



Dionex IonPac AS32-Fast-4 μ m Columns

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Product Manual

for

Dionex IonPac AS32-Fast-4 μ m Analytical Columns

4 \times 150 mm (Item # 303151)

2 \times 150 mm (Item # 303153)

Dionex IonPac AG32-Fast-4 μ m Guard Columns

4 \times 30 mm (Item # 303152)

2 \times 30 mm (Item # 303154)

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Safety and special notices include the following:



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



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



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.



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 |
| 1.1 | Dionex IonPac AS32-Fast-4µm/Dionex IonPac AG32-Fast-4µm Column Packing Specifications | 7 |
| 2. | Installation | 9 |
| 2.1 | Thermo Scientific Dionex High Pressure Ion Chromatography Systems | 9 |
| 2.2 | System Requirements | 10 |
| 2.3 | System Void Volume..... | 10 |
| 2.4 | Column Start-Up | 10 |
| 2.5 | Dionex IonPac Guard Columns | 10 |
| 2.6 | Sample Concentrators..... | 11 |
| 2.7 | The Injection Loop..... | 11 |
| 2.8 | Dionex Anion Dynamically Regenerated Suppressor Requirements | 12 |
| 2.9 | Dionex Anion Chemically Regenerated Suppressor Requirements | 12 |
| 2.10 | Using Dionex Displacement Chemical Regeneration (DCR) with the Chemical Suppression Mode | 12 |
| 3. | Operation..... | 13 |
| 3.1 | General Operating Conditions | 13 |
| 3.2 | General Operational Precautions | 14 |
| 3.3 | Quality Assurance Reports | 14 |
| 3.4 | Chemical Requirements and Solvent Compatibility | 14 |
| 3.5 | Eluent Preparation..... | 15 |
| 3.5.1 | Eluent Generation | 15 |
| 3.5.2 | Making Eluents that Contain Solvents | 15 |
| 3.5.3 | Manually Prepared Eluents | 16 |
| 4. | Example Applications..... | 17 |
| 4.1 | Isocratic Elution With and Without a Guard Column | 18 |
| 4.2 | Analysis of Polythionates | 19 |
| 4.3 | Analysis of Aromatic Sulfonates, Pigments, and Dyes | 20 |
| 4.4 | Analysis of Perchlorate in High Chloride and Sulfate Matrices..... | 22 |
| 4.5 | Analysis of Persulfate | 24 |

| | | |
|-----------|----------------------------------------------------------------------------|-----------|
| 5. | Troubleshooting..... | 25 |
| 5.1 | High Back Pressure | 27 |
| 5.1.1 | Finding the Source of High System Pressure..... | 27 |
| 5.1.2 | Replacing Column Bed Support Assemblies (4 mm and 2 mm columns only) | 27 |
| 5.1.3 | Filter Eluent..... | 28 |
| 5.1.4 | Filter Samples | 28 |
| 5.2 | High Background | 28 |
| 5.2.1 | Preparation of Eluents | 28 |
| 5.2.2 | A Contaminated Trap Column..... | 29 |
| 5.2.3 | Contaminated Dionex CR-TC Column | 29 |
| 5.2.4 | A Contaminated Guard or Analytical Column | 29 |
| 5.2.5 | Contaminated Hardware..... | 29 |
| 5.2.6 | A Contaminated Suppressor..... | 29 |
| 5.3 | Inconsistent Retention Times | 29 |
| 5.3.1 | Drifting to Shorter Retention Time..... | 30 |
| 5.3.2 | Drifting to Longer Retention Time..... | 30 |
| 5.3.3 | Oscillating Retention Times..... | 30 |
| 5.4 | Poor Peak Resolution | 31 |
| 5.4.1 | Loss of Column Efficiency | 31 |
| 5.4.2 | Analyte Specific Efficiency Loss | 32 |
| 5.4.3 | Shortened Retention Times..... | 33 |
| 5.4.4 | Loss of Front-End Resolution..... | 34 |
| 5.5 | Spurious Peaks..... | 34 |
| 6. | Appendix A – Column Care..... | 35 |
| 6.1 | Recommended Operation Pressures..... | 35 |
| 6.2 | Column Start-Up | 35 |
| 6.3 | Column Storage | 35 |
| 6.4 | Chemical Purity Requirements..... | 35 |
| 6.4.1 | Inorganic Chemicals | 36 |
| 6.4.2 | Deionized Water | 36 |
| 6.4.3 | Solvents | 36 |
| 6.5 | Column Cleanup..... | 37 |
| 6.5.1 | Choosing the Appropriate Cleanup Solution | 38 |
| 6.5.2 | Column Cleanup Procedure | 39 |
| 7. | Appendix B – Quality Assurance Reports..... | 40 |
| 8. | Appendix C – Additional Information..... | 42 |
| 8.1 | General Information on PEEK™ Tubing | 42 |

1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ AS32-Fast-4µm column is designed for analysis of low polarity anions, polysulfonated aromatics, aromatic dyes, and pigments. Its selectivity allows for sub-ppb quantification of perchlorate in high chloride and sulfate matrices using suppressed conductivity and mass spectrometry detection. The Dionex IonPac AS32-Fast-4µm column is available in standard bore (4 mm i.d.) and microbore (2 mm i.d) formats, each 150 mm long. The Dionex IonPac AS32-Fast-4µm column is compatible with pH 0-14 eluents and eluents containing organic solvents from 0–100% in concentration. The Dionex IonPac AS32-Fast-4µm column can be used with any suppressible ionic eluent that does not exceed the capacity of the Thermo Scientific Dionex Anion Dynamically Regenerated Suppressor 600 (Dionex ADRS 600). The Dionex IonPac AS32-Fast-4µm column has nominal efficiency for perchlorate of at least 7500 plates/column using standard operating conditions.

