Dionex IonPac AS31 Columns

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thermo scientific

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Product Manual

for

Dionex IonPac AS31 Analytical Column

Analytical Column, 2 × 250 mm (Item # 303147)

Dionex IonPac AG31 Guard Column

Guard Column, 2 × 50 mm (Item # 303148)

For Research Use Only. Not for Use in Diagnostic Procedures.

Revision History:

Revision 01, December 2018, Original Publication.

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

The Dionex IonPac® AS31 2-mm Analytical and IonPac AG31 2mm Guard Columns are hydroxide-selective anion-exchange columns designed for faster separation of several environmental ions including haloacetic acids (HAAs), dalapon, and bromate in drinking water. The Dionex IonPac AS31 column is a low bleed column designed to be used for ion chromatography separations coupled with Mass Spectrometry (IC/MS), or (IC/MS/MS). It is a high capacity column, which allows relatively large injection volumes. Water samples can be directly injected into an ion chromatography system coupled to a mass spectrometer for sensitive detection. The use of a Dionex IonPac AS31 column for IC-MS and IC-MS/MS eliminates the need for sample pretreatment and preconcentration that is typically needed to eliminate sample matrix effects on analyte peak efficiency and resolution such as in HAA determination or other environmental ions in relatively high matrix samples.

The high capacity and compatibility of this column with mass spectroscopy makes it useful with Reagent Free Ion Chromatography (RFIC™) systems for several environmental ions. The Dionex IonPac AS31 is compatible with pH 0-14 eluents and samples. The Dionex IonPac AS31 can be used with any suppressible ionic eluent that does not exceed the capacity of the suppressor. The Dionex IonPac AS31 has nominal efficiency of at least 4500 plates/column for bromide using Quality Assurance test conditions. The maximum operating pressure should be less than 5,000 psi (34.47 MPa).

1.1 Dionex IonPac AS31/Dionex IonPac AG31 Column Packing Specifications

Resin Characteristics:

Nominal Particle Size:	6 µm (Analytical Column*)
Nominal Particle Size:	11 µm (Guard Column**)
Particle Cross-linking:	55%
Ion exchange capacity:	116 µeq per 2 × 250 mm column
	1.5 µeq per 2 × 50 mm column

Functional Characteristics:

Functional Group:	Alkanol quaternary ammonium ion
Hydrophobicity:	Low

*Analytical Column resin composition: supermacroporous ethylvinylbenzene polymer cross-linked with divinylbenzene.
**Guard Column resin composition: microporous ethylvinylbenzene polymer cross-linked with divinylbenzene.

Table 1 Dionex IonPac AS31/Dionex IonPac AG31 Operating Parameters

Column(s)	Typical Back Pressure psi (MPa[a]), 15°C[b]	Standard Flow Rate mL/min	Maximum Flow Rate mL/min[c]
Dionex IonPac AS31 2 mm Analytical column	~ 3000 (20.68)	0.30	0.40
Dionex IonPac AG31 2 mm Guard column	~ 200 (1.38)	0.30	0.40
Dionex IonPac AG31 and AS31 2 mm columns	~ 3200 (22.06)	0.30	0.40

Note: 1MPa = 145.04 psi

[b] Total backpressure at standard flow rates

[c] In all cases, flow rate should not result in pressures over 5,000 psi

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S., call the Customer Care Center for Dionex Products at 1-800-346-6390.
Outside the U.S., call the nearest Thermo Fisher Scientific office.

2. Installation

NOTE

Read the instrument manuals. This manual assumes that you are using Thermo Scientific Dionex instrumentation and are familiar with the installation and operation of the Thermo Scientific Dionex Ion Chromatograph (IC). If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

The proper configuration of an Ion Chromatography System (ICS) is dependent on the column format. Although they can typically use the same system, the use of a 2 mm or 4 mm column requires different set up to ensure optimum performance. The selected format and analysis type will affect the type of pump recommended. A gradient pump is designed to blend and pump isocratic, linear, or gradient mixtures of up to four mobile phase components at precisely controlled flow rates. An isocratic pump is for applications not requiring gradient or multi-eluent proportioning capabilities. For high pressure applications (over 3000 psi), the use of high-pressure consumables, components and fittings is required.

2.1 Thermo Scientific Dionex High Pressure Ion Chromatography Systems

A minimum of a Dionex High Pressure Ion Chromatography (HPIC) System is recommended when running Dionex IonPac AS31 columns due to the higher backpressures generated at typical operational flow rates. Systems should have the capability to operate up to at least 5000 psi. Standard IC systems, with an upper limit of 3000 psi, are insufficient for proper column operation.

All systems should allow 100% metal-free operation to prevent column damage. This includes pump heads and all flow paths.

WARNING

Care should always be taken not to exceed the maximum operating pressure of the system components. IC systems with lower backpressure capabilities are not recommended as reduced flow rates may result in loss of performance.

NOTE

Contact your local representative for information on how to customize your system to your application needs.

2.2 System Requirements

The Dionex IonPac Columns are designed to run on Dionex Ion Chromatographs equipped with suppressed conductivity detection. We recommend the use of ferrules and fittings rated with a pressure of >5000 psi. The use of Thermo Scientific Dionex IC PEEK Viper™ fittings is recommended to achieve consistent low dead volume connections and ensure optimum chromatographic performance. Dionex IC PEEK Viper fittings are available in convenient kits for Dionex ICS-5000^{+} and ICS-6000 systems with conductivity detectors (Item # 088803).

2.2.1 System Void Volume

When using 2 mm columns, it is particularly important to minimize system void volume. The system void volume should be scaled down to at least 1/4 of the system volume in a standard 4 mm system. For best performance, all tubing installed between the injection valve and detector should be 0.005" i.d. PEEK tubing. In order to reduce system backpressure at higher flow rates, 0.007" i.d. PEEK tubing may be used for connections *before* the injection valve, but peak efficiency will be compromised if used for post injector connections, which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.3 Column Start-Up

The column is shipped using sodium bicarbonate as the storage solution.

Prepare the eluent shown on the Quality Assurance Report. To remove the storage solution, flush the column to waste with the 80 mM KOH eluent for at least 45 minutes and then 15 minutes with QAR eluent before attaching the column outlet to the suppressor. Install the column in the column module and 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.

IMPORTANT ***When making any tubing connections, first turn off the pump. This will avoid any slippage of the ferrule under high pressure conditions.***

2.4 Eluents

2.4.1 Eluent Generation

It is recommended that Dionex IonPac columns are used with Dionex HPIC Systems equipped with a Thermo Scientific Dionex Eluent Generator (EG). The use of eluent generation provides a more stable baseline, improved performance, and increased reproducibility over the use of manually prepared eluents.

The Dionex Eluent Generator is used to automatically produce eluents either isocratically or as gradients from deionized water. Please refer to the Dionex EG manual for information on the operation of the Dionex eluent generators.

CAUTION

Only Dionex Eluent Generator Cartridges (EGCs) rated for 5,000 psi or higher should be used when running Dionex IonPac AS31 column due to the higher backpressures generated at typical operational flow rates.

2.5 Dionex IonPac Guard Columns

A Dionex IonPac AG31 Guard Column is normally used with the Dionex IonPac AS31 Analytical Column. A guard is placed in front of the analytical column to prevent sample contaminants from damaging the analytical column. It is easier to clean or replace the guard column than it is the analytical column. Placing a guard column in front of the analytical column will cause retention times to increase by approximately 1.5% under isocratic test conditions. Replacing the guard column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the analytical column.

2.6 Trap Columns

For gradient operation a trap column is installed between the gradient pump and the injection valve and takes the place of the gradient mixer if present. The trap column is filled with high capacity ion exchange resin which helps to minimize the baseline shift caused by increasing ionic contaminants as the eluent ionic strength increases over the course of gradient analysis.

2.6.1 Continuously Regenerated Anion Trap Columns for Use with Eluent Generator Cartridges

For applications using eluent generation on a Dionex ICS-5000^{+} HPIC system, a Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column (Item # 075550) should be installed at the Dionex EGC 500 eluent outlet to remove trace level ionic contaminants from the carrier deionized water. For applications using eluent generation on a Dionex ICS-6000 HPIC system, use a Dionex CR-ATC 600 (Item # 088662). See the Dionex Continuously Regenerated Trap Column Product Manual (Document No. 079684) for instructions.

As an alternative to a Dionex CR-ATC 500, a Thermo Scientific Dionex ATC 500 Trap Column (Item # 079018) can be installed between the pump outlet and the inlet of the Dionex EGC 500 to remove anionic contaminants from the carrier deionized water. Note that use of the Dionex ATC 500 will require off-line regeneration. See the Dionex Anion Trap Columns Product Manual (Document No. 032697) for instructions.

CAUTION

Only trap columns rated for 5,000 psi pressure should be used when running Dionex IonPac AS31 columns due to the higher backpressures generated at typical operational flow rates.

2.7 Sample Concentrators

The function of a concentrator column is to strip ions of interest 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 at the start of the analysis.

Dionex Concentrator columns or the Dionex IonPac Guard Column can be used for trace anion concentration work with Dionex IonPac columns. A pump is used to load the sample onto the concentrator column in the OPPOSITE direction of the eluent flow. Once concentration is complete the eluent flow is then directed through the concentrator to the analytical column. When using concentration techniques, care should be taken not to overload the concentrator column by concentrating an excessive amount of sample. If an excessive amount of sample is used, inaccurate results may be obtained. It is possible during the concentration step for the stronger binding polyvalent ions to elute the weakly retained ions from the concentrator column. For more detailed information on sample concentration techniques for high sensitivity work and a detailed discussion of concentration techniques refer to the appropriate concentrator manual for your application.

CAUTION

Dionex IonPac Concentrator Columns are designed for use with specific eluent systems. Use only concentrator columns designed for the eluent system you are using.

3. Operation

3.1 General Operating Conditions

The following conditions should be used as a starting point towards method development.

Injection Volume:	2.5 µL (QAR) 100 µL (EPA Method 557)
Column:	Dionex IonPac AG31 2-mm Guard Column + Dionex IonPac AS31 2-mm Analytical Column
Eluent:	24 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge with Dionex CR-ATC 500 (Dionex ICS-5000^{+}) or Dionex CR-ATC 600 (Dionex ICS-6000)
Eluent Flow Rate:	0.30 mL/min
Temperature:	15 ºC
Suppressor:	Dionex ADRS 600 Anion Dynamically Regenerated Suppressor, AutoSuppression, External Water Mode
Expected Background Conductivity:	< 1 µS
Long-term Storage Solution	(> 1 week): 100 mM sodium bicarbonate
Short-term Storage Solution	(< 1 week): Eluent

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S., call the Customer Care Center for Dionex Products at 1-800-346-6390.
Outside the U.S., call the nearest Thermo Fisher Scientific office.

3.2 General Operational Precautions

The following precautions should always be adhered to when using Dionex IonPac columns.

- Samples and manually prepared eluents should always be filtered and degassed to protect the system and column from particulates and ensure a stable background.
- Eluents and samples used should be used within the allowable limits for the column.
- Do not exceed the operational pressure of the system.
- Take care not to exceed the maximum operational flow rate and pressure of the column. If the pressure approached the maximum allowed pressure, reduce the operational flow rate.

Table 2 Operational Limits for the Dionex IonPac AS31 Column

Eluent pH	Between 0 and 14
Sample pH	Between 0 and 14
Maximum Flow Rate for 2 mm columns	0.40 mL/min
Maximum Operating Pressure	5,000 psi (34.47MPa)

3.3 Quality Assurance Reports

Each column is qualified to ensure it meets specifications. Example copies of these Quality Assurance Reports (QARs) can be found in Section 7. The QAR supplied with the column should be used as a guide to ensure system performance.

3.4 Chemical Requirements and Solvent Compatibility

Chemical purity can influence separation performance. Only chemicals of the highest purity should be used. Refer to Section 6.4, Chemical Purity Requirements for more details.

Some solvents may be used for cleaning or eluent modification.

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 3 Typical HPLC Solvents for Cleaning and Use with Dionex IonPac Columns

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 ADRS 600 must be operated in the AutoSuppression External Water Mode when using eluents containing organic solvents. Refer to the Suppressor Product Manual (Document No. 031956) for operation limitations in the electrolytic mode (power on).

3.5 Eluent Preparation

A Dionex Eluent Generator (EG) is recommended for use with Dionex IonPac columns.

When preparing eluents manually, ensure all chemicals and water are of the highest purity.

3.5.1 Manually Prepared Eluents

Eluents should be stored in plastic bottles under a helium atmosphere to ensure contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents). Contamination from carbon dioxide when basic eluents are exposed to the air can cause performance variability such as retention time shifts.

3.5.2 Making Eluents that Contain Solvents

Mixing solvents with water should be done on a volume to volume basis. For example, if a procedure requires an eluent of 40% acetonitrile, prepare the eluent by adding 400 mL of acetonitrile to an eluent reservoir. Then add 600 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 will evaporate or be "boiled" off from the solution under reduced pressure.

NOTE

Always degas and store all eluents in 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 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.

3.6 Regenerant Preparation for the Thermo Scientific Dionex Chemically Regenerated Suppressor (Dionex CRS 500)

The Dionex Chemically Regenerated Suppressor requires the use of a regenerant solution. Refer to the product manual (Document No. 031727) for operating instructions.

3.7 Recommendations for Method Development

The Dionex IonPac AS31 is designed for the determination of haloacetic acids in less than 35 minutes using a hydroxide gradient eluent delivered by a Dionex Eluent Generator. 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. Potassium hydroxide is converted to water in the suppressor, making it the preferred source of 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 17 mM KOH and end at 85 mM KOH, with a resulting total baseline change of 1 to 2 µS.

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

You can increase the sensitivity of your system by using sample concentration techniques (see Section 2.7, “Sample Concentrators”).

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.

4. Example Applications

4.1 Recommendations for Optimum System Performance

The chromatograms in this section were obtained using columns that reproduced section 4.2 (*Isocratic Elution With and Without a Guard Column*) on optimized Ion Chromatographs. 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 AS31 is designed for the determination of haloacetic acids using a hydroxide gradient eluent delivered by an Eluent Generator. 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. The best choice for an eluent is potassium hydroxide as it is converted to water in the suppressor. 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 can begin at 17 mM KOH and end at 85 mM KOH, with a resulting total baseline change of 1 to 2 µS.

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

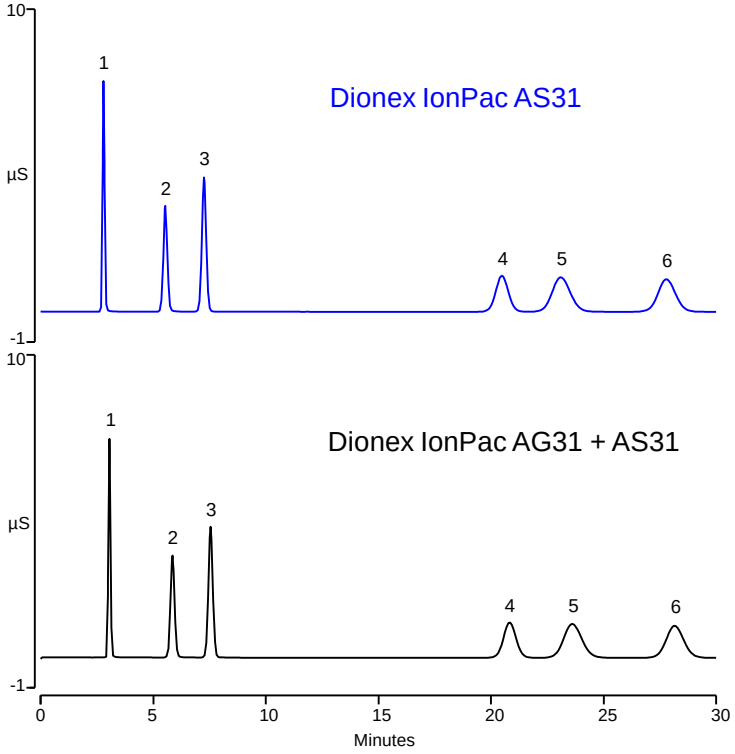
You can increase the sensitivity of your system by using sample concentration techniques. However, none have been used for the test chromatograms.

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.

4.2 Isocratic Elution With and Without a Guard Column

Isocratic elution of inorganic anions on the Dionex IonPac AS31 column has been optimized utilizing a hydroxide eluent. By using this eluent, common inorganic anions can be used to test the performance (QAR) of the Dionex IonPac AS31 column. The Dionex IonPac AS31 analytical column should always be used with the Dionex IonPac AG31 guard column. An operating temperature of 15 °C is used to ensure reproducible resolution and retention. Note that the Dionex IonPac AG31 guard column is packed with a microporous resin of proportionally lower capacity and contributes approximately 1.5% increase in retention time when placed in-line prior to the analytical column under isocratic test conditions.

Figure 1 Dionex IonPac AS31 With and Without Dionex IonPac AG31 Guard Column



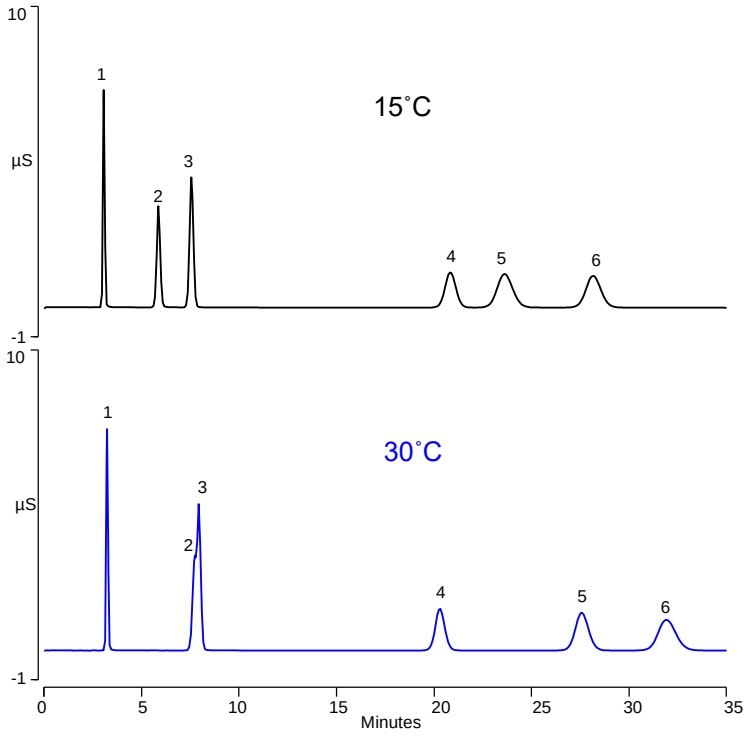
Column:	See Chromatogram
Eluent:	24 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.30 mL/min
Inj. Volume:	2.5 µL
Temperature:	15˚C
Detection:	Suppressed Conductivity, Dionex ADRS 600 (2 mm) AutoSuppression, recycle mode

Peaks (Standard):	mg/L
1. Fluoride	5.0
2. Sulfate	10.0
3. Chloride	10.0
4. Bromide	20.0
5. Thiosulfate	20.0
6. Nitrate	20.0

4.3 Effect of Temperature on the Dionex IonPac AS31 Selectivity

The following chromatograms demonstrate the effect of temperature on the Dionex IonPac AS31 selectivity. Notice there is a greater effect on the divalent anions (sulfate and thiosulfate) relative to monovalent anions (fluoride, chloride, bromide, and nitrate) when temperature is changed from 15ºC to 30ºC.

Figure 2 Effect of Temperature on Dionex IonPac AS31 Selectivity



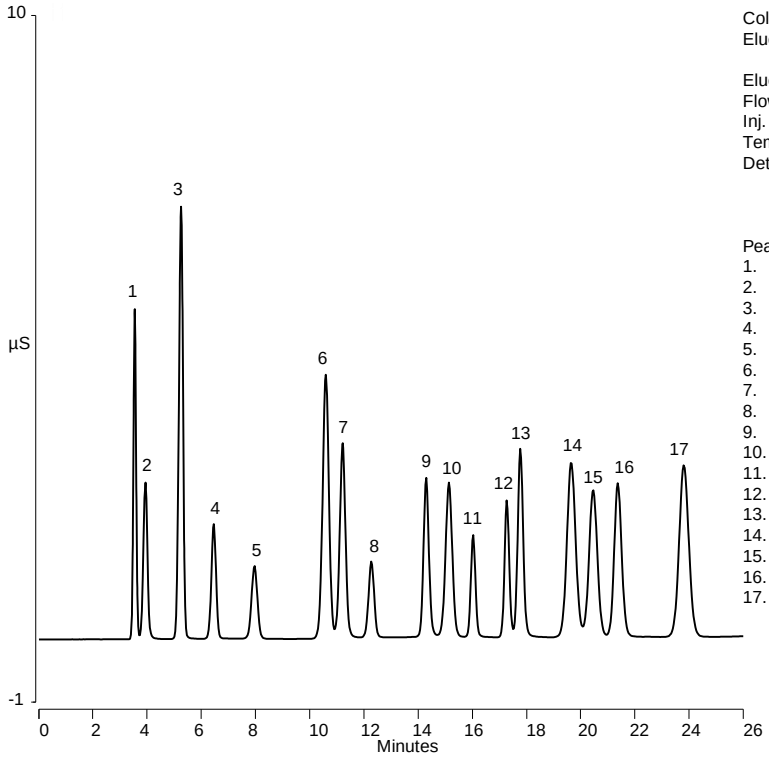
Column:	Dionex IonPac AG31/AS31, 2mm
Eluent:	24 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.30 mL/min
Inj. Volume:	2.5 µL
Temperature:	See Chromatogram
Detection:	Suppressed Conductivity, Dionex ADRS 600 (2 mm) AutoSuppression, recycle mode

Peaks (Standard):	mg/L
1. Fluoride	5.0
2. Sulfate	10.0
3. Chloride	10.0
4. Bromide	20.0
5. Thiosulfate	20.0
6. Nitrate	20.0

4.4 Separation of Various Inorganic and Organic Anions

The Dionex IonPac AS31 column provides excellent separation of a variety of environmental anions including inorganic anions, oxyhalides, oxyanions, and organic acids. When developing a method, keep in mind that if there is a big difference in the hydrophobicity and valencies of the two analytes, variation in column capacities will have significant effect on the peak resolution.

Figure 3 Separation of Various Inorganic and Organic Anions Using the Dionex IonPac AS31 Column



Column:	Dionex IonPac AG31/AS31, 2mm
Eluent:	15 mM KOH from 0 to 6 minutes, 15-70 mM KOH from 6 to 26 minutes
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.30 mL/min
Inj. Volume:	2.5 µL
Temperature:	15˚C
Detection:	Suppressed Conductivity, Dionex ADRS 600 (2 mm) AutoSuppression, recycle mode

Peaks (Standard):	mg/L
1. Fluoride	3.0
2. Acetate	10.0
3. Formate	10.0
4. Chlorite	10.0
5. Bromate	10.0
6. Chloride	10.0
7. Sulfate	10.0
8. Malonate	10.0
9. Oxalate	10.0
10. Nitrite	10.0
11. Phosphate	10.0
12. Arsenate	20.0
13. Citrate	20.0
14. Chlorate	20.0
15. Bromide	20.0
16. Chromate	20.0
17. Nitrate	20.0

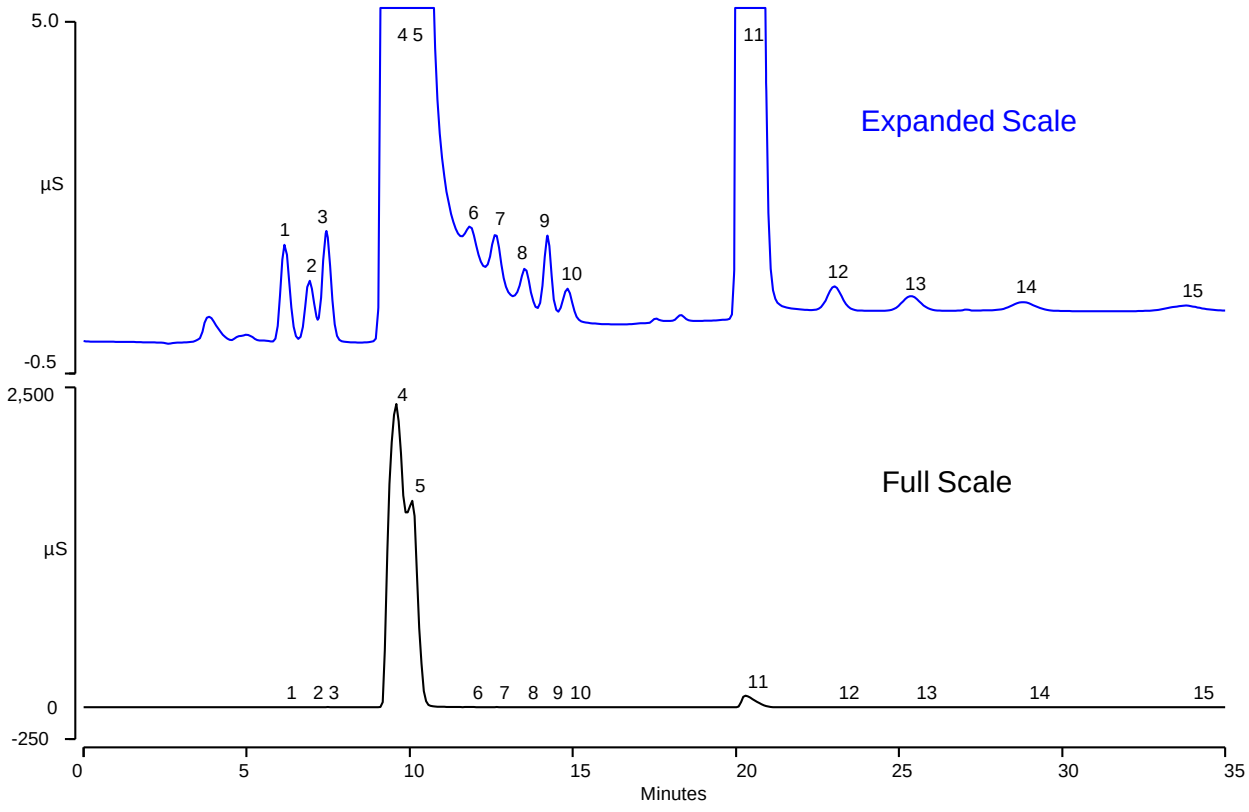
4.5 Separation and Detection of 9 Haloacetic Acids, Bromate, and Dalapon using IC Method and EPA Method 557 Laboratory Synthetic Sample Matrix

The following chromatograms demonstrate the Dionex IonPac AS31 column's selectivity and capacity using IC method and EPA Method 557 LSSM matrix.

Figure 4 Analysis of Dalapon, Bromate, and Haloacetic Acids in LSSM (EPA Method 557) Matrix Using the Dionex IonPac AG31 and AS31 Columns

Column:	Dionex IonPac AG31/AS31, 2mm
Eluent:	17 mM KOH from 0 to 7 minutes, 17-85 mM KOH from 7 to 18 minutes, 85 mM KOH from 18 to 35 minutes
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.30 mL/min
Inj. Volume:	100 µL
Temperature:	15˚C
Detection:	Suppressed Conductivity, Dionex ADRS 600 (2 mm) AutoSuppression, recycle mode

Peaks (Standard):	mg/L
1. Monochloroacetate	0.5
2. Monobromoacetate	0.5
3. Bromate	0.5
4. Chloride	316
5. Sulfate+carbonate	250+150
6. Dalapon	0.5
7. Dichloroacetate	0.5
8. Bromochloroacetate	0.5
9. Nitrite	0.15
10. Dibromoacetate	0.5
11. Nitrate	20.0
12. Trichloroacetate	0.5
13. Bromodichloroacetate	0.5
14. Chlorodibromoacetate	0.5
15. Tribromoacetate	0.5



4.6 Analysis of Municipal Drinking Water using Dionex IonPac AS31 column

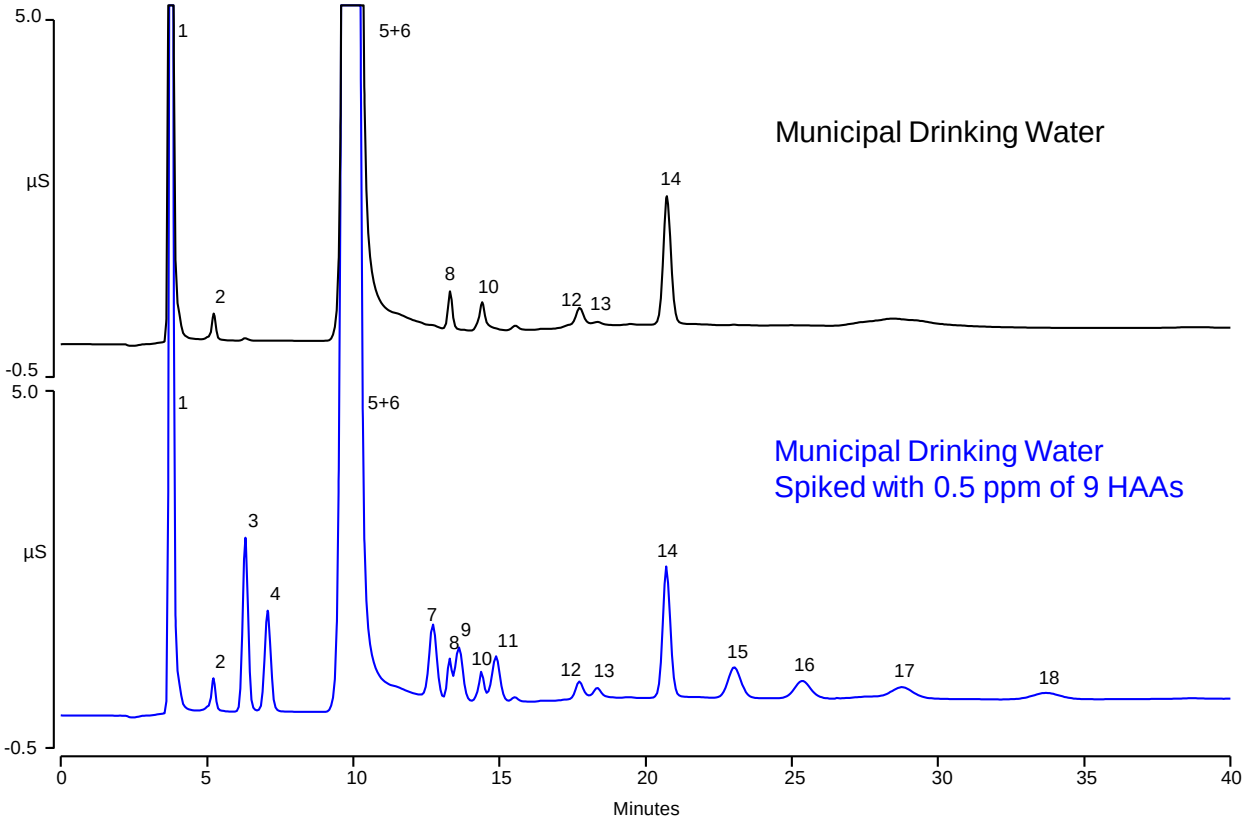
The following chromatograms show the analyses of municipal drinking water with and without a spike of 9HAAs.

Figure 5 Analysis of Municipal Drinking Water Spiked with Nine Haloacetic Acids Using the Dionex IonPac AG31/AS31 Columns

Column:	Dionex IonPac AG31/AS31, 2mm
Eluent:	17 mM KOH from 0 to 7 minutes, 17-85 mM KOH from 7 to 18 minutes, 85 mM KOH from 18 to 35 minutes
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.30 mL/min
Inj. Volume:	100 µL
Temperature:	15˚C
Detection:	Suppressed Conductivity, Dionex ADRS 600 (2 mm) AutoSuppression, recycle mode
Sample	Municipal Drinking Water Spiked with 9HAAs

Peaks (Standard):	mg/L
1. Fluoride	NQ
2. Unknown	NQ
3. Monochloroacetate	0.5
4. Monobromoacetate	0.5
5. Chloride	NQ
6. Sulfate+carbonate	NQ
7. Dichloroacetate	0.5
8. Unknown	NQ
9. Bromochloroacetate	0.5
10. Unknown	NQ
11. Dibromoacetate	0.5
12. Chlorate	NQ
13. Bromide	NQ
14. Nitrate	NQ
15. Trichloroacetate	0.5
16. Bromodichloroacetate	0.5
17. Chlorodibromoacetate	0.5
18. Tribromoacetate	0.5

NQ: Not Quantified



4.7 Separation and Detection of 9 HAAs, Bromate, and Dalapon using IC-MS

The Dionex IonPac AS31 provides good separation of common inorganic anions, monochloroacetic acid, monobromoacetic acid, dichloroacetic acid, chlorobromoacetic acid, dibromoacetic acid, trichloroacetic acid, dichlorobromoacetic acid, chlorodibromoacetic acid, tribromoacetic acid, bromate, and dalapon using a potassium hydroxide eluent and mass spectrometric detection. Please note that IC analysis was performed on a Thermo Scientific Dionex ICS-5000^{+} HPIC System and matrix ions were diverted to waste, with only the ions of interest sent to the Mass Spectrometer. The separation of all nine haloacetic acids, bromate, and dalapon in the EPA Method 557 LSSM matrix is shown in Figures 6 and 7.

For these analyses, we used negative ion, Full Scan Mode with 330 ms max inject time. Good extracted ion chromatograms were produced from both SIM and Full Scan Modes. S/N values for MCAA and MBAA were better in SIM mode, while S/N values for all other ions were better in Full Scan mode. In both modes, we observed highly accurate mass values allowing resolution of molecular ions differing in m/z by 0.032 (Bromate and DCAA). With minor *In-Source* fragmentation, we observed diagnostic fragments for all components.

The following table can provide initial guidance on setting up time windows for matrix diversion based on the retention times of chloride, carbonate/sulfate, and nitrate. The recommendations below can be adjusted based on the gradient delay volume of the HPIC system and the retention times of the Dionex IonPac AG31/AS31 columns to ensure analytes of interest are not diverted to waste. Please note that on some occasions, chloride, carbonate, and sulfate could co-elute as a single peak. Example 2 below shows how to setup the matrix diversion window when this occurs.

	Retention Time Chloride	**Retention Time Carbonate/Sulfate**	**Retention Time Nitrate**
Matrix diversion window plus		+ 1.0 min	+ 1.5 min
Matrix diversion window minus	– 1.0 min		– 1.5 min

Example 1:	**Retention Time Chloride**	**Retention Time Carbonate/Sulfate**	**Retention Time Nitrate**
Dionex IonPac AG31/AS31 set #1	9.58 min	10.08 min	20.3 min
Start diverting flow to waste	8.58 min		18.8 min
Stop diverting flow to waste		11.08 min	21.8 min

Example 2:	**Retention Time Chloride**	**Retention Time Carbonate/Sulfate**	**Retention Time Nitrate**
Dionex IonPac AG31/AS31 set #2	9.93 min	9.93 min	20.7 min
Start diverting flow to waste	8.93 min		19.2 min
Stop diverting flow to waste		10.93 min	22.2 min

4.7.1 System, Conditions, and Consumables

Thermo Scientific™ Dionex ICS-5000+ with Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

Column:	Dionex IonPac AG31/AS31 (2 mm)
Eluent:	KOH Gradient (see timed events)
Suppressor:	Dionex ADRS 600, 2mm, external water, 0.3 mL/min
Suppressor Current:	64 mA
Analytical Flow Rate:	0.3 mL/min
Column Temp:	15 ° C
Injection Volume:	100 µL
Detector:	CD, Q Exactive

Q Exactive Tune Parameters:

Type:	Full MS (50-300 m/z) or t-SIM (with Inclusion List)
Resolution:	60,000 (Negative Polarity)
Max Inject Time:	330 ms (AGC Target 5e5)
Sheath Gas:	40 (Temp 100 ° C)
Aux Gas:	20 (Temp 100 ° C, No Sweep Gas)
Spray Voltage:	2.00 KV
Funnel RF:	40.0

Timed Events

Time		[KOH]	Divert Valve
-0.5	Begin	17.0	Eluent to Waste
0.0		17.0	
4.0		17.0	Eluent to MS
7.0		17.0	
8.6			Eluent to Waste
11.1			Eluent to MS
18.0		85.0	
18.73			Eluent to Waste
21.73			Eluent to MS
40.0		85.0	Eluent to Waste
40.1		17.0	
47.0	End		

4.7.2 Targeted SIM Inclusion List

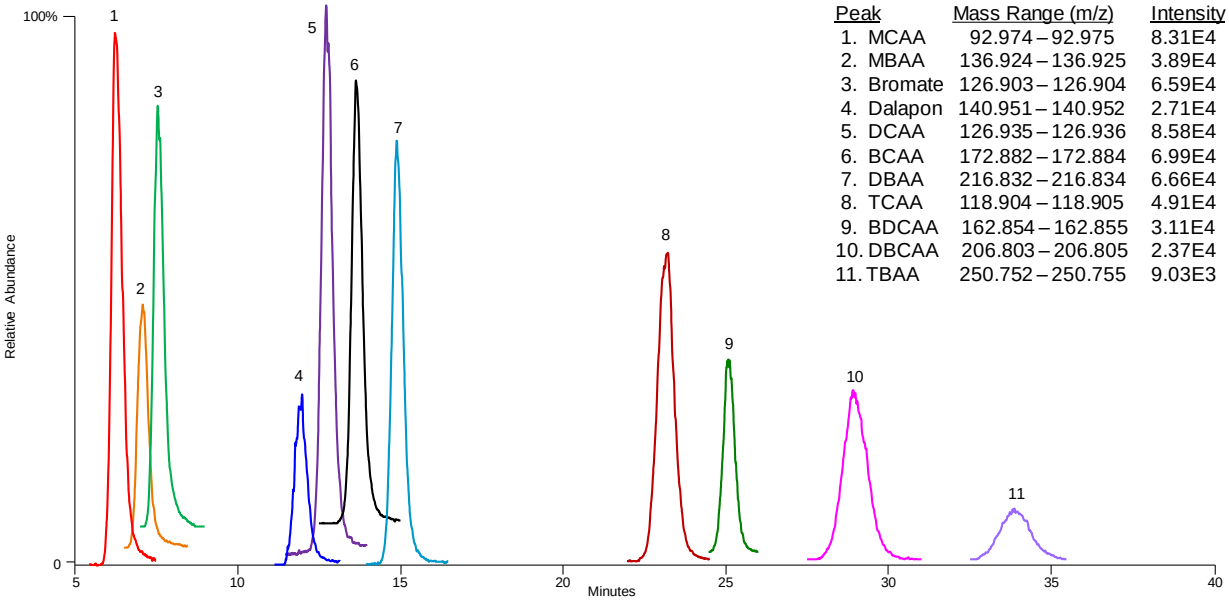
Full Scan (50-300 m/z, 60000 Res, Max IT = 330 ms) during non-SIM segments.

SIM Segments:

Time Range (min)	Analyte	SIM m/z	ID of m/z (i=Isotope)	Diagnostic Fragments
5.7 – 6.6	MCAA	92.974	$[M\text{-}H^+]^{-1}$	50.9819 ($CH_2{}^{37}Cl$)
6.7 – 7.35	MBAA	136.924	$[M\text{-}H^+]^{-1}$	78.919, 80.917 (Br^- ions)
7.35 – 8.4	Bromate	126.903	$[M\text{-}H^+]^{-1}$	110.909, 112.986 (BrO_2^- ions)
11.1 – 12.3	Dalapon	140.952	$[M\text{-}H^+]^{-1}$	96.9602 $[M\text{-}CO_2\text{-}H^+]^{-1}$
12.3 – 13.1	DCAA	126.936	$[M\text{-}H^+]^{-1}$	80.975, 82.946 ($CHCl_2^-$)
13.1 – 14.3	BCAA	172.883	$[M\text{-}H^+]^{-1}$ abundant Br^- i	128.893 $[M\text{-}CO_2\text{-}H^+]^{-1}$
14.3 – 15.5	DBAA	214.835	$[M\text{-}H^+]^{-1}$ abundant Br^- i	172.843 $[M\text{-}CO_2\text{-}H^+]^{-1}$
22.0 – 24.3	TCAA	118.904	$[M\text{-}CO_2\text{-}H^+]^{-1}$ abundant Cl^- i	160.897 $[M\text{-}H^+]^{-1}$
24.5 – 26.2	BDCAA	162.854	$[M\text{-}CO_2\text{-}H^+]^{-1}$ abundant Br^- i	78.919, 80.917 (Br^-)
27.7 – 30.3	DBCAA	206.804	$[M\text{-}CO_2\text{-}H^+]^{-1}$ abundant Br^- i	78.919, 80.917 (Br^-)
32.4 – 35.5	TBAA	250.754	$[M\text{-}CO_2\text{-}H^+]^{-1}$ abundant Br^- i	78.919, 80.917 (Br^-)

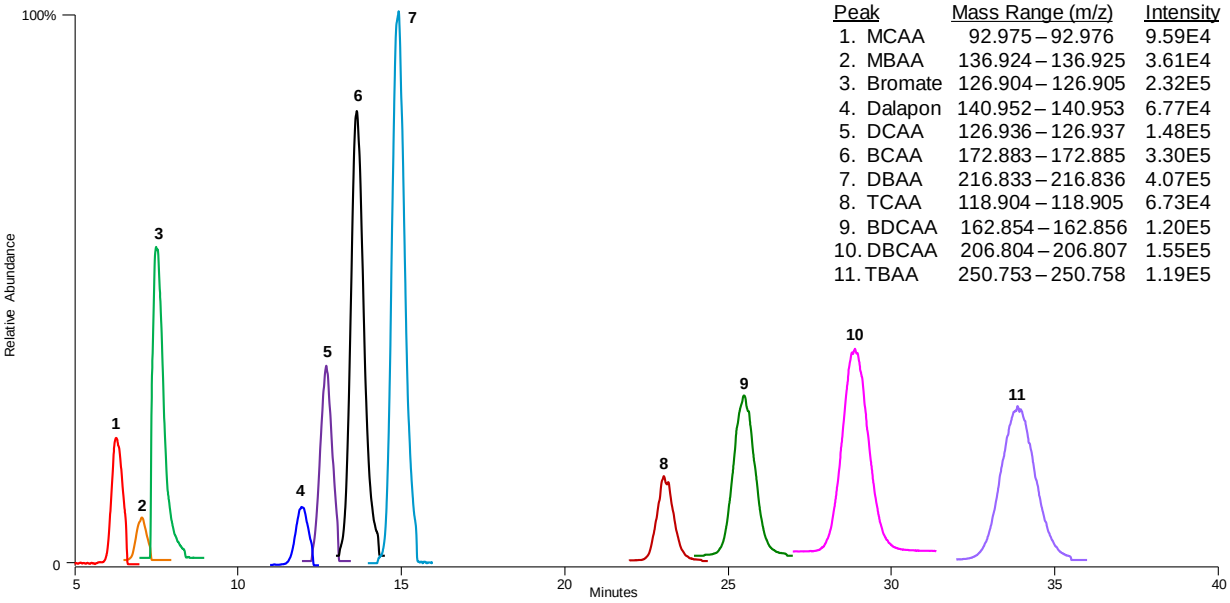
4.7.3 Example Chromatograms

Figure 6 4 ppb HAAs, Bromate, and Dalapon in EPA Method 557 LSSM Matrix (Full Scan)



Peak	Mass Range (m/z)	Intensity
1. MCAA	92.974 – 92.975	8.31E4
2. MBAA	136.924 – 136.925	3.89E4
3. Bromate	126.903 – 126.904	6.59E4
4. Dalapon	140.951 – 140.952	2.71E4
5. DCAA	126.935 – 126.936	8.58E4
6. BCAA	172.882 – 172.884	6.99E4
7. DBAA	216.832 – 216.834	6.66E4
8. TCAA	118.904 – 118.905	4.91E4
9. BDCAA	162.854 – 162.855	3.11E4
10. DBCAA	206.803 – 206.805	2.37E4
11. TBAA	250.752 – 250.755	9.03E3

Figure 7 4ppb HAAs, Bromate, and Dalapon in EPA Method 557 LSSM Matrix (Targeted SIM XIC)



Peak	Mass Range (m/z)	Intensity
1. MCAA	92.975 – 92.976	9.59E4
2. MBAA	136.924 – 136.925	3.61E4
3. Bromate	126.904 – 126.905	2.32E5
4. Dalapon	140.952 – 140.953	6.77E4
5. DCAA	126.936 – 126.937	1.48E5
6. BCAA	172.883 – 172.885	3.30E5
7. DBAA	216.833 – 216.836	4.07E5
8. TCAA	118.904 – 118.905	6.73E4
9. BDCAA	162.854 – 162.856	1.20E5
10. DBCAA	206.804 – 206.807	1.55E5
11. TBAA	250.753 – 250.758	1.19E5

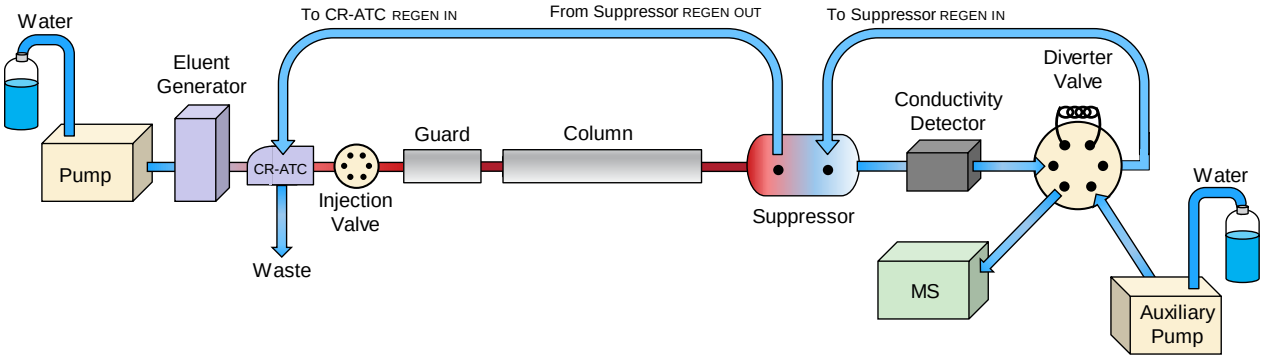
4.7.4 Signal to Noise Comparison

Figure 8 Signal to Noise (S/N) Comparison for 4 ppb HAAs, Bromate, and Dalapon in EPA Method 557 LSSM Matrix (SIM vs. Full Scan)

Peak (Analyte)	DI H_2O Signal	MS S/N (SIM)	MS S/N (Full Scan)
MCAA	560	1.71E+02	1.99E+01
MBAA	4.8	7.52E+03	1.93E+03
Bromate	26.6	8.72E+03	5.81E+04
Dalapon	24.4	2.77E+03	2.90E+04
DCAA	127	1.17E+03	1.70E+04
BCAA	44.2	7.47E+03	3.20E+04
DBAA	4.5	9.04E+04	3.69E+05
TCAA	3.3	2.74E+04	7.27E+05
BDCAA	211	4.58E+04	5.05E+05
DBCAA	2.46	3.22E+04	2.61E+05
TBAA	2.62	1.24E+04	6.26E+04

4.8 System Setup

Figure 9 Flow diagram for IC-MS Analysis



The setup described in this section provides general guidance for the required connections for optimal performance. For information about individual modules, refer to the installation guides and manuals available for these modules. The ion chromatograph is configured for microbore operation. Red PEEK tubing (0.005" ID) should be used for all chromatographic connections. The Dionex CR-ATC is installed between the eluent generator and the injection valve as described in the Dionex CR-ATC manual. The eluent generator and cartridge are purged and operated according to the eluent generator installation guide for low-flow rate operation. The column and suppressor are plumbed in the normal configuration. Referring to the Figure 9 above, the column outlet is connected to the suppressor. For MS detection, the suppressor is operated in the external water mode and regen water is provided via the auxiliary pump at 0.3 mL/min. The matrix diversion valve switches the regen mode between external water and recycle modes to provide continuous flow of water to the suppressor and mass spectrometer during matrix diversion.

The suppressor eluent out is connected to the conductivity cell inlet. The cell outlet is connected to a 6-port valve that is used for matrix diversion.

Figure 10 Connections from Ion Chromatograph to Mass Spectrometer through a Matrix Diversion Valve

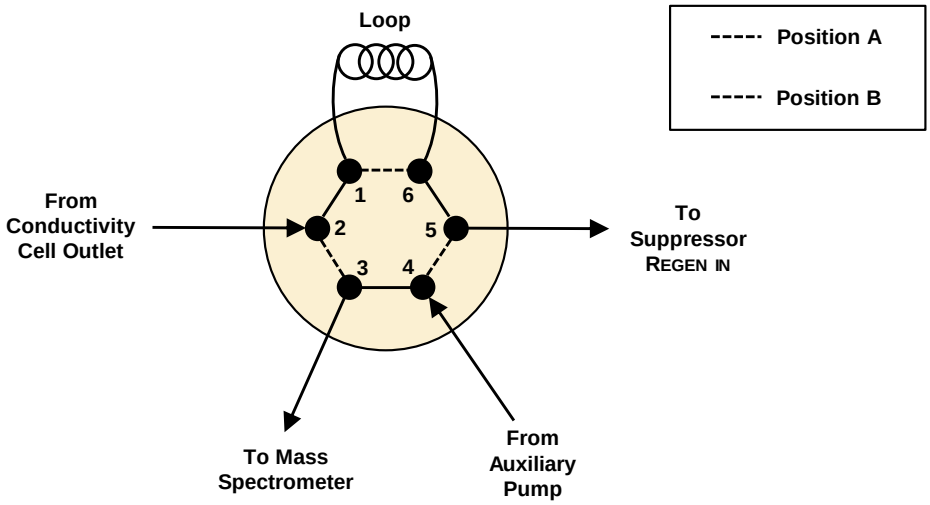


Figure 10 shows the connections for the matrix diversion valve. During target analyte elution, the 6-port valve is set to position A, where flow from the conductivity cell is directed to the Mass Spectrometer for gas-phase ionization and analysis. When matrix ions such as sulfate, chloride, nitrate, and carbonate elute, the valve is switched to position B to direct the flow from the cell outlet to the suppressor for "recycle mode". Matrix diversion is necessary to prevent contamination of the mass spectrometer with matrix ions (present at high concentration in some samples). Poor sensitivity and poor recovery (ion-suppression in the mass spectrometer) may result if sample matrix ions present at ppm (mg/L) levels are not diverted to waste. The mass spectrometer source may occasionally require physical cleaning followed by several hours of vacuum pumping to regain pristine performance. Note that the IC eluent line should not be connected to the MS inlet until the background conductivity is below ~3 µS.

NOTE

If an ICS-5000$^+$ or ICS-6000 is used, any of the 6 or 10-port valves in the automation manager (AM) module can be used for matrix diversion.

Makeup DI water can be added to the analytical flow during matrix diversion to keep the system equilibrated. If an ICS-5000$^+$ with a DP pump module is used, the second pump can be used to provide the makeup flow. Otherwise, a second (auxiliary) pump is used for this purpose. A Dionex AXP (Item # 063973) auxiliary pump was used in this example. The flow of makeup solvent is 0.3 mL/min in the IC/MS example above. To avoid tubing constriction at connections and its potential for causing elevated pressures, we employed 0.005" ID Viper® tubing for IC/MS operation.

NOTE

Grounding Connection: A grounding adaptor is needed when the liquid line connection from the IC is made directly to the mass spectrometer electrospray probe. However, if the connection is made through a grounded adapter present on the MS, there is no need for another ground.

5. Troubleshooting

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex IonPac columns. For more information on problems that originate with the Ion Chromatograph (IC) or other consumables such as the suppressor, trap or concentrator columns, refer to the Troubleshooting Guide in the appropriate operator's manual.

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com
Or call the Customer Care Center for Dionex Products at 1-800-346-6390
Outside the U.S., call the nearest Thermo Fisher Scientific office.

Table 4 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	5.1.1
	Plugged Column Bed Supports	Replace Bed Supports, Filter Eluents, and Filter Samples	5.1.2, 5.1.3, 5.1.4
	Other System Components	Unplug, Replace, Filter Eluents and Samples	Component Manual
High Background Conductivity	Contaminated Eluents	Remake Eluents	5.2.1
	Contaminated Trap Column	Clean or Replace Trap Column	5.2.2,5.2.3
	Contaminated Guard or Analytical Column	Clean or Replace Guard and Analytical Column	5.2.4
	Contaminated Suppressor	Clean or Replace Suppressor	5.2.6, Component Manual
	Contaminated Hardware	Clean Component	5.2.5, Component Manual
Poor Resolution	Gradient method not optimized	Optimize method	5.4
Poor Efficiency	Large System Void Volumes	Replumb System	Component Manual, 5.4.1B
	Column Headspace	Replace Column	5.1.2, 5.4.1A
	Improper connections	Replumb system	5.4.1B, 5.4.1C
	Leaks in the system	Check for leaks, Replumb system	5.4.1D, 5.4.1C
	Contaminated Suppressor	Clean or Replace Suppressor	5.4.1E, Component Manual
Short Retention Times	Flow Rate Too fast	Check Flow Rate, Recalibrate Pump	5.4.3B
	Conc. Incorrect Eluents	Remake Eluents	5.2.1, 5.4.3C
	Column Contamination	Clean Column	5.2.4
	Insufficient Equilibration	Extend Equilibration Time at the Start of the Gradient Run	5.4.3A

Retention Time Drift	Contaminated sample or eluent, Poorly mixed eluent	Remake Sample/Eluents	5.2.1, 5.3.1A, E, 5.3.2A
	Temperature variability	Use a thermostatted oven, check oven operation	5.3.1C,D, 5.3.2D, Component Manual
	Poor pump priming or loss of prime	Prime Pump	5.3.1B, 5.3.2B, Component Manual
	Inconsistent flow due to leaking pump	Repair pump	5.3.2C,Component Manual
Oscillating Retention Time	Pump Problems	Recalibrate/Repair Pump	5.3.3A, Component Manual
	Temperature variability	Use a thermostatted oven, check oven operation	5.3.3B, Component Manual
	Sluggish Injection valve	Service Valve	5.3.3C, Component Manual
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	5.2.1, 5.4.4A
	Column Overloading	Reduce Sample Size	5.4.4B
	Large System Void Volumes	Replumb System	Component Manual, 5.4.4C
Spurious Peaks	Sample Contaminated	Pretreat Samples	5.5A
	Sluggish Injection Valve	Service Valve	5.5B, Component Manual
Analyte Specific Efficiency Loss	Column Contamination from sample or system	Purge contamination, employ a trap or guard column, clean or replace column	5.4.2

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex IonPac AG31 Guard Column plus the Dionex IonPac AS31 Analytical Column when using the test chromatogram conditions should be less than 3800 psi. If the system pressure at the standard flow rate is higher than the maximum operational pressure for the system, 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 stopwatch and graduated cylinder to confirm flow rate is correct. Pre-weigh the graduated cylinder and calculate the weight of eluent collected to obtain a more accurate measure of flow.

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 guard or separator column with particulates clogging the bed support, a clogged High-Pressure In-Line Filter, the suppressor, or the detector cell.

C. 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 be at its lowest (< 50 psi) with everything disconnected. The pressure with the eluent generator connected should be < 200 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 and Analytical columns are connected.

D. Measure the system back pressure by attaching a short piece of new 0.010” tubing in place of the column.

E. The Dionex Dynamically Regenerated Suppressor with backpressure loops 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.

F. A Dionex High-Pressure In-Line Filter positioned between the Pump and Eluent Generator (or injection valve if an EG is not installed) should be installed to prevent particulates from blocking the system.

5.1.2 Replacing Column Bed Support Assemblies

If the column or guard 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.

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.

NOTE

Replacement of the 2 mm outlet bed support is not recommended.

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 bench top 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.

E. Screw the end fitting back onto the column. Tighten it finger-tight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

F. Reconnect the column to the system and resume operation.

Table 5 Dionex IonPac AG31/AS31 Column Spare Parts

Bed Support Assembly	044689
End Fitting	043278

5.1.3 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.

5.1.4 Filter Samples

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

5.2 High Background

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

Table 6 Typical Background Conductivity for Anion Columns

Eluent	Expected Background Conductivity
17 mM KOH	0.5 – 0.8 µS
85 mM KOH	0.8 – 1.5 µS
60 mM KOH/15% Methanol	1-3 µS

5.2.1 Preparation of Eluents

A. Make sure that the eluents prepared manually 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.

5.2.2 A Contaminated Trap Column

Please refer to the Product Manual for the Trap column in use.

5.2.3 Contaminated Dionex CR-ATC 500 or CR-ATC 600 Trap Column

A. A Dionex CR-ATC 500 or CR-ATC 600 Trap Column should be installed if using a Dionex Eluent Generator Cartridge.

B. If there is high baseline shift during gradient, the Dionex CR-ATC 500 or CR-ATC 600 may be contaminated or too old, please replace Dionex CR-ATC 500 or CR-ATC 600 and/or refer to the Clean-Up Procedure, in the Dionex CR-TC Product Manual (Document No. 079684).

5.2.4 A Contaminated Guard or Analytical Column

A. Remove the Dionex IonPac Guard and Dionex IonPac Analytical Columns from the system.

B. Install a backpressure coil that generates approximately 1500 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 caused by fouling, a guard column should be used. Clean or replace the Dionex IonPac Guard Column at the first sign of column performance degradation. The columns can be cleaned as instructed in Section 6.5.2, “Column Cleanup Procedure”.

5.2.5 Contaminated Hardware

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

A. Bypass the columns and the suppressor.

B. Install a backpressure coil that generates approximately 1,500 psi.

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 µS. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

5.2.6 A Contaminated Suppressor

If the above items have been checked and the problem persists, the Dionex Dynamically Regenerated Suppressor or the Dionex Chemically Regenerated Suppressor is probably causing the problem. For details on Dionex Dynamically Regenerated Suppressor operation, refer to the Dionex Dynamically Regenerated Suppressor Product Manual (Document No. 031956). For details on Dionex Chemically Regenerated Suppressor operation, refer to the Product Manual (Document No. 031727).

a. Check the suppressor settings in the instrument method and look for any suppressor-related errors in the audit trail.
b. Check the regenerant flow rate at the REGEN OUT port of the suppressor if operating in the Auto Suppression External Water Mode.
c. Check the eluent flow rate.
d. Replace the regenerant water.

5.2.7 Contaminated Regenerant Water

If the suppressor is used with external water mode, especially in IC-MS or IC-MS/MS applications, contaminated water can affect the system background and elevate conductivity and background.

5.3 Inconsistent Retention Times

Inconsistent or shifting retention times could be due to one or several different factors. These should be checked to determine the cause and address the issue.

5.3.1 Drifting to Shorter Retention Time

A. Contamination of the sample or eluent. Poorly mixed eluent. Remake sample and/or eluent.

B. Insufficient pump priming. Prime the pump. Refer to the Operator's Manual for correct pump operation.

C. Changes in temperature will also cause peaks to drift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment and calibrate/service as needed. Refer to the Operator's Manual for correct operation of the column compartment.

D. Excessive temperatures may cause column degradation. Check the temperature of the column compartment. Reduce the operational temperature. Refer to the Operator's Manual for correct operation of the column compartment. Replace the column.

E. Oxidizing eluent may cause hydrolysis or degradation of the column resulting in decreased analyte retention. Remove oxidizing agent, remake eluents, and replace the column.

5.3.2 Drifting to Longer Retention Time

A. Pump flow rate is too high. Pump flow rate impacts the eluent concentration generated by the EG. Verify the pump flow rate and have the pump repaired if pump flow rate is an issue.

B. If eluents are prepared manually, poorly mixed eluent can cause longer retention time. Remake eluent.

C. A pump losing prime will cause retention time drift. Prime the pump. Refer to the Operator's Manual for correct pump operation.

D. A leaking pump will cause longer retention times. Check the pump flow. Refer to the Operator's Manual for correct pump operation.

E. Changes in temperature will also cause peaks to drift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment and calibrate/service as needed. Refer to the Operator's Manual for correct operation of the column compartment.

5.3.3 Oscillating Retention Times

A. Pump problems can cause retention time to shift to longer and shorter time, run to run. Check the pump flow rate and prime the pump. Refer to the Operator's Manual for correct pump operation.

B. Temperature fluctuation will also cause peaks to shift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment and calibrate/service as needed. Refer to the Operator's Manual for correct operation of the column compartment.

C. A sluggish injection valve will cause peaks to shift if the injection time varies. The injection valve may need maintenance. When an injection valve is actuated, the timing is critical for consistent retention times. This will occur when the injection valve needs to be cleaned or serviced. Refer to the Operator's Manual for troubleshooting and service procedures.

5.4 Poor Peak Resolution

When carrying out separations using gradient analysis the column must be sufficiently equilibrated with the eluent concentration used at the start of the analysis. The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration. Typically, equilibration takes place in 3-5 column volumes of eluent. Depending on flow rate and concentration change, equilibration times range from 3 to 10 minutes.

A. If increased separation is needed for the first group of peaks, reduce the concentration of the starting eluent (E1). This part of the chromatogram is run isocratically with E1.

B. Due to different system configurations, the observed 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 proportions.

C. Keep the concentrations of E1 and E2 (final eluent concentration) constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.

D. Change the proportions of E1 and E2 and adjust the gradient time. This approach requires more time to develop and more knowledge in methods development work. The 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 that poor peak resolution can be due to any or all of the following factors.

5.4.1 Loss of Column Efficiency

When chromatographic efficiency is lost, peak resolution may decrease to an unacceptable level as the peaks broaden.

A. Check to see if headspace has developed in the guard or analytical column. This is usually due to improper use of the column such as exposing it to high pressures. Remove the column's inlet end fitting according to Section 5.1.2, "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 elution of the peaks broader resulting in reduced efficiency. Make sure connections are made correctly with 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. Cut the tubing lengths as short as possible, checking to ensure a smooth, 90° cut.

C. If tubing is not connected properly at the column inlet and outlet, it can cause dispersion resulting in low efficiency numbers.

    a. When installing the columns, it is recommended to turn off the pump while making connections. This will avoid any slippage of the ferrule under high pressure conditions which can result in a void in the fitting. Dispersion results in chromatograms with tailing peaks as shown in Figure 11 below.

    b. Before connecting the fittings, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette while the flow

is off. This will result in faster equilibration by preventing air from entering the system.

c. When making connections, make sure the ferrule and fitting bolt are at least 2 mm (0.1 inch) 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 12 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing.

Figure 11 Tailing Peaks Caused by Incorrectly Installed Tubing Fittings

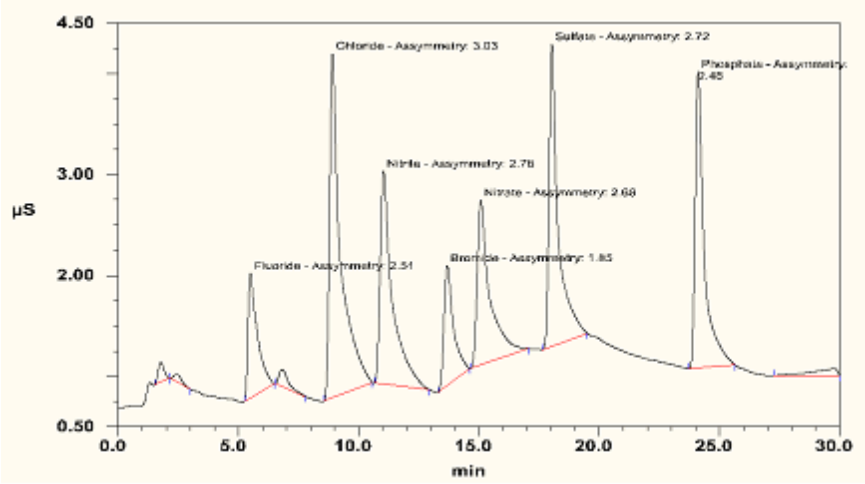
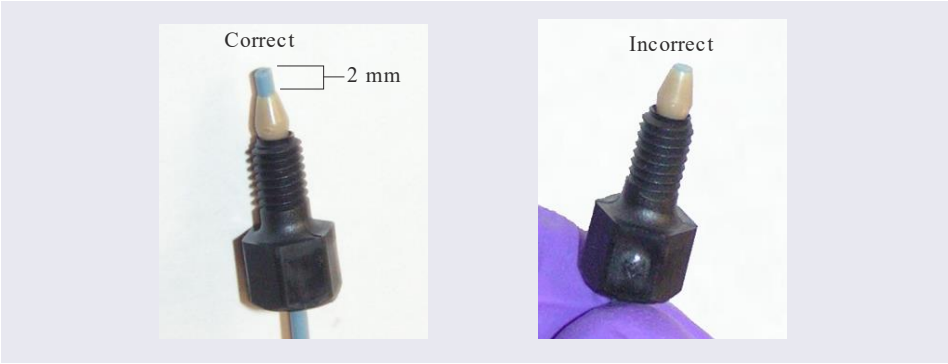


Figure 12 Correct and Incorrect Ferrule and Fitting Bolt Placement for Tubing Connections



D. Check for leaks. This can be done by visual inspection at elevated flow rates. Use of a paper towel or KimWipe™ touched to connection will show noticeable wetness even with micro liter volume leaks.

E. Suppressor contamination may be the cause of efficiency loss. Typically, such contamination is analyte-dependent so if all analytes are equally affected, it is most likely due to one of the causes above. Refer to the Suppressor Product Manual for cleaning protocols.

5.4.2 Analyte Specific Efficiency Loss

If a specific analyte exhibits an efficiency problem this could be due to contamination. For example, iron oxide causes sulfate and phosphate to tail; aluminum causes poor recovery and peak shape for phosphate; magnesium and calcium hydroxide precipitation in the suppressor cause poor recovery and peak shape for magnesium and calcium (refer to the Product Manual for the suppressor for product specific troubleshooting); nonionic surfactants can cause sulfate to tail.

A. Purge system of suspected contamination. Refer to the Operator's Manual for the system for details.

B. Check sample as source of contamination.

C. Employ the use of an appropriate guard or trap column to remove contaminants. Contact your local representative for current product specific information.

5.4.3 Shortened Retention Times

Shortened retention times will cause peaks to elute closer together and may be due to one or more factors. Fast eluent flow will cause quicker elution of analytes. Higher eluent concentration or a contaminated column (which results in loss of capacity) will reduce the analyte retention causing peaks to elute early.

NOTE

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast due to elevated flow rate or eluent concentration.

A. During gradient analysis the column must be equilibrated with the starting eluent prior to analysis. The time required is dependent on the difference in concentration between the start and the end of the gradient. If the ion concentration on the column is higher than the starting eluent concentration, then peaks may elute early or with inconsistent retention time. Typically, 3-5 column volumes of eluent are suggested for equilibration. Increase the length of time the column is in the starting eluent prior to injection to ensure adequate equilibration.

B. Check the flow rate. Ensure the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Confirm the eluent flow rate after the column using a stopwatch and graduated cylinder.

C. Ensure the eluent compositions and concentrations are correct. An eluent that is too concentrated will cause the peaks to elute sooner. Prepare fresh eluent.

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, Section 6, "Column Care").

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 resolves the problem. There may be a problem when one of the proportioned eluents is less than 5%.

Column contamination can lead to a loss of column capacity. This is because all of the ion exchange sites will no longer be available for the sample ions. For example, polyvalent ions or metals from the sample may concentrate on the column. Refer to Section 6.5 "Column Cleanup Procedure" 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.

E. After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is sufficiently 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.

5.4.4 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. Eluent concentration: Incorrect eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity. The eluent concentration maybe incorrect if the flow delivered to the eluent generator cartridge is incorrect. Check pump flow rate.

B. Sample concentration and injection volume: Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical column by either diluting the sample or injecting a smaller volume onto the column.

C. System plumbing: Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem. Reconnect components one at a time in the system prior to the columns, and test for front-end resolution after every system change.

5.5 Spurious Peaks

In a system where peaks are observed where none are expected, both the column and the system should be checked.

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 Section 7 "Column Cleanup".

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 serviced. 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 so long as they do not interfere with the quantification of the peaks of interest.

6. Column Care

6.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for the Dionex IonPac AS31 Column is listed in Sections **Error! Reference source not found.**, "Introduction" and 3.1, " General Operating Conditions".

6.2 Column Start-Up

The column is shipped using sodium bicarbonate as the storage solution.

Prepare the eluent shown on the Quality Assurance Report. To remove the storage solution, flush the column to waste with 80 mM KOH for at least 45 minutes and then 15 minutes with the QAR eluent before attaching the column outlet to the suppressor. Install the column in the column module and 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.

IMPORTANT ***When making any tubing connections, first turn off the pump. This will avoid any slippage of the ferrule under high pressure conditions.***

6.3 Column Storage

For short-term storage (< 1 week), use eluent; for long-term storage (> 1 week), store in eluent in a refrigerator or use 100 mM sodium bicarbonate for the column storage solution when stored at room temperature. With the column outlet directed to waste, flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

6.4 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.

Currently at Thermo Fisher Scientific, we have obtained consistent results using Optima® Solvents by Fisher Scientific. We have found that bottled HPLC-grade water from Burdick & Jackson contains acceptably low levels of impurities.

6.4.1 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.

6.4.2 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 ionized 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.

6.4.3 Solvents

In most cases, unless otherwise noted in Section 3.2 "General Operational Precautions", solvents can be added to the ionic eluents used with Dionex IonPac columns 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.

When using a solvent in an ionic eluent, the back pressure generated 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. Do not exceed the maximum operating backpressure of the Dionex IonPac column.

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.

6.5 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.

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 viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.***
- ***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 by solvents during column rinses can result in very high-pressure zones.***

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.

6.5.1 Choosing the Appropriate Cleanup Solution

Table 7 Cleaning Solutions for Anion Exchange Columns

Contamination	Anion Solutions
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.
Hydrophilic Contamination of High Valence	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.
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.
Nonionic and Hydrophobic Contamination	Although this is extremely rare, nonionic and hydrophobic contaminants can contaminate an ion exchange column. The symptoms include reduced chromatographic efficiency, elevated pressure or tailing for a subset of analyte ions. Retention is unaffected by this type of 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.

6.5.2 Column Cleanup Procedure

A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in the table above.

B. Disconnect the outlet of the Dionex IonPac column from the suppressor. If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path. Double check that the eluent flows in the correct direction as designated on each of the column labels.

CAUTION

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

C. For aqueous cleaning solutions; set the pump flow rate to 0.25 mL/min for a Dionex IonPac 2 mm Analytical or Guard Column.

D. For cleaning solutions containing organic solvents; set the pump flow rate 0.12 mL/min for a Dionex IonPac 2 mm Analytical or Guard Column.

E. Rinse the column for 10 minutes with deionized water before pumping the chosen cleanup solution over the column or before switching cleanup solutions.

F. Unless otherwise noted, pump the cleanup solution through the column for at least 60 minutes (pump for longer time if column is highly contaminated). A step gradient is used for column cleanup when switching between solutions.

G. Rinse the column for at least 10 minutes with deionized water before pumping eluent over the column.

H. Equilibrate the column(s) with eluent, still directing the effluent to waste, for at least 30 minutes before resuming normal operation.

I. Reconnect the Dionex IonPac analytical column to the suppressor and if your system was originally configured with a guard column, place the guard column in line between the injection valve and the analytical column.

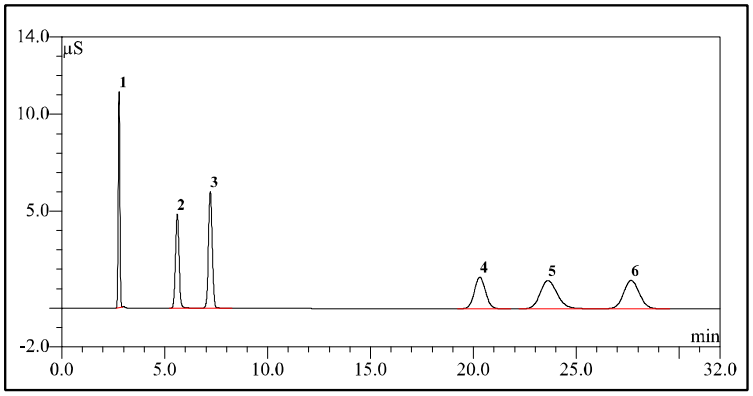
7. Quality Assurance Report

Device Monitoring Enabled
and Viper Fitting Ready

Dionex IonPac™ AS31
Analytical (2 x 250 mm)
Product No. 303147

Date: 01-Dec-18 10:11
Serial No. : 181201002
Lot No. : 01824029

Eluent:	24 mM KOH
Eluent Source:	Dionex EGC 500 KOH Cartridge
Flow Rate:	0.30 mL/min
Temperature:	15 °C
Detection:	Suppressed Conductivity
Suppressor:	Dionex Anion Dynamically Regenerated Suppressor (Dionex ADRS™ 600 2mm), AutoSuppression™ Recycle Mode
Applied Current:	18 mA, Constant Current Mode
Injection Volume:	2.5 µL
Storage Solution:	100 mM Sodium Bicarbonate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	2.78	1.00	12.54	6092	5.0
2	Sulfate	5.60	1.09	5.02	5261	10.0
3	Chloride	7.21	1.04	19.80	7440	10.0
4	Bromide	20.31	1.08	2.68	6712	20.0
5	Thiosulfate	23.62	1.28	2.81	4012	20.0
6	Nitrate	27.66	1.14	n.a.	6309	20.0

QA Results:

Analyte	Parameter	Specification	Results
Thiosulfate	Efficiency	>=2700	Passed
Bromide	Efficiency	>=4500	Passed
Thiosulfate	Asymmetry	1.0-2.0	Passed
Bromide	Asymmetry	1.0-1.8	Passed
Nitrate	Retention Time	25.3-29.7	Passed
(Thiosulfate-Bromide)/ (Nitrate-Thiosulfate)	Retention Time Ratio	0.5-1.0	Passed
	Pressure	<=3630	2996

Production Reference:

Datasource: Resin
Directory: RTC\RTC_5
Sequence: AS31_2X250MM_Col_Con_Nov 15_2018
Sample No.: 78

6.80 SR15 Build 4656 (243203)

Chromeleon ™ Thermo Fisher Scientific

8. Additional Information

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com
From the U.S. call the Customer Care Center for Dionex Products at 1-800-346-6390
Outside the U.S., call the nearest Thermo Fisher Scientific office.

8.1 General Information on PEEK™ Tubing

Dionex brand PEEK™ tubing is available. Note that tubing from different suppliers varies with respect to color and i.d. Care should be taken to ensure the correct i.d. is being used to avoid problems caused by sample dispersion.

Table 8 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 1mL/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

NOTE

Blue and Light blue are very similar in color. Additionally, tubing from different suppliers may differ to that stated above.