



Thermo Scientific

Dionex IonPac AS11-HC-4 μ m

Product Manual

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Product Manual

for

Dionex IonPac AS11-HC-4 μ m Capillary Column

(0.4 x 250 mm, P/N 078031)

Dionex IonPac AG11-HC-4 μ m Capillary Guard Column

(0.4 x 50 mm, P/N 078032)

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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.

Contents

1.	Introduction	7
2.	Ion Chromatography Systems.....	9
3.	Installation	10
3.1	Column Start-Up	10
3.2	Column Storage.....	10
3.3	System Requirements for 0.4mm Operation	10
3.4	System Void Volume	10
3.5	The Sample Concentrator.....	11
3.6	The 0.4 mm System Injection Loop, 0.4 µL Internal Loop.....	11
3.7	The Dionex IonPac AG11-HC-4µm Capillary Guard Column.....	11
3.8	Installing the Dionex CR-ATC Trap Column for Use with Dionex EGC.....	12
3.9	Eluent Storage	12
3.10	Dionex Anion Capillary Electrolytic Suppressor Requirements.....	12
3.11	Using the Dionex EGC-KOH with Dionex IonPac AS11-HC-4µm Column.....	13
3.12	Detector Requirements.....	13
3.13	Installation of the Capillary Column.....	13
4.	Operation	17
4.1	General Operating Conditions.....	17
4.2	Dionex IonPac AS11-HC-4µm Column Operating Precautions	17
4.3	Chemical Purity Requirements.....	18
4.3.1	Inorganic Chemicals.....	18
4.3.2	Deionized Water	18
4.3.3	Solvents	18
4.4	Making Eluents that Contain Solvents	19
4.5	Eluent Preparation.....	19
5.	Example Applications	20
5.1	Recommendations for Optimum System Performance	20
5.2	Dionex IonPac AS11-HC-4µm Capillary Column With and Without Capillary Guard Column.....	21
5.3	Gradient Analysis of a Large Number of Anions using Aqueous KOH Eluent.....	22
5.4	Comparison of Dionex IonPac AS11-HC and AS11-HC-4µm Capillary Columns.....	23
5.5	Analysis of Beer Samples	24
5.6	Analysis of Fruit Juice Samples.....	26
5.7	Analysis of a Wine Sample	28

6.	Troubleshooting Guide	29
6.1	High Back Pressure.....	30
6.1.1	Finding the Source of High System Pressure	30
6.1.2	Filter Eluent.....	31
6.1.3	Filter Samples.....	31
6.2	High Background.....	31
6.2.1	Preparation of Eluents	31
6.2.2	Contaminated Dionex CR-ATC Column.....	31
6.2.3	A Contaminated Capillary Guard or Capillary Column	31
6.2.4	Contaminated Hardware	32
6.2.5	A Contaminated Dionex ACES 300 Suppressor	32
6.3	Poor Peak Resolution	33
6.3.1	Loss of Column Efficiency.....	33
6.3.2	Poor Resolution Due to Shortened Retention Times	33
6.3.3	Loss of Front End Resolution	34
6.3.4	Spurious Peaks.....	35
6.3.5	Poor Efficiency Using Capillary Columns	36
Appendix A	– Column Care	37
A.1	Recommended Operation Pressures.....	37
A.2	Column Start-Up.....	37
A.3	Column Storage.....	37
A.4	Column Cleanup	38
A.4.1	Choosing the Appropriate Cleanup Solution	39
A.4.2	Column Cleanup Procedure.....	40
Appendix B	– System Configuration	41
Appendix C	– Quality Assurance Reports	42

1. Introduction

The Dionex IonPac AS11-HC-4 μ m 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-4 μ m 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-4 μ m 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-4 μ m column is available in 0.4 x 250 mm format. The Dionex IonPac AS11-HC-4 μ m column is stable between pH 0 and 14 and is compatible with eluents containing 0-100% organic solvents. The Dionex IonPac AG11-HC-4 μ m 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-4 μ m Capillary Column (0.4 x 250 mm) requires only one-hundredth (1/100) the eluent flow rate of a typical 4mm application. The capillary format has the advantage of less eluent consumption, providing reduced costs.

Table 1 – Dionex IonPac AS11-HC-4 μ m/Dionex IonPac AG11-HC-4 μ m Column Packing Specifications

Column	Particle Diameter μ m	Substrate X-linking %	Latex Diameter nm	Column Capacity μ eq/column	Functional Group	Hydrophobicity
Dionex IonPac AS11-HC-4 μ m* 0.4 x 250 mm	4	55%	70	2.90	Alkanol quaternary ammonium	Medium-Low
Dionex IonPac AG11-HC-4 μ m** 0.4 x 50 mm	13	55%	70	0.07	Alkanol quaternary ammonium	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 2 – Dionex IonPac AS11-HC-4 μ m/Dionex IonPac AG11-HC-4 μ m Column Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Dionex IonPac AS11-HC-4 μ m 0.4-mm Capillary Column	\leq 3800 (26.20)	0.015	0.020
Dionex IonPac AG11-HC-4 μ m 0.4-mm Capillary Guard Column	\leq 150 (1.03)	0.015	0.020
Dionex IonPac AS11-HC-4 μ m and AG11-HC-4 μ m 0.4-mm Capillary and Capillary Guard Column	\leq 3950 (27.23)	0.015	0.020

**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 μ m Capillary Column can only be operated under standard conditions using a Thermo Scientific Dionex ICS-5000 Capillary HPIC System or a Dionex ICS-4000 Capillary HPIC System. Both of these systems are capable of operating up to 5000 psi to support the back pressure generated by the Dionex IonPac AS11-HC-4 μ m Capillary Column under standard conditions.

See Appendix B, "System Configuration" for specific recommended settings and parts including pumps, eluent flow rate, Thermo Scientific Dionex Capillary Electrolytic Suppressor (CES 300), injection loop, system void volume, detectors, and tubing back pressure.



NOTE

Do not operate suppressors over 20 °C. It is highly recommended to use ACES-300 at lower temperature (15°C) for optimum performance. Use of a Dionex EG with a Dionex EGC III KOH (P/N 074532) 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, 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 and 6.3.5 Poor Efficiency using Capillary Columns for information regarding proper connections.

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 0.4mm Operation

The Dionex IonPac AS11-HC-4 μ m Capillary Guard and Capillary Column are designed to be run on a capillary ion chromatograph system equipped with suppressed conductivity detection with the capability of running at 5000 psi continuously. It is required to run the capillary column only on the Dionex ICS-5000 Capillary HPIC system or the Dionex ICS-4000 Capillary HPIC system for best performance.

3.4 System Void Volume

The Dionex ICS-5000 Capillary HPIC and the Dionex ICS-4000 Capillary HPIC systems have preconfigured tubing to minimize the system void volume. This 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 method may be required to ensure equivalent retention time and peak resolution.

3.5 The Sample Concentrator

For trace anion concentration work with the Dionex IonPac AS11-HC-4 μ m Capillary Column, use the Dionex IonSwift MAC-100 Concentrator Column or the Dionex IonPac AG11-HC-4 μ m Capillary Guard Column. 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.

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 Dionex IonSwift Monolith Anion Concentrator 100 (Dionex MAC-100) Column Product Manual (Document No. 065387).

3.6 The 0.4 mm System Injection Loop, 0.4 μ L Internal Loop

For most applications on a 0.4 mm capillary system, a 0.4 μ 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. The standard 4 port injection valve must be changed to the 6 port injection valve (P/N 061947), to accommodate the external loop.

3.7 The Dionex IonPac AG11-HC-4 μ m Capillary Guard Column

A Dionex IonPac AG11-HC-4 μ m Capillary Guard Column is normally used with the Dionex IonPac AS11-HC-4 μ m Capillary Column. Retention times will increase by approximately 5% when a guard column is placed in-line prior to the capillary column under isocratic test conditions. A guard is placed prior to the capillary column to prevent sample contaminants from eluting onto the capillary column. It is easier to clean or replace a guard column than it is a capillary column. Replacing the Dionex IonPac AG11-HC-4 μ m 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 μ m Capillary Column.

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

For Dionex IonPac AS11-HC-4 μ m applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC Continuously Regenerated Trap Column (Capillary) (P/N 072078) 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.

3.9 Eluent Storage

The Dionex IonPac AS11-HC-4 μ m column is designed to be used with hydroxide eluent systems. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

3.10 Dionex Anion Capillary Electrolytic Suppressor Requirements

A Dionex Anion Capillary Electrolytic Suppressor (ACES 300) 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 ACES 300 modes of operation.



CAUTION

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

For Dionex IonPac AS11-HC-4 μ m Capillary Column, use a Dionex ACES 300 (P/N 072052).

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”.

3.11 Using the Dionex EGC-KOH with Dionex IonPac AS11-HC-4 μ m Column

The Dionex IonPac AS11-HC-4 μ m column is recommended for use with Dionex ICS-5000 Capillary HPIC or Dionex ICS-4000 Capillary HPIC Systems equipped with a Dionex Eluent Generator. The Dionex Eluent Generator is used to automatically produce potassium hydroxide gradients from deionized water. Please refer to the Dionex ICS-5000 Capillary HPIC system (Document No. 065342) or the Dionex ICS-4000 Capillary HPIC system (Document No. 065468) manual for information on the operation of the Eluent Generator.

3.12 Detector Requirements

See Section 2, “Ion Chromatography Systems,” for capillary system detector, cell and thermal stabilizer requirements.

3.13 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

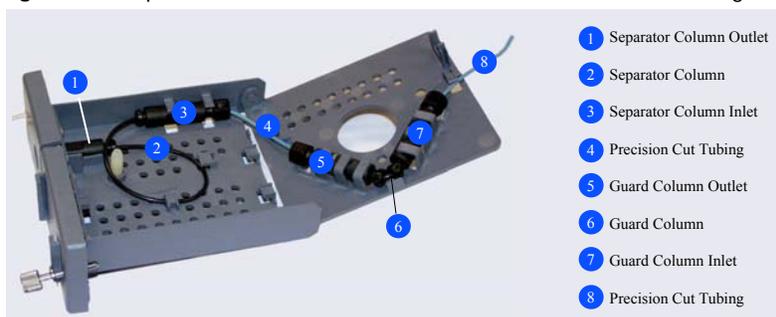
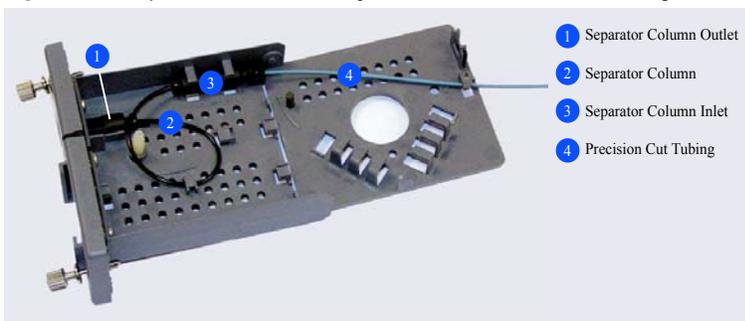


Figure 2 – Separator Column Only Installed in Column Cartridge



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:

3 – Installation

Table 3 – 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

3 – Installation

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

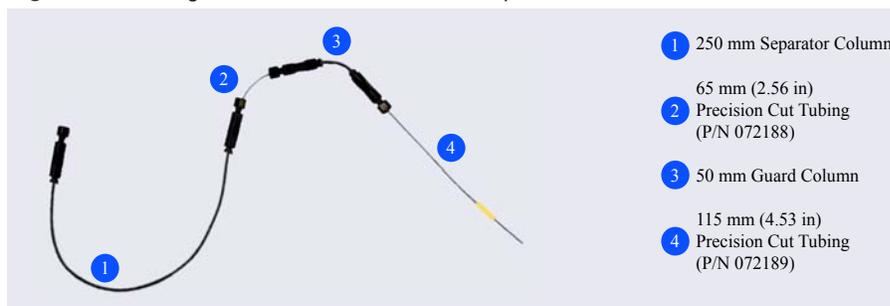
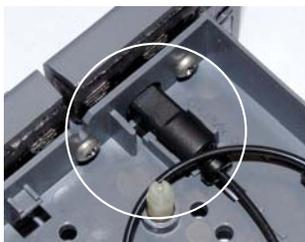


Figure 4 – Tubing Connections for Separator Column Only



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



4. Operation

4.1 General Operating Conditions

Sample Volume:	0.4-mm: 0.4 μ L Loop
Column:	0.4-mm: Dionex IonPacAG11-HC-4 μ m Capillary Guard Column+ Dionex IonPac AS11-HC-4 μ m Capillary Column
Eluent:	30 mM KOH (for test chromatogram)
Eluent Source:	Dionex EGC III KOH Capillary Cartridge
Eluent Flow Rate:	0.4-mm: 0.015 mL/min
SRS Suppressor:	Dionex Anion Capillary Electrolytic Suppression, ACES 300 AutoSuppression Recycle Mode
Expected Background Conductivity:	< 2 μ S
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent

4.2 Dionex IonPac AS11-HC-4 μ m Column Operating Precautions

Table 4 – 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	0.020 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 μm . 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 μm 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 μm column is 5,000 psi (34.47MPa).

The Dionex IonPac AS11-HC-4 μm capillary 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 5 – HPLC Solvents for Use with Dionex IonPac AS11-HC-4 μ 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.

**CAUTION**

The Dionex ACES 300 must be operated in the AutoSuppression External Water Mode when using eluents containing solvents. Do not use > 40% solvent on the Dionex ACES 300 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.

**NOTE**

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.

**NOTE**

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.

**NOTE**

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.

**SAFETY**

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

4.5 Eluent Preparation

The Dionex Eluent Generator is used to automatically produce potassium hydroxide gradients from deionized water. Please refer to the Dionex ICS-5000 Capillary HPIC system (Document No. 065342) or the Dionex ICS-4000 Capillary HPIC system (Document No. 065468) manual for information on the operation of the Eluent Generator.

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 μ m capillary 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 μ 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 capillary guard column to protect the capillary column. If column performance deteriorates and it is determined that the guard and 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 3.5, “The Sample Concentrator”).



CAUTION

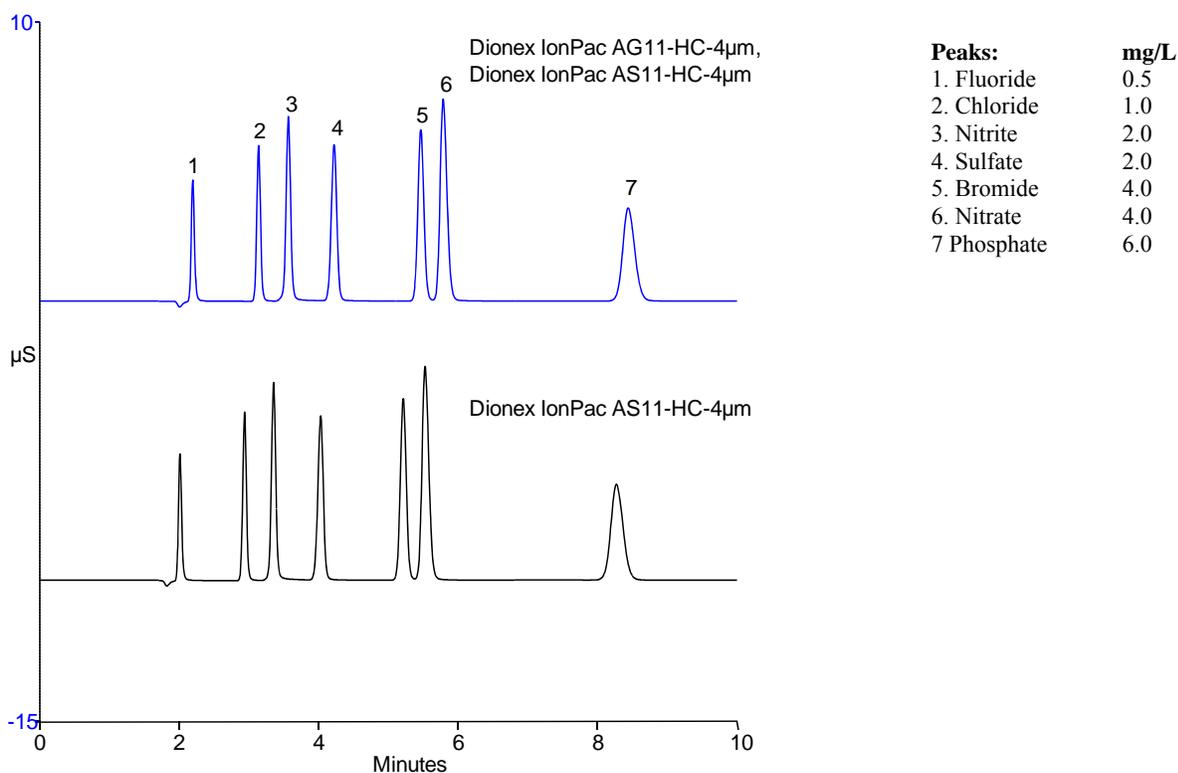
Carbon dioxide readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can affect the retention times of the anions being analyzed. Eluents should be maintained under an inert helium atmosphere to avoid carbonate contamination.

5.2 Dionex IonPac AS11-HC-4 μ m Capillary Column With and Without Capillary Guard Column

Isocratic elution of common anions using the Dionex IonPac AS11-HC-4 μ m 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 μ m Capillary Column. The Dionex IonPac AS11-HC-4 μ m Capillary Column should always be used with the Dionex IonPac AG11-HC-4 μ m Capillary Guard Column. An operating temperature of 30°C is used to ensure reproducible resolution and retention of analytes. Note that the Dionex IonPac AG11-HC-4 μ m Capillary Guard is packed with a microporous resin of proportionally lower capacity and contributes approximately **5%** increase in retention times when a guard column is placed in-line prior to the capillary column under isocratic test conditions.

Column: See Chromatogram
 Eluent : 30mM KOH
 Eluent Source: Dionex EGC III KOH capillary cartridge
 Flow Rate: 0.015mL/min
 Inj. Volume: 0.4 μ L
 Temperature: 30°C
 Detection: Suppressed Conductivity, Dionex Anion Capillary Electrolytic Suppressor (Dionex ACES 300)
 AutoSuppression recycle mode

Figure 7 – Dionex IonPac AS11-HC-4 μ m Capillary Column With and Without Capillary Guard Column

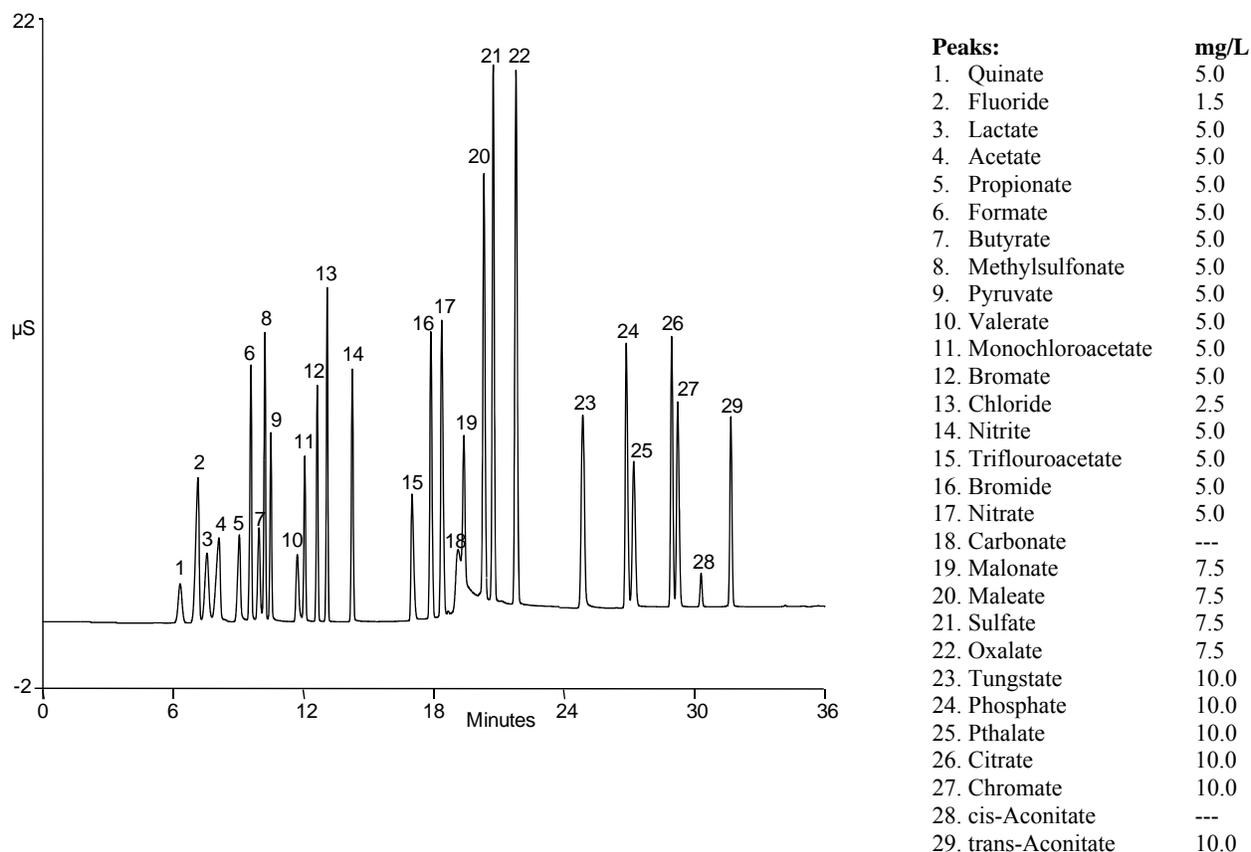


5.3 Gradient Analysis of a Large Number of Anions using Aqueous KOH Eluent

Figure 8 illustrates the separation of a large number of inorganic anions and organic acids using the Dionex IonPac AS11-HC-4 μ m 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.

Column: Dionex IonPac AG11-HC-4 μ m/
AS11-HC-4 μ m (0.4 \times 250 mm)
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 μ L
Temperature: 30 $^{\circ}$ C
Detection: Suppressed conductivity, Dionex ACES 300 AutoSuppression recycle mode

Figure 8 – Gradient Analysis of a Large Number of Anions using Aqueous KOH Eluent

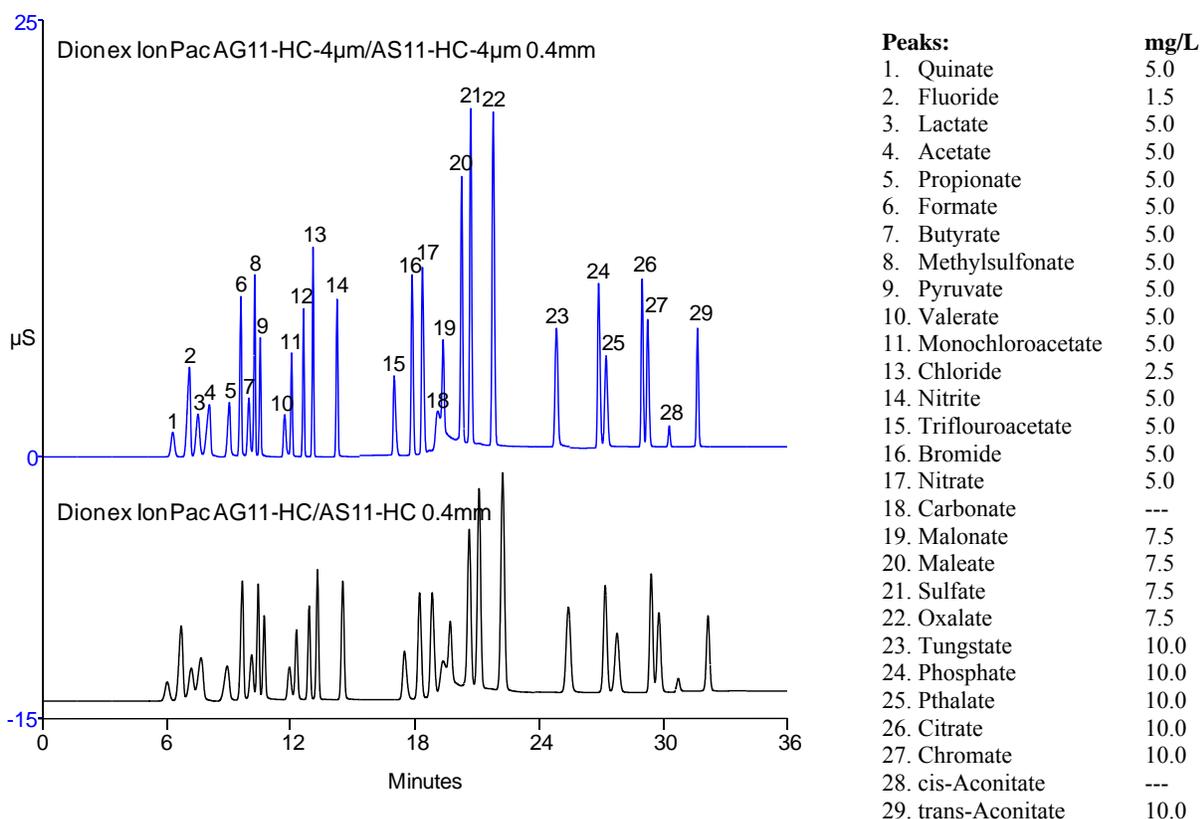


5.4 Comparison of Dionex IonPac AS11-HC and AS11-HC-4µm Capillary Columns

The new Dionex IonPac AS11-HC-4µm capillary column is packed with smaller resin particles than the current Dionex IonPac AS11-HC capillary column. The smaller resin particles produce more efficient peaks as shown in Figure 9 below. Note the improved resolution of the early eluting peaks when using the Dionex IonPac AS11-HC-4µm column. In general, all the peaks are sharper and taller, providing better resolution and sensitivity when using the Dionex IonPac AS11-HC-4µm column.

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 µL
 Temperature: 30 °C
 Detection: Suppressed conductivity, Dionex ACES 300
 AutoSuppression recycle mode

Figure 9 – Comparison of Dionex IonPac AS11-HC and AS11-HC-4µm Capillary Columns



5.5 Analysis of Beer Samples

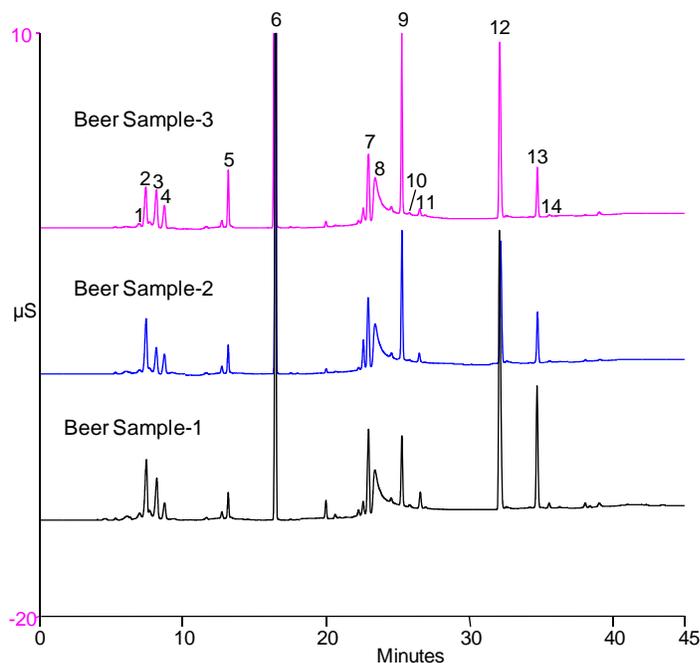
Figure 10 uses an optimized potassium hydroxide gradient (Figure 10A) as well as a potassium hydroxide gradient with methanol (Figure 10B) for analysis of beer samples. The beer samples were diluted 1:25 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge. Note that, under aqueous eluent conditions, succinate and malate co-elute. However, by adding 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 (0.12mL/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.

Column: Dionex IonPac AG11-HC-4 μ m/AS11-HC-4 μ m (0.4 \times 250 mm)
 Eluent: 1mM KOH for 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 III KOH capillary cartridge
 Flow Rate: 0.015mL/min
 Inj. Volume: 0.4 μ L
 Temperature: 30°C
 Detection: Suppressed Conductivity, Dionex ACES 300
 AutoSuppression recycle mode
 Sample: Beer with 1:25 sample dilution

Figure 10A – Analysis of Beer Samples Using an Aqueous Gradient with the Dionex IonPac AS11-HC-4 μ m Capillary Column



Peaks:

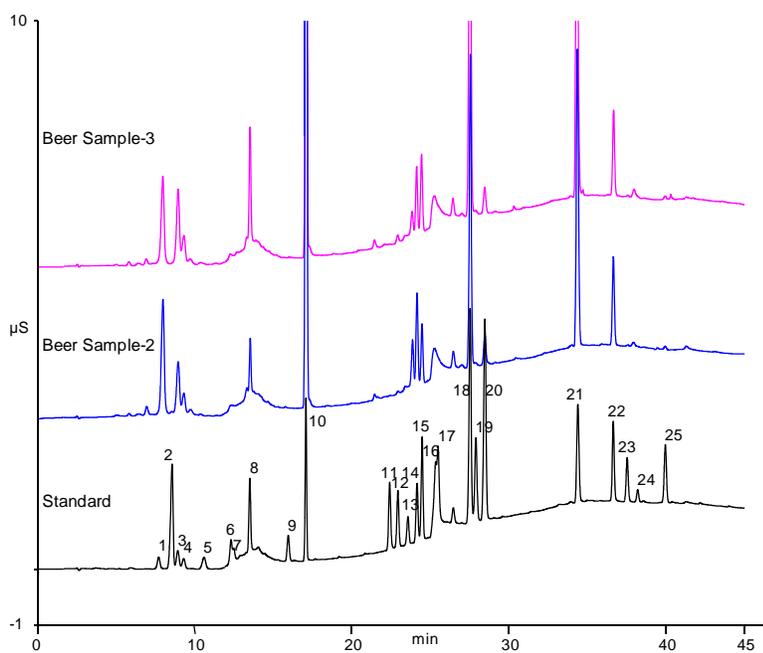
1. Quinate
2. Fluoride
3. Lactate
4. Acetate
5. Pyruvate
6. Chloride
7. Succinate+Malate*
8. Carbonate
9. Sulfate
10. Oxalate
11. Fumarate
12. Phosphate
13. Citrate
14. Isocitrate

*Succinate and Malate co-elute under aqueous conditions

5 – Example Applications

Column: Dionex IonPac AG11-HC-4 μ m/AS11-HC-4 μ m (0.4 \times 250 mm)
 Eluent + Methanol*: 1mM KOH for 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 III KOH capillary cartridge
 Flow Rate: 0.015mL/min
 Inj. Volume: 0.4 μ L
 Temperature: 30 $^{\circ}$ C
 Detection: Suppressed Conductivity, Dionex ACES 300
 AutoSuppression external water mode
 Sample: Beer with 1:25 sample dilution

Figure 10B – Analysis of Beer Samples Using an Aqueous Gradient with Solvent and the Dionex IonPac AS11-HC-4 μ m Capillary Column



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. cis-Aconitate	--
25. trans-Aconitate	3

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

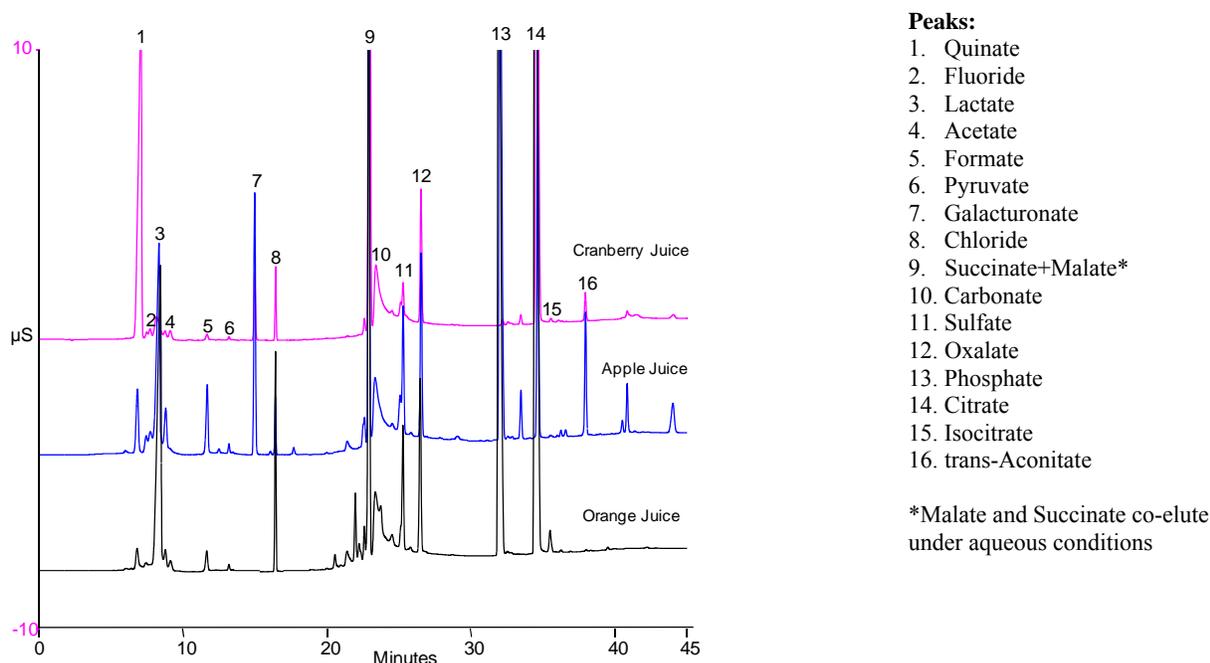
5.6 Analysis of Fruit Juice Samples

Figure 11 uses an optimized potassium hydroxide gradient (Figure 11A) as well as a potassium hydroxide gradient with methanol (Figure 11B) for analysis of various juice samples. The juice samples were diluted 1:50 with deionized water and filtered through a 0.45 mm 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 (0.12mL/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.

Column: Dionex IonPac AG11-HC-4 μ m/AS11-HC-4 μ m (0.4 \times 250 mm)
 Eluent: 1mM KOH for 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 III KOH capillary cartridge
 Flow Rate: 0.015mL/min
 Inj. Volume: 0.4 μ L
 Temperature: 30°C
 Detection: Suppressed Conductivity, Dionex ACES 300
 AutoSuppression recycle mode
 Sample: Juice sample diluted 1:50

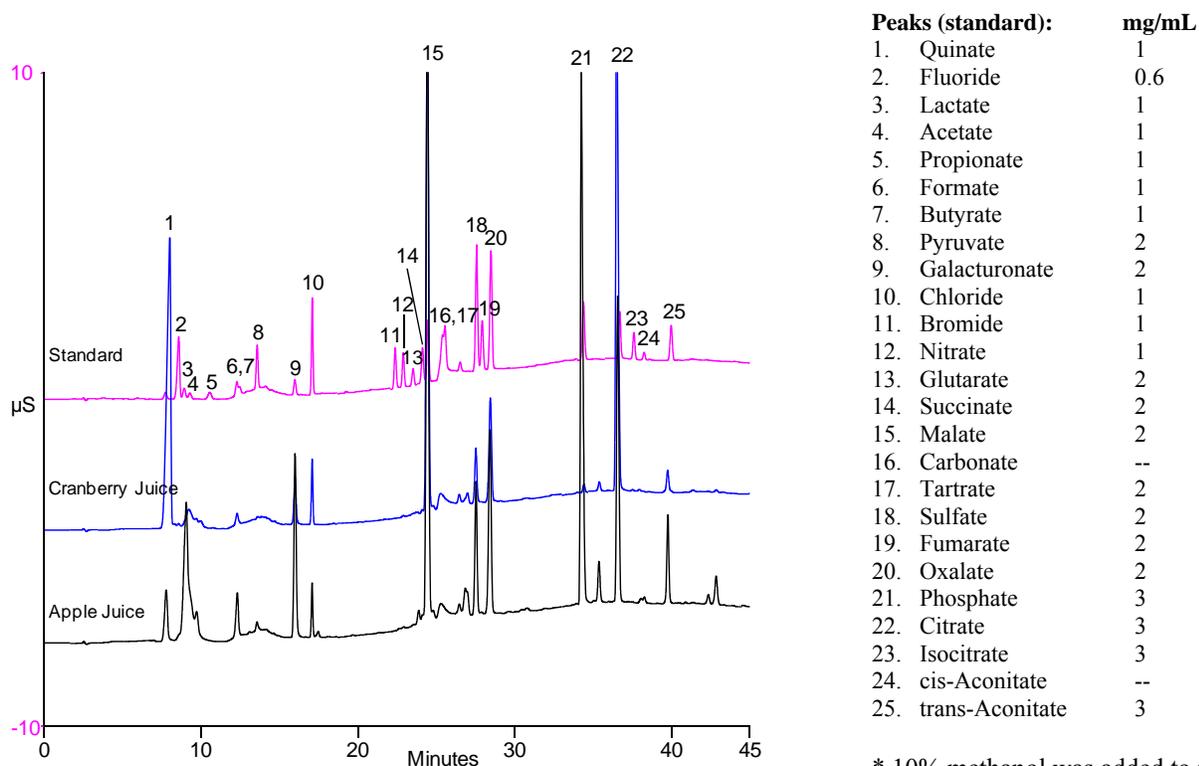
Figure 11A – Analysis of Juice Samples Using an Aqueous Gradient with the Dionex IonPac AS11-HC-4 μ m Capillary Column



5 – Example Applications

Column: Dionex IonPac AG11-HC-4 μ m/AS11-HC-4 μ m (0.4 \times 250 mm)
Eluent + Methanol*: 1mM KOH for 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 III KOH capillary cartridge
Flow Rate: 0.015mL/min
Inj. Volume: 0.4 μ L
Temperature: 30°C
Detection: Suppressed Conductivity, Dionex ACES 300
 AutoSuppression recycle mode
Sample: Juice sample diluted 1:50

Figure 11B – Analysis of Juice Samples Using an Aqueous Gradient with Solvent and the Dionex IonPac AS11-HC-4 μ m Capillary Column



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

5.7 Analysis of a Wine Sample

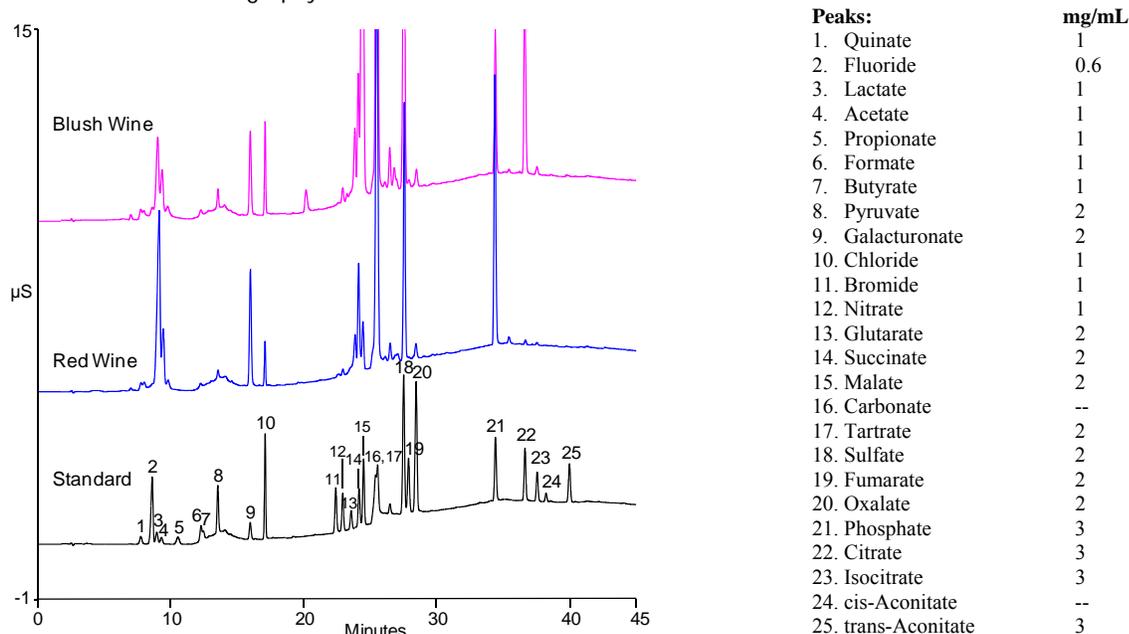
Figure 12 uses an optimized potassium hydroxide gradient with methanol for analysis of a wine sample. The wine sample was diluted 1:100 with deionized water and treated with a Thermo Scientific Dionex OnGuard RP cartridge. Under aqueous eluent conditions, succinate and malate co-elute. However, by adding 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 (0.12mL/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.

Column: Dionex IonPac AG11-HC-4 μ m/AS11-HC-4 μ m (0.4 \times 250 mm)
 Eluent + Methanol*: 1mM KOH for 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 III KOH capillary cartridge
 Flow Rate: 0.015mL/min
 Inj. Volume: 0.4 μ L
 Temperature: 30°C
 Detection: Suppressed Conductivity, Dionex ACES 300
 AutoSuppression external water mode
 Sample: Wine sample diluted 1:100

Figure 12 – Analysis of Wine Samples Using the Dionex IonPac AS11-HC-4 μ m Capillary Column and Gradient Chromatography



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

6. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operational problems that may arise while using the Dionex IonPac AS11-HC-4 μ m column. 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 6 – Dionex IonPac AS11-HC-4 μ m/Dionex IonPac AG11-HC-4 μ m Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	6.1.1
	Plugged Column Bed Supports	Filter Eluents, and Filter Samples	6.1.2, 6.1.3
	Other System Components	Unplug, Replace	Component Manual
High Background Conductivity	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
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.3.1.A, Component Manual
Short Retention Times	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
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.3.3.A
	Column Overloading	Reduce Sample Size	6.3.3.B
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.3.3.D, Component Manual
Spurious Peaks	Column Contaminated	Pretreat Samples	6.3.4.A
	Sluggish Injection Valve	Service Valve	6.3.4.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 μ m Capillary Guard Column plus the Dionex IonPac AS11-HC-4 μ m Capillary Column when using the test chromatogram conditions should be equal or less than 4000 psi. If the system pressure is higher than 4000 psi, it is advisable to determine the cause of the high system pressure.

- 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 plugged tubing or tubing with collapsed 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 50 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 Capillary Guard and Capillary columns are connected (see Table 7, “Typical Dionex IonPac AS11-HC-4 μ m/Dionex IonPac AG11-HC-4 μ m Column Operating Back Pressures”).

The Dionex Anion Capillary Electrolytic Suppressor 300 may add up to 100 psi (0.69 MPa). 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 7 – Typical Dionex IonPac AS11-HC-4 μ m/Dionex IonPac AG11-HC-4 μ m Column Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Dionex IonPac AS11-HC-4 μ m 0.4-mm Capillary Column	\leq 3800 (26.20)	0.015	0.020
Dionex IonPac AG11-HC-4 μ m 0.4-mm Capillary Guard Column	\leq 150 (1.03)	0.015	0.020
Dionex IonPac AS11-HC-4 μ m and AG11-HC-4 μ m 0.4-mm Capillary and Capillary Guard Column	\leq 3950 (27.23)	0.015	0.020

6.1.2 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.

6.1.3 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 ₃ OH	1-3 μS

6.2.1 Preparation of Eluents

- Make sure that the eluents and the regenerant are made correctly.
- Make sure that the eluents are made from chemicals with the recommended purity.
- 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

- Install a Dionex CR-ATC Capillary Anion Trap Column (P/N 072078) if using a Dionex Eluent Generator with Dionex EGC KOH cartridge.
- If the Dionex CR-ATC becomes contaminated, please refer to the Dionex CR-ATC Product Manual (Document No. 031910).

6.2.3 A Contaminated Capillary Guard or Capillary Column

- Remove the Dionex IonPac AG11-HC-4 μm Capillary Guard and Dionex IonPac AS11-HC-4 μm Capillary Columns from the system.
- 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.
- To eliminate downtime, clean or replace the Dionex IonPac AG11-HC-4 μm Capillary Guard 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

- A. Eliminate the hardware as the source of the high background conductivity.
- B. Bypass the columns and the suppressor.
- C. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.
- D. Pump deionized water with a specific resistance of 18.2 megohm-cm through the system.
- E. The background conductivity should be less than 2 μS . 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 Dionex ACES 300 Suppressor

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

- A. Check the power level and alarms on the Dionex ACES 300 Control.
- B. Check the regenerant flow rate at the REGEN OUT port of the Dionex ACES 300 if operating in the AutoSuppression External Waster mode.

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 μ 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, start with a more dilute eluent. This part of the chromatogram is run isocratically.

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 gradient eluent concentrations.

- A. Keep the concentrations of the gradient eluent 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 concentration of the gradient 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. Extra-column effects can result in sample band dispersion, making the peaks' elution less efficient. Make sure you are only using precut tubing and that all your fittings are tightly connected. Make sure the ferrule is 2 mm from the end of the tube and push the tubing in hard, holding it in place while tightening the fitting. See Section 6.3.5 for more information. Check for leaks.

6.3.2 Poor Resolution Due to 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.
- 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. 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. Contaminated eluent may be the problem. Ensure that the water and chemicals used are of the required purity.
- B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the 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 capillary columns may be the problem. Swap components, one at a time, in the system prior to the 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 spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in Appendix A “Column Care”.



NOTE

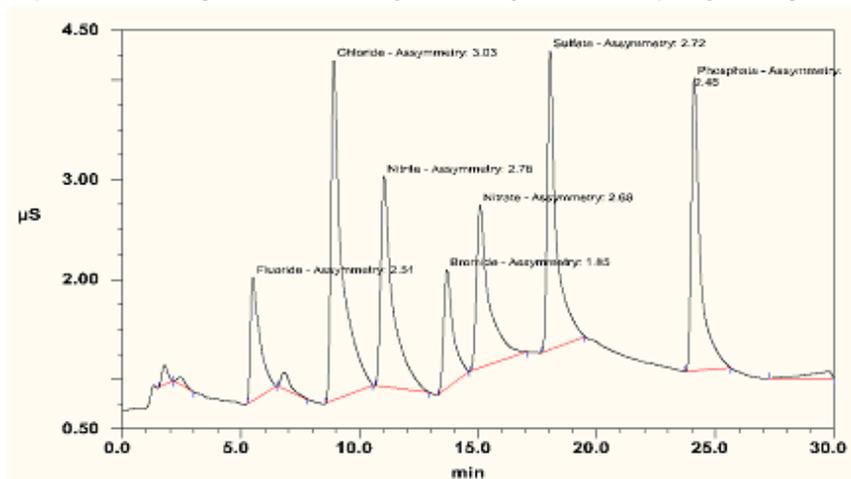
For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

- 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.

6.3.5 Poor Efficiency Using Capillary Columns

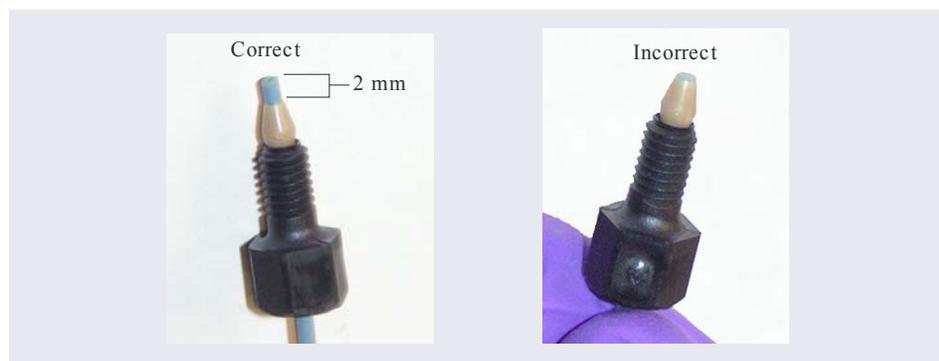
Incorrectly installed fittings on capillary tubing can increase void volumes, causing chromatograms with tailing peaks.

Figure 13 – 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. Insert the tubing hard and hold it in place while tightening the fitting. Figure 14 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing. If necessary to hold the ferrule and nut securely, turn the pump off while making capillary connections.

Figure 14 – Correct and Incorrect Ferrule and Fitting Bolt Placement for Capillary Tubing Connections



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 μ 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, 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 and 6.3.5 Poor Efficiency using Capillary Columns for information regarding proper connections.

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.



- *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.*

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.

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 100 mL solution of the appropriate cleanup solution using the guidelines in Section A.4.1, "Choosing the Appropriate Cleanup Solution."
- B. Disconnect the Dionex ACES 300 from the Dionex IonPac AS11-HC-4 μ m Capillary Column. If your system is configured with both a guard column and a capillary column, reverse the order of the guard and 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 a capillary column and a guard column in series, ensure that the guard column is placed after the capillary column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the capillary column and irreversibly damage it. If in doubt, clean each column separately.

- C. Set the pump flow rate to 0.010 mL/min for a Dionex IonPac AS11-HC-4 μ m Capillary 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 a minimum of 60 minutes. **Note: Additional time may be required (up to 16 hours) to remove contaminants from the column.**
- F. Rinse the column for 10 minutes with deionized water before pumping eluent over the column.
- G. Equilibrate the column(s) with eluent for at least 30 minutes before resuming normal operation.
- H. Reconnect the Dionex ACES 300 to the Dionex IonPac AS11-HC-4 μ m Capillary Column and place the guard column in line between the injection valve and the capillary column if your system was originally configured with a guard column.

Appendix B – System Configuration

Table B1 – Configuration

CONFIGURATION	0.4 mm
Eluent Flow Rate	0.015 $\mu\text{L}/\text{min}$
SRS Suppressor	N/A
MMS Suppressor	N/A
ACES Suppressor	Dionex ACES 300 (P/N 072052)
Injection Loop	0.4 μL (typical)
System Void Volume	Use only in an IC system equipped for capillary analysis.
Pumps	Use only a pump designed for capillary flow rates such as the Dionex ICS-5000 Capillary HPIC or Dionex ICS-4000 Capillary HPIC pump.
NOTE: <i>Use of a Dionex EGC-KOH cartridge (P/N 072076) in conjunction with a Dionex CR-ATC (P/N 072078) for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.</i>	
Chromatographic Module	A thermally controlled column compartment such as the Dionex ICS-5000 DC or Dionex IC-Cube.
Detectors	Use only a conductivity detector designed for capillary flow rates such as the Dionex ICS-5000 Capillary CD.

Table B2 – Tubing Back Pressures

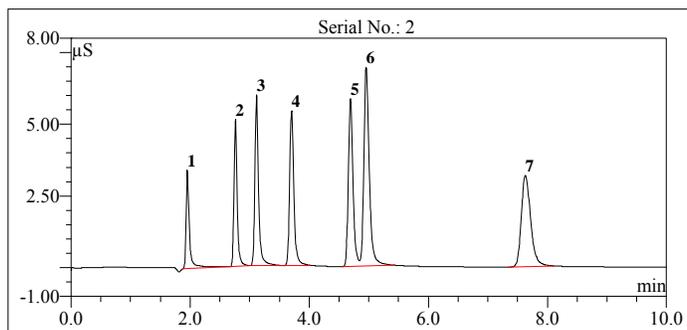
Color	Part Number	I.D. inch	I.D. cm	Volume mL/ft	Back Pressure Psi/ft. at 1 mL/min	Back Pressure Psi/ft. at 0.25 mL/min	Back Pressure Psi/cm. at 1 mL/min
Green	044777	0.030	0.076	0.137	0.086	0.021	0.003
Orange	042855	0.020	0.051	0.061	0.435	0.109	0.015
Blue	049714	0.013	0.033	0.026	2.437	0.609	0.081
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: 02-May-12 09:20
Serial No. : 000002
Lot No. : 2011-13-184#2

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 (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	1.95	1.6	9.05	8180	0.5
2	Chloride	2.76	1.4	3.60	14295	1.3
3	Nitrite	3.11	1.4	5.13	14197	2.5
4	Sulfate	3.70	1.3	7.49	13813	2.5
5	Bromide	4.69	1.3	1.78	18223	5.0
6	Nitrate	4.96	1.5	12.40	15965	5.0
7	Phosphate	7.63	1.5	n.a.	12264	7.5

QA Results:

Analyte	Parameter	Specification	Results
Sulfate	Efficiency	>=9900	Passed
Sulfate	Asymmetry	1.0-1.8	Passed
Sulfate	Retention Time	3.36-4.35	Passed
	Pressure	<=4400	3201

Production Reference:

Datasource: Column
 Directory Capillary/Cap_Anion-3
 Sequence: AS11HC_0P4X250_4µM_VAL_04-03-12
 Sample No.: 216

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

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078944-01 (QAR)