1.1 Dionex IonPac AS32-Fast-4µm/Dionex IonPac AG32-Fast-4µm Column Packing Specifications

Resin Characteristics:

| | |
|-------------------------|------------------------------------|
| Nominal Particle Size: | 4 µm (Analytical and Guard column) |
| Particle Cross-linking: | 55% |
| Ion exchange capacity: | 126 µeq per 4 × 150 mm column |
| | 31.5 µeq per 2 × 150 mm column |
| | 25 µeq per 4 × 30 mm column |
| | 8 µeq per 2 × 30 mm column |

Functional Characteristics:

| | |
|-------------------|---------------------------------|
| Functional Group: | Alkanol quaternary ammonium ion |
| Hydrophobicity: | Ultralow |

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

Table 1 **Dionex IonPac AS32-Fast-4µm/Dionex IonPac AG32-Fast-4µm Operating Parameters**

| Column(s) | Typical Back Pressure psi (MPa ^a), 30°C ^b | Standard Flow Rate mL/min | Maximum Flow Rate mL/min ^{c, d} |
|----------------------------------------------------------------|---------------------------------------------------------------------|------------------------------|------------------------------------------|
| Dionex IonPac AS32-Fast-4µm, 4 mm Analytical column | ~ 2500 (17.24) | 1.0 | 1.0 |
| Dionex IonPac AG32-Fast-4µm, 4 mm Guard column | ~ 500 (3.44) | 1.0 | 1.0 |
| Dionex IonPac AS32-Fast-4µm and AG32-Fast-4µm, 4 mm columns | ~ 3000 (20.68) | 1.0 | 1.0 |
| Dionex IonPac AS32-Fast-4µm, 2 mm Analytical column | ~ 2200 (15.17) | 0.25 | 0.25 |
| Dionex IonPac AG32-Fast-4µm, 2 mm Guard column | ~ 500 (3.44) | 0.25 | 0.25 |
| Dionex IonPac AS32-Fast-4µm and AG32-Fast-4µm, 2 mm columns | ~ 2700 (18.62) | 0.25 | 0.25 |

^a Note: 1 MPa = 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^d When developing a method for multiple column formats or long term, widespread use, it is important to operate within the maximum flow rate listed above to ensure a reproducible method.**NOTE**

*For assistance, visit UnityLab 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.*

**WARNING**

Exceeding the maximum flow rates listed in the above table can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column.

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 are required.

2.1 Thermo Scientific Dionex High Pressure Ion Chromatography Systems

A minimum of a Dionex High Pressure Ion Chromatography System (HPIC) is recommended when running Dionex IonPac 4µm 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. ICS 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

Dionex IonPac columns are designed to be 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 Thermo Scientific Dionex Integrion™ systems with conductivity detectors (Item # 088798) and Dionex ICS-6000 systems with conductivity detectors (Item # 088803).

2.3 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 of the 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, 0.010" 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.

2.4 Column Start-Up

The column is shipped using 4 mM KOH as the storage solution. Flush the column to waste with the QAR eluent for at least 30 minutes 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.

2.5 Dionex IonPac Guard Columns

A Dionex IonPac Guard Column is normally used with the Dionex IonPac Analytical Column. A guard is placed before 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 20% 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 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 a 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.

2.7 The Injection Loop

2.7.1.1 The 2 mm System Injection Loop, 2 – 15 μ L

For most applications on a 2 mm analytical system, a 2 – 15 μ L injection loop is sufficient. Generally, you should not inject more than 12.5 nanomoles of any one analyte onto a 2 mm analytical column. Injecting larger numbers of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. The Dionex IonPac AS32-Fast-4 μ m 2 mm columns require a microbore system configuration. Install an injection loop one-fourth or less (<15 μ L) of the loop volume used with a 4 mm analytical system.

2.7.1.2 The 4 mm System Injection Loop, 10 – 50 μ L

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

2.8 Dionex Anion Dynamically Regenerated Suppressor Requirements

A Dionex Anion Dynamically Regenerated Suppressor should be used for applications that require suppressed conductivity detection.



CAUTION

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

For 4 mm Dionex IonPac AS32-Fast-4 μ m columns, use a Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (4 mm, Item # 088666).

For 2 mm Dionex IonPac AS32-Fast-4 μ m columns, use a Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm, Item # 088667).

For detailed information on the operation of the Dionex Anion Dynamically Regenerated Suppressor, see Document No. 031956, the “Product Manual for the Dionex DRS 600.”

2.9 Dionex Anion Chemically Regenerated Suppressor Requirements

A Thermo Scientific Dionex Anion Chemically Regenerated Suppressor (Dionex ACRS 500) may be used instead of a Dionex ADRS 600 for applications that require suppressed conductivity detection. Use a 4 mm Dionex ACRS 500 (4 mm, Item # 085090) with the 4 mm Dionex IonPac AS32-Fast-4 μ m column. For 2 mm operation, use the 2 mm Dionex ACRS 500 (2 mm, Item # 085091). These suppressors are compatible with all solvents and concentrations with which the systems and columns are compatible.

For detailed information on the operation of the Dionex Anion Chemically Regenerated Suppressor, see Item # 031727, the “Product Manual for the Dionex Anion Chemically Regenerated Suppressor 500, the Dionex ACRS 500”.

2.10 Using Dionex Displacement Chemical Regeneration (DCR) with the Chemical Suppression Mode

The Dionex Displacement Chemical Regeneration (Dionex DCR) Mode is recommended for chemical suppression using sulfuric acid and the Dionex Anion Chemically Regenerated Suppressor (Dionex ACRS 500). See the Dionex DCR kit manual, Document P/N 031664, for details.



SAFETY

Use proper safety precautions in handling acids and bases.

3. Operation

3.1 General Operating Conditions

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

Table 2 General Operating Conditions for the Dionex IonPac AS32-Fast-4 μ m Column

| | | |
|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| Sample Volume: | 2 mm: | 2.5 μ L Loop |
| | 4 mm: | 10 μ L Loop |
| Column: | 2 mm: | Dionex IonPac AS32-Fast-4 μ m 2 mm Analytical Column + Dionex IonPac AG32-Fast-4 μ m 2 mm Guard Column |
| | 4 mm: | Dionex IonPac AS32-Fast-4 μ m 4 mm Analytical Column + Dionex IonPac AG32-Fast-4 μ m 4 mm Guard Column |
| Eluent: | 4.0 mM KOH (QAR eluent) | |
| Eluent Flow Rate: | 2 mm: | 0.25 mL/min |
| | 4 mm: | 1.0 mL/min |
| DRS Suppressor: | 2 mm and 4 mm: Dionex Anion Dynamically Regenerated Suppressor, Dionex ADRS 600, AutoSuppression Recycle Mode | |
| or CRS Suppressor: | 2 mm and 4 mm: Dionex Anion Chemically Regenerated Suppressor, Dionex ACRS 500 | |
| CRS Regenerant: | 50 mN H ₂ SO ₄ | |
| Expected Background Conductivity: | <1 μ S | |
| Long-term Storage Solution (> 1 week): | Eluent | |
| Short-term Storage Solution (< 1 week): | Eluent | |



NOTE

*For assistance, visit UnityLab 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 3 Operational Limits for the Dionex IonPac AS32-Fast-4 μ m Columns

| | |
|------------------------------------|----------------------|
| Eluent pH | Between 0 and 14 |
| Sample pH | Between 0 and 14 |
| Maximum Flow Rate for 2 mm Columns | 0.25 mL/min |
| Maximum Flow Rate for 4 mm Columns | 1.0 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 [Appendix B – Quality Assurance Reports](#). 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 4 Typical HPLC Solvents for Cleaning and Use with Dionex IonPac Columns

| Solvent | Maximum Operating Concentration |
|--------------|---------------------------------|
| Acetonitrile | 100% |

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



Use of a Dionex AERS 500e operated in the AutoSuppression External Water Mode is recommended when using eluents containing organic solvents. Refer to the Suppressor Product Manual for operation limitations in the electrolytic mode (power on).

3.5 Eluent Preparation

3.5.1 Eluent Generation

It is recommended that Dionex IonPac columns are used with Dionex HPIC Systems equipped with a Thermo Scientific Dionex Eluent Generator Cartridge. The use of eluent generation provides a more stable baseline, improved performance, and increased reproducibility compared to 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.



Only Dionex EGC 500 cartridges rated for 5,000 psi or higher should be used when running Dionex IonPac 4µm columns due to the higher backpressures generated at typical operational flow rates.

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.



When purging or degassing eluents containing solvents, do not purge or degas the eluent excessively since it is possible that a volatile solvent can 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.

3.5.3 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.

4. Example Applications



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.*

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.

Ensure that your system is properly configured and that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water with a specific resistance of 18.2 megohm-cm. For chemical purity requirements, see [Section 6.4, “Chemical Purity Requirements”](#). After running synthetic standards to calibrate your system, you may find that real sample matrices foul your columns. For this reason, it is always advisable to use a guard column to protect the analytical column. If column performance deteriorates and it is determined that the guard or the analytical column has been fouled, refer to the column cleanup protocols in [“Appendix A – Column Care”](#). If your sample matrices are relatively low in ionic concentration, you may be able to increase the sensitivity of your system by using sample concentration techniques.

4.1 Isocratic Elution With and Without a Guard Column

Isocratic elution of anions on the Dionex IonPac AS32-Fast-4 μ m Analytical Column has been optimized utilizing a hydroxide eluent. By using this eluent, mono- and divalent anions can be isocratically separated and quantitated in a single injection. The Dionex IonPac AS32-Fast-4 μ m Analytical Column should always be used with the Dionex IonPac AG32-Fast-4 μ m Guard Column. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column.

Figure 1 Dionex IonPac AS32-Fast-4 μ m Column (4 x 150 mm) With and Without Guard Column

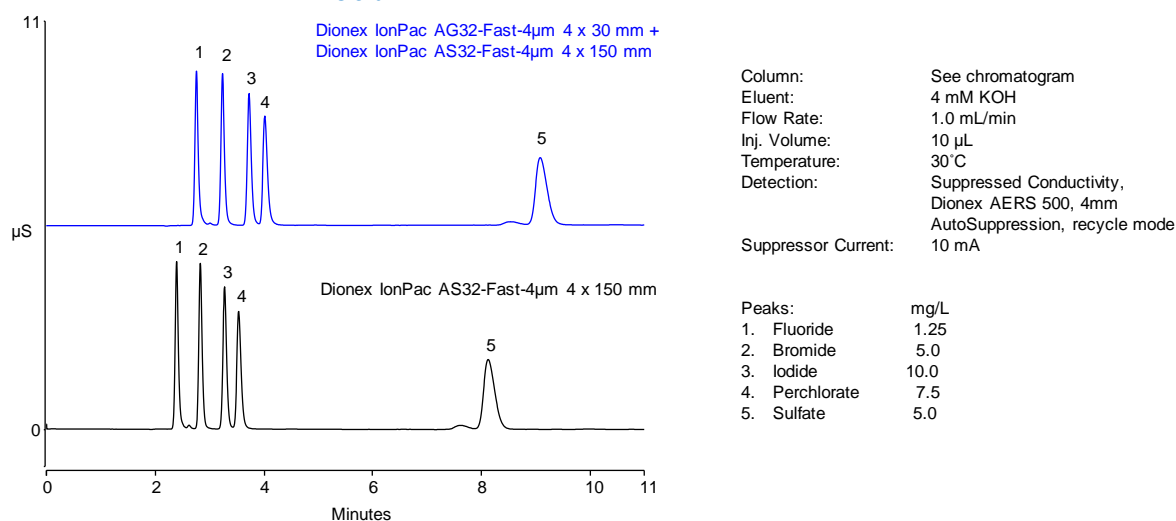
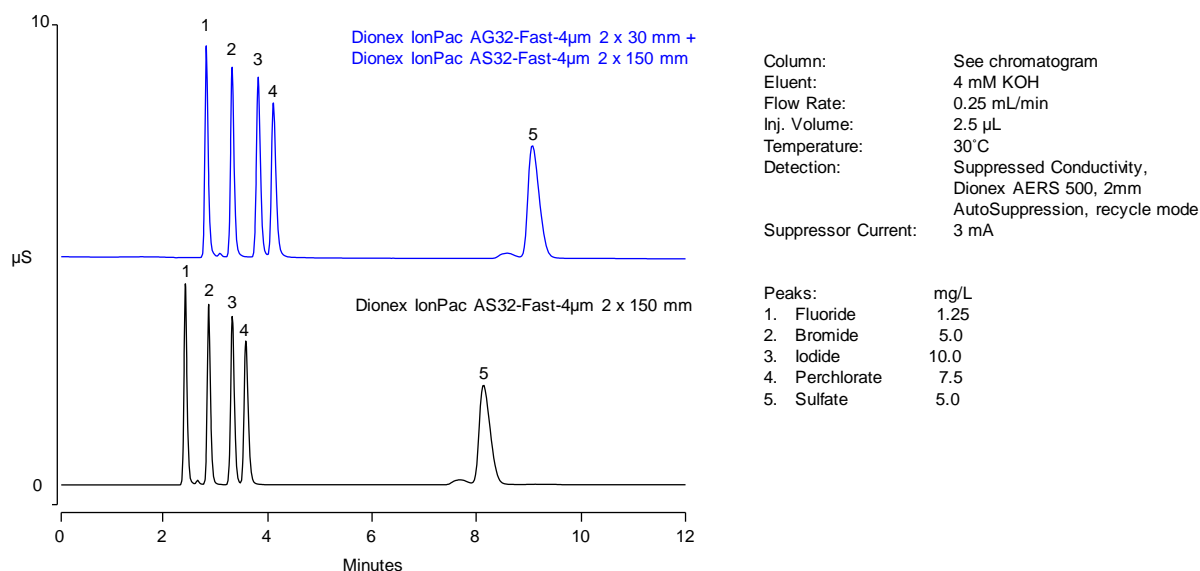


Figure 2 Dionex IonPac AS32-Fast-4 μ m Column (2 x 150 mm) With and Without Guard Column



4.2 Analysis of Polythionates

The following chromatograms demonstrate the separation of polythionates on the Dionex AS32-Fast-4 μ m column.

Figure 3 Analysis of Polythionate Samples Using the Dionex IonPac AS32-Fast-4 μ m Column (4 x 150 mm)

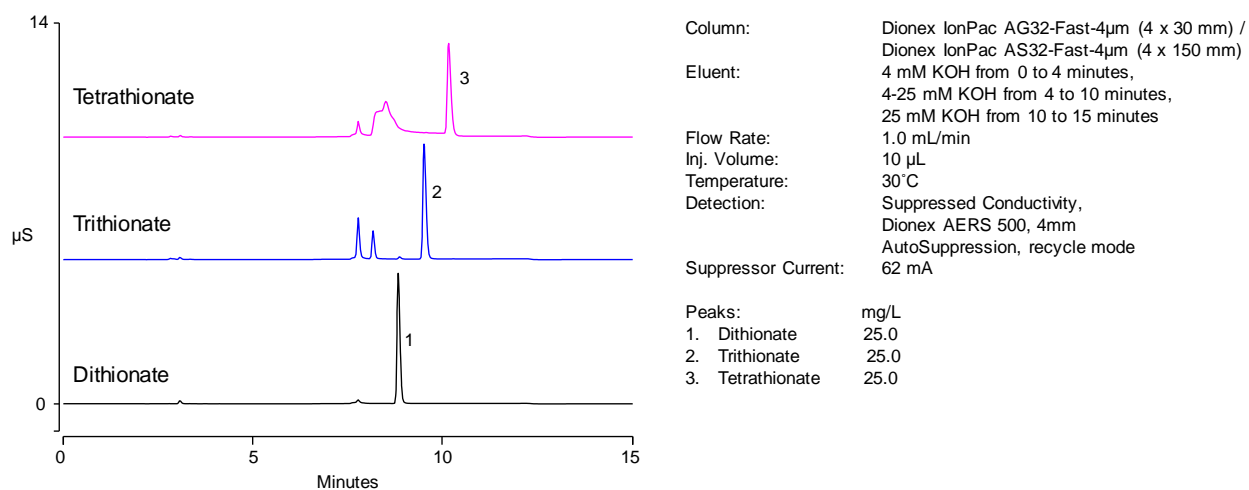
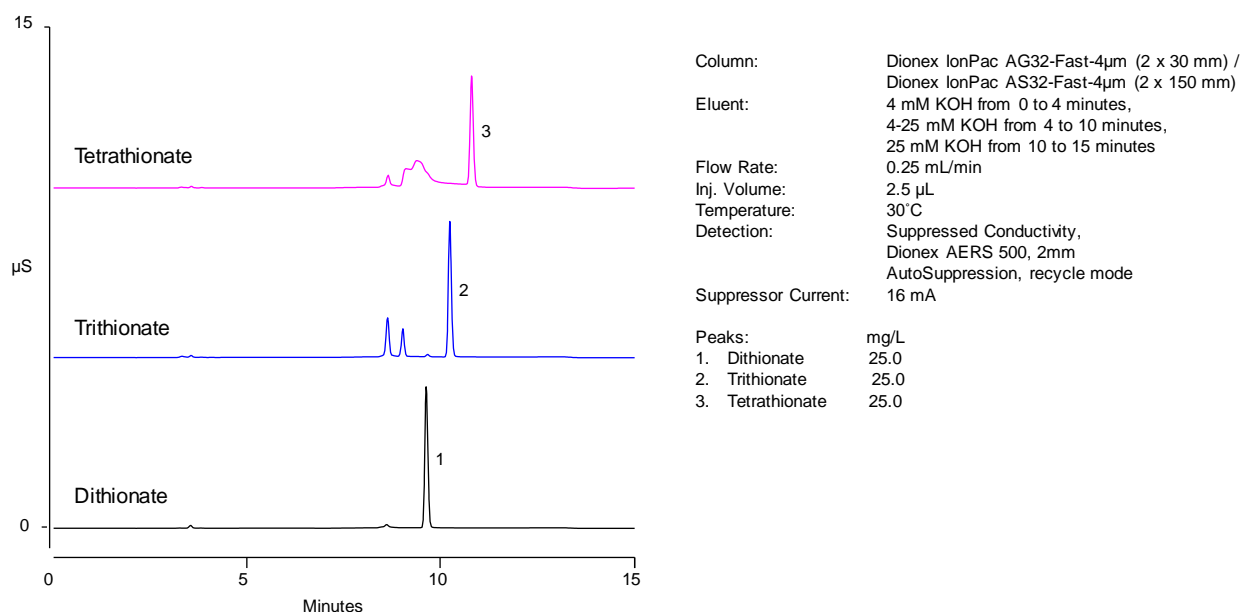


Figure 4 Analysis of Polythionate Samples Using the Dionex IonPac AS32-Fast-4 μ m Column (2 x 150 mm)



4.3 Analysis of Aromatic Sulfonates, Pigments, and Dyes

The following chromatograms show the separation of various aromatic sulfonates, pigments, and dyes on the Dionex IonPac AS32-Fast-4 μ m column.

Figure 5 Analysis of Aromatic Sulfonates, Pigments, and Dyes Using the Dionex IonPac AS32-Fast-4 μ m Column (4 x 150 mm)

| | | | |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|------|
| Column: | Dionex IonPac AG32-Fast-4 μ m (4 x 30 mm) / Dionex IonPac AS32-Fast-4 μ m (4 x 150 mm) | Peaks | mg/L |
| Eluent: | 4 mM KOH from 0 to 4 minutes, 4-40 mM KOH from 4 to 10 minutes, 40-80 mM KOH from 10 to 14 minutes, 80 mM KOH from 14 to 20 minutes | 1. p-Toluenesulfonic acid | 25 |
| Flow Rate: | 1.0 mL/min | 2. 2-Naphthalenesulfonic acid | 25 |
| Inj. Volume: | 10 μ L | 3. 4-Sulfobenzoic acid | 25 |
| Temperature: | 30°C | 4. 1-Naphthol-4-sulfonic acid | 25 |
| Detection: | Suppressed Conductivity, Dionex AERS 500, 4mm AutoSuppression, recycle mode | 5. Naphthalene-disulfonic acid | 25 |
| Suppressor Current: | 198 mA | 6. Naphthalene-trisulfonic acid | 25 |
| | | 7. FDC Yellow #5 | 25 |
| | | 8. FDC Yellow #6 | 25 |
| | | 9. FDC Red #40 | 25 |

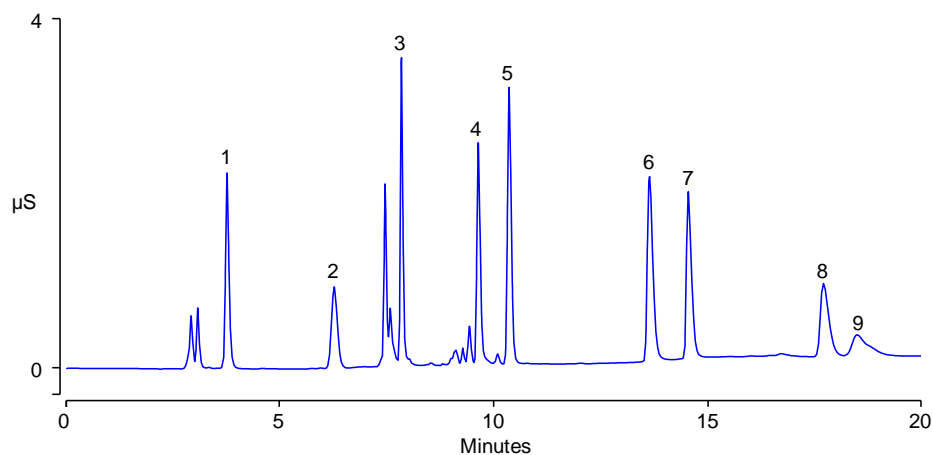
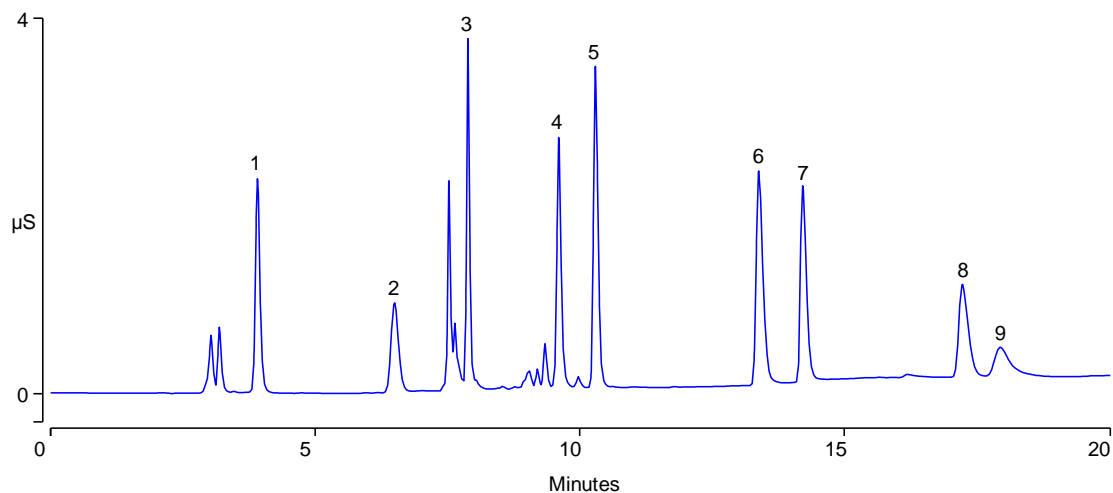


Figure 6 Analysis of Aromatic Sulfonates, Pigments, and Dyes Using the Dionex IonPac AS32-Fast-4 μ m Column (2 x 150 mm)

| | | | |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|------|
| Column: | Dionex IonPac AG32-Fast-4 μ m (2 x 30 mm) / Dionex IonPac AS32-Fast-4 μ m (2 x 150 mm) | Peaks | mg/L |
| Eluent : | 4 mM KOH from 0 to 4 minutes, 4-40 mM KOH from 4 to 10 minutes, 40-80 mM KOH from 10 to 14 minutes, 80 mM KOH from 14 to 20 minutes | 1. p-Toluenesulfonic acid | 25 |
| Flow Rate: | 0.25 mL/min | 2. 2-Naphthalenesulfonic acid | 25 |
| Inj. Volume: | 2.5 μ L | 3. 4-Sulfobenzoic acid | 25 |
| Temperature: | 30°C | 4. 1-Naphthol-4-sulfonic acid | 25 |
| Detection: | Suppressed Conductivity, Dionex AERS 500, 2mm AutoSuppression, recycle mode | 5. Naphthalene-disulfonic acid | 25 |
| Suppressor Current | 50 mA | 6. Naphthalene-trisulfonic acid | 25 |
| | | 7. FDC Yellow #5 | 25 |
| | | 8. FDC Yellow #6 | 25 |
| | | 9. FDC Red #40 | 25 |



4.4 Analysis of Perchlorate in High Chloride and Sulfate Matrices

A unique feature of the Dionex IonPac AS32-Fast-4 μ m column selectivity is its ability to separate perchlorate in the presence of high chloride and sulfate. The following chromatograms demonstrate this capability. Trace amounts of perchlorate can be determined using suppressed conductivity and mass spectrometry detection.

Figure 7 Analysis of Perchlorate in High Chloride and Sulfate Matrices Using the Dionex IonPac AS32-Fast-4 μ m Column (4 x 150 mm)

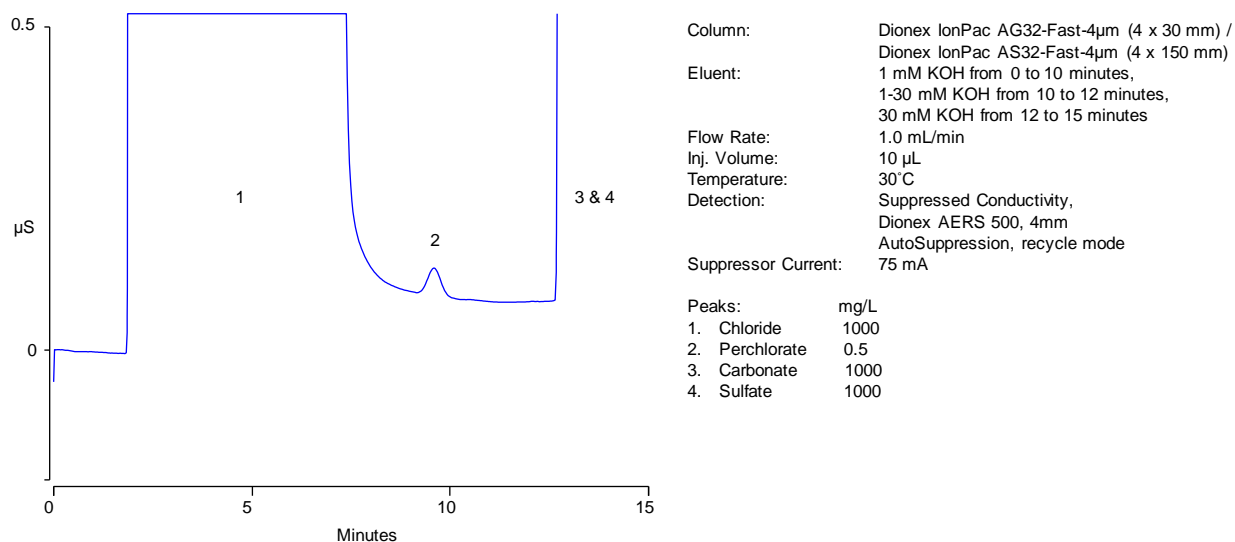
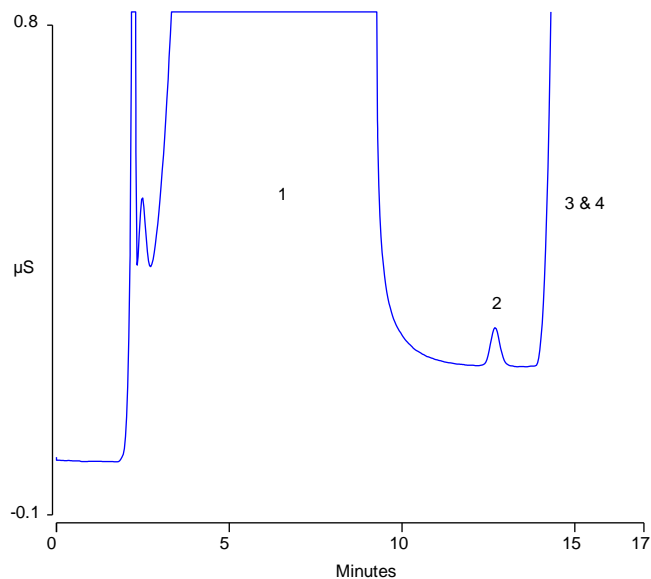


Figure 8 Analysis of Perchlorate in High Chloride and Sulfate Matrices Using the Dionex IonPac AS32-Fast-4 μ m Column (2 x 150 mm)



Column: Dionex IonPac AG32-Fast-4 μ m (2 x 30 mm) /
Dionex IonPac AS32-Fast-4 μ m (2 x 150 mm)
Eluent: 1 mM KOH from 0 to 10 minutes,
1-30 mM KOH from 10 to 12 minutes,
30 mM KOH from 12 to 15 minutes
Flow Rate: 0.25 mL/min
Inj. Volume: 2.5 μL
Temperature: 30°C
Detection: Suppressed Conductivity,
Dionex AERS 500, 2mm
AutoSuppression, recycle mode
Suppressor Current: 19 mA

Peaks: mg/L
1. Chloride 1000
2. Perchlorate 0.5
3. Carbonate 1000
4. Sulfate 1000

4.5 Analysis of Persulfate

The following chromatograms demonstrate the separation of persulfate on the Dionex IonPac AS32-Fast-4 μ m column.

Figure 9 Analysis of Persulfate Using the Dionex IonPac AS32-Fast-4 μ m Column (4 x 150 mm)

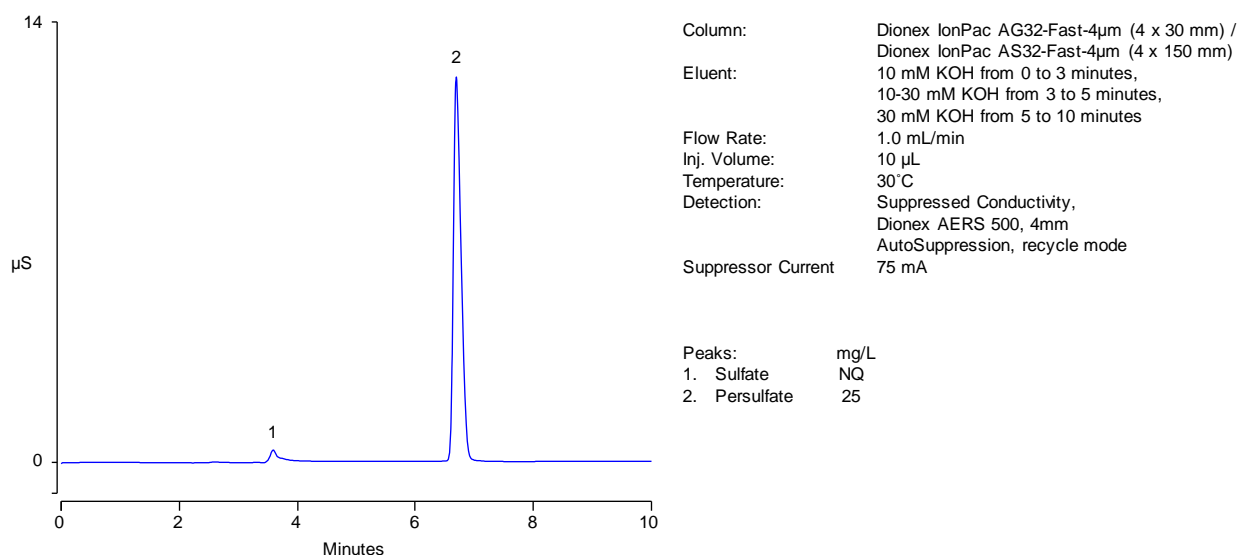
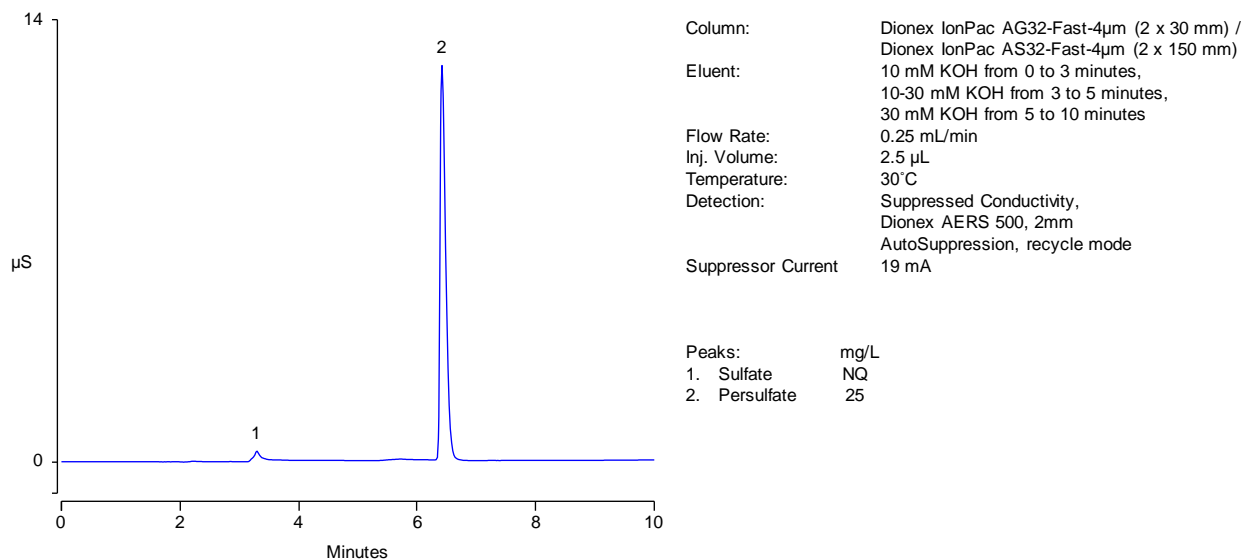


Figure 10 Analysis of Persulfate Using the Dionex IonPac AS32-Fast-4 μ m Column (2 x 150 mm)



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 UnityLab 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 5 Troubleshooting Summary

| Observation | Cause | Action | Reference Section |
|-------------------------------------|-----------------------------------------|------------------------------------------------------------|--------------------------|
| High Back Pressure | Unknown | Isolate Blocked Component | 5.1.1 |
| | Plugged Column Bed | 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 |
| | Incorrect Eluent Concentration | 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 |

5 – Troubleshooting

| Observation | Cause | Action | Reference Section |
|-----------------------------------------|----------------------------------------------------|-----------------------------------------------------------------------------|------------------------------------|
| 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 | Incorrect Eluent Concentration | 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 4 μ m Guard Column plus the Dionex IonPac 4 μ m Analytical Column when using the test chromatogram conditions should be less than 3500 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 stop watch 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 inline 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 components (Dionex EGC 500 and HPIC degasser) connected should be <400 psi at 1.0 mL/min. 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 EGC is not installed) should be installed to prevent particulates from blocking the system.

5.1.2 Replacing Column Bed Support Assemblies (4 mm and 2 mm columns only)

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.



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 or 4 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.

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

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 DionexCR-TC Column

- A. A Dionex CR-TC Trap Column should be installed if using a Dionex Eluent Generator KOH Cartridge.
- B. If the Dionex CR-TC becomes contaminated, please refer to the Clean-Up Procedure, in the Dionex CR-TC Product Manual.

5.2.4 A Contaminated Guard or Analytical Column

- A. Remove the Dionex IonPac guard and analytical columns from the system.
- B. Install a back-pressure coil that generates approximately 1,500 psi and continue to pump eluent. If the background conductivity decreases, the columns 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 back-pressure 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 μ S. 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. For details on Dionex Chemically Regenerated Suppressor operation, refer to the Product Manual.

5.3 Inconsistent Retention Times

Inconsistent or shifting retention times could be due to one of 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 Product 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 Product 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 Product 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. Poorly mixed eluent. Remake eluent.
- B. A pump losing prime will cause retention time drift. Prime the pump. Refer to the Product Manual for correct pump operation.
- C. A leaking pump will cause longer retention times. Check the pump flow. Refer to the Product Manual for correct pump operation.
- D. 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 Product Manual for correct operation of the column compartment.

5.3.3 Oscillating Retention Times

- A. Pump problems can cause retention times to shift to longer and shorter times, run to run. Check pump flow rate, prime the pump. Refer to the Product 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, calibrate/service as needed. Refer to the Product 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 retorqued. Refer to the valve 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 poor peak resolution can be due to any or all of the following factors.

5.4.1 Loss of Column Efficiency

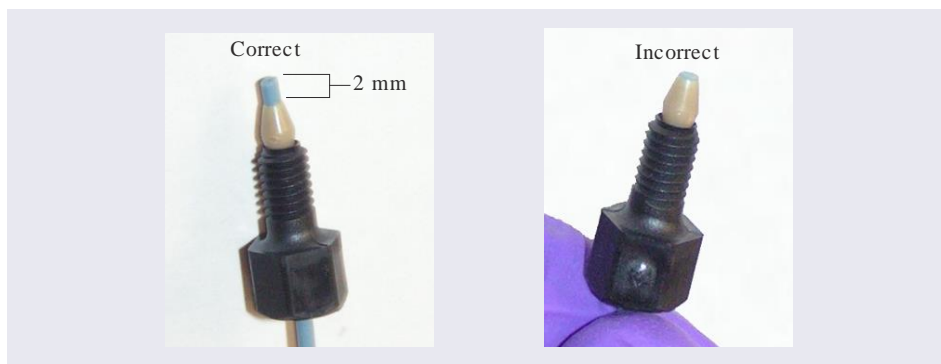
When chromatographic efficiency is lost, peak resolution may decrease to an unacceptable level as the peaks broaden.

- A. Check to see if headspace (2 and 4 mm columns only) 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 top end fitting according to [Section 5.1.2, "Replacing Column Bed Support Assemblies \(4 mm and 2 mm columns only\)"](#). 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. The use of Thermo Scientific Dionex IC PEEK Viper fittings is recommended to achieve consistent low dead volume connections and ensure optimum chromatographic performance.
- 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.
 - 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. Do not place the ferrule and fitting bolt flush with the end of the tubing. Figure 11 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing.

Figure 11 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 Product 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 Appendix A – 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](#) 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 may be 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

If 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 be spurious, inefficient (broad) peaks that can show up at unexpected times. Clean the column as indicated in [Section 6.5, “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 retorqued. 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. Appendix A – 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 this Dionex IonPac Column is listed in [Sections 1, “Introduction”](#) and [3.1, “General Operating Conditions”](#).

6.2 Column Start-Up

The column is shipped using Eluent 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 QAR eluent for at least 30 minutes 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 (4mM KOH); for long-term storage (> 1 week), store in eluent (4mM KOH). With the column outlet 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 backpressure 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 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 6 **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 0.2M 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 Dionex 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**

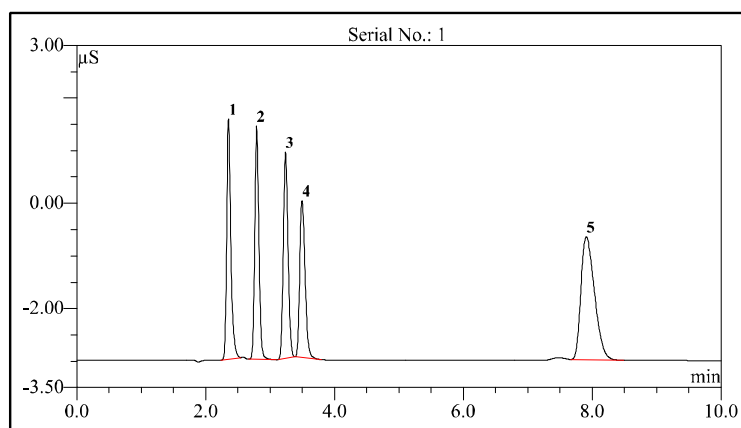
When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each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

- C. For aqueous cleaning solutions; set the pump flow rate to 1.0 mL/min for a Dionex IonPac 4 mm Analytical or Guard Column or 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 to 0.5 mL/min for a Dionex IonPac 4 mm Analytical or Guard Column or 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 Dionex 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. Appendix B – Quality Assurance Reports

Dionex IonPac™ AS32-Fast-4μm **Date:** 08-Feb-19 15:09
Analytical (2 x 150 mm) **Serial No. :** 1
Product No. 303153 **Lot No. :** Val lot #2
 Device Monitoring Enabled
 and Viper Fitting Ready

Eluent: 4 mM KOH
Flow Rate: 0.25 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrolytically Regenerated Suppressor (Dionex AERS™ 500 2mm)
 AutoSuppression™ Recycle Mode
Applied Current: 3 mA
Injection Volume: 2.5 μL
Storage Solution: Eluent



| No. | Peak Name | Ret.Time (min) | Asymmetry (AIA) | Resolution (EP) | Efficiency (EP) | Concentration (mg/L) |
|-----|-------------|-------------------|--------------------|--------------------|--------------------|-------------------------|
| 1 | Fluoride | 2.35 | 1.5 | 3.87 | 7299 | 1.25 |
| 2 | Bromide | 2.79 | 1.2 | 3.60 | 9235 | 5.0 |
| 3 | Iodide | 3.24 | 1.2 | 1.80 | 9279 | 10.0 |
| 4 | Perchlorate | 3.50 | 1.4 | 16.31 | 8519 | 7.5 |
| 5 | Sulfate | 7.91 | 1.7 | n.a. | 6543 | 5.0 |

QA Results:

| Analyte | Parameter | Specification | Results |
|-------------|----------------|---------------|---------|
| Perchlorate | Efficiency | >=7650 | Passed |
| Perchlorate | Asymmetry | 1.0-1.8 | Passed |
| Sulfate | Retention Time | 7.4-9.1 | Passed |
| | Pressure | <=3080 | 2038 |

Production Reference:

Datasource: Resin
 Directory: RTC\RTC_5
 Sequence: RQ_AS32_2X150_VALIDATION_MJ_HG
 Sample No.: 23

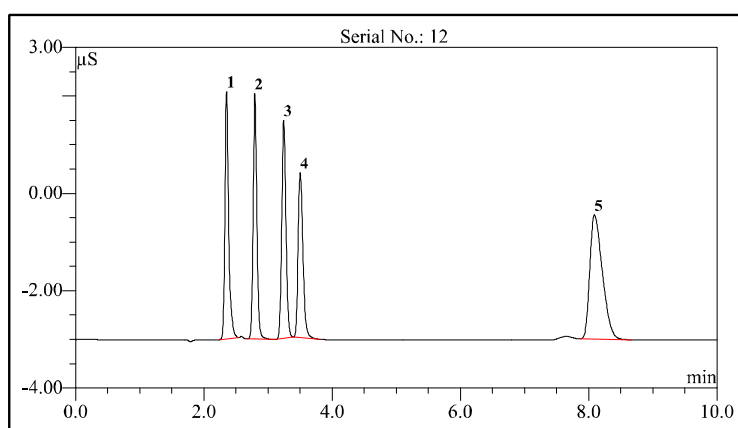
6.80 SR16 Build 5387 (291597)

Chromleon™ Thermo Fisher Scientific

7 – Appendix B – Quality Assurance Reports

Dionex IonPac™ AS32-Fast-4µm **Date:** 09-Feb-19 08:10
Analytical (4 x 150 mm) **Serial No. :** 12
Product No. 303151 **Lot No. :** Val lot #3
 Device Monitoring Enabled
 and Viper Fitting Ready

Eluent: 4 mM KOH
Flow Rate: 1.0 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrolytically Regenerated Suppressor (Dionex AERS™ 500 4mm)
 AutoSuppression™ Recycle Mode
Applied Current: 10 mA
Injection Volume: 10 µL
Storage Solution: Eluent



| No. | Peak Name | Ret.Time (min) | Asymmetry (AIA) | Resolution (EP) | Efficiency (EP) | Concentration (mg/L) |
|-----|-------------|-------------------|--------------------|--------------------|--------------------|-------------------------|
| 1 | Fluoride | 2.35 | 1.5 | 4.39 | 9173 | 1.25 |
| 2 | Bromide | 2.79 | 1.2 | 4.06 | 11810 | 5.0 |
| 3 | Iodide | 3.24 | 1.2 | 2.03 | 11837 | 10.0 |
| 4 | Perchlorate | 3.50 | 1.5 | 18.54 | 10828 | 7.5 |
| 5 | Sulfate | 8.09 | 1.9 | n.a. | 7992 | 5.0 |

QA Results:

| Analyte | Parