6   | Nitrate   | 5.32              | 1.3                | 8.15               | 16816              | 5.0                     |
| 7   | Phosphate | 7.06              | 1.3                | n.a.               | 11434              | 7.5                     |

## QA Results:

| Analyte | Parameter      | Specification | Results |
|---------|----------------|---------------|---------|
| Sulfate | Efficiency     | >=9450        | Passed  |
| Sulfate | Asymmetry      | 1.0-1.8       | Passed  |
| Sulfate | Retention Time | 3.36-4.35     | Passed  |
|         | Pressure       | <=4400        | 2985    |

## Production Reference:

Datasource: QAR  
 Directory Cap\AS11-HC-4µm  
 Sequence: AS11HC\_0p4X250\_4µm  
 Sample No.: 2

6.80 SR11 Build 3161 (184582) (Demo-Installation)

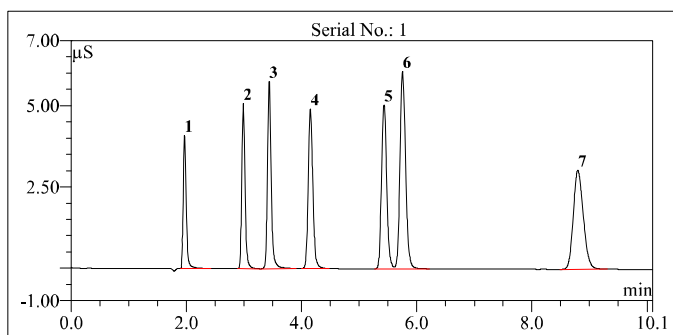
Chromeleon™ Thermo Fisher Scientific

078944-01 (QAR)

**Dionex IonPac™ AS11-HC-4μm**  
**Analytical (2 x 250 mm)**  
**Product No. 078035**

**Date:** 21-Sep-12 08:45  
**Serial No. :** 000001  
**Lot No. :** 006-09-133A

**Eluent:** 30 mM Potassium Hydroxide (KOH)  
**Eluent Source:** Dionex EGC 500 KOH  
**Flow Rate:** 0.38 mL/min  
**Temperature:** 30 °C  
**Detection:** Suppressed Conductivity  
**Suppressor:** Dionex Anion Self-Regenerating Suppressor (Dionex ASRS™ 300 2mm)  
 AutoSuppression™ Recycle Mode  
**Applied Current:** 29 mA  
**Injection Volume:** 2.5 μL  
**Storage Solution:** 100 mM Sodium Tetraborate



| No. | Peak Name | Ret.Time<br>(min) | Asymmetry<br>(AIA) | Resolution<br>(EP) | Efficiency<br>(EP) | Concentration<br>(mg/L) |
|-----|-----------|-------------------|--------------------|--------------------|--------------------|-------------------------|
| 1   | Fluoride  | 1.96              | 1.5                | 10.88              | 8052               | 2.0                     |
| 2   | Chloride  | 2.99              | 1.3                | 4.18               | 13959              | 5.0                     |
| 3   | Nitrite   | 3.44              | 1.2                | 5.55               | 14164              | 10.0                    |
| 4   | Sulfate   | 4.15              | 1.2                | 8.38               | 13544              | 10.0                    |
| 5   | Bromide   | 5.43              | 1.1                | 1.87               | 17685              | 20.0                    |
| 6   | Nitrate   | 5.75              | 1.3                | 12.35              | 16424              | 20.0                    |
| 7   | Phosphate | 8.80              | 1.3                | n.a.               | 12485              | 30.0                    |

**QA Results:**

| Analyte | Parameter      | Specification | Results |
|---------|----------------|---------------|---------|
| Sulfate | Efficiency     | >=9450        | Passed  |
| Sulfate | Asymmetry      | 1.0-1.8       | Passed  |
| Sulfate | Retention Time | 3.36-4.35     | Passed  |
|         | Pressure       | <=4400        | 3366    |

*Production Reference:*

Datasource: QAR  
 Directory: Anion\AS11\_HC-4μm  
 Sequence: AS11HC\_2X250MM\_4μM  
 Sample No.: 2

6.80 SR11 Build 3161 (184582) (Demo-Installation)

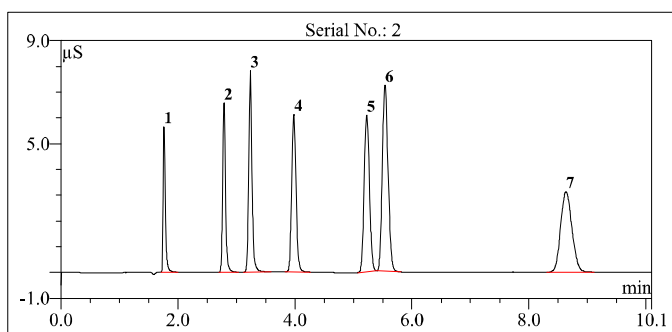
Chromeleon™ Thermo Fisher Scientific

078946-01 (QAR)

**Dionex IonPac™ AS11-HC-4μm**  
**Analytical (4 x 250 mm)**  
**Product No. 082313**

**Date:** 25-Sep-12 08:17  
**Serial No. :** 000002  
**Lot No. :** 006-09-116D

**Eluent:** 30 mM Potassium Hydroxide (KOH)  
**Eluent Source:** Dionex EGC 500 KOH  
**Flow Rate:** 1.50 mL/min  
**Temperature:** 30 °C  
**Detection:** Suppressed Conductivity  
**Suppressor:** Dionex Anion Self-Regenerating Suppressor (Dionex ASRS™ 300 4mm)  
 AutoSuppression™ Recycle Mode  
**Applied Current:** 112 mA  
**Injection Volume:** 10 μL  
**Storage Solution:** 100 mM Sodium Tetraborate



| No. | Peak Name | Ret.Time<br>(min) | Asymmetry<br>(AIA) | Resolution<br>(EP) | Efficiency<br>(EP) | Concentration<br>(mg/L) |
|-----|-----------|-------------------|--------------------|--------------------|--------------------|-------------------------|
| 1   | Fluoride  | 1.76              | 1.7                | 13.29              | 10999              | 2.0                     |
| 2   | Chloride  | 2.79              | 1.2                | 4.70               | 16116              | 5.0                     |
| 3   | Nitrite   | 3.24              | 1.2                | 6.21               | 15667              | 10.0                    |
| 4   | Sulfate   | 3.98              | 1.1                | 8.23               | 13387              | 10.0                    |
| 5   | Bromide   | 5.23              | 1.2                | 1.80               | 15965              | 20.0                    |
| 6   | Nitrate   | 5.54              | 1.3                | 11.45              | 14614              | 20.0                    |
| 7   | Phosphate | 8.63              | 1.2                | n.a.               | 9260               | 30.0                    |

**QA Results:**

| Analyte | Parameter      | Specification | Results |
|---------|----------------|---------------|---------|
| Sulfate | Efficiency     | >=9450        | Passed  |
| Sulfate | Asymmetry      | 1.0-1.8       | Passed  |
| Sulfate | Retention Time | 3.36-4.35     | Passed  |
|         | Pressure       | <=4400        | 3988    |

*Production Reference:*

Datasource: QAR

Directory Anion\AS11\_HC-4μm

Sequence: AS11HC\_4X250MM\_4μm

Sample No.: 2

6.80 SR11 Build 3161 (184582) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

078945-02 (QAR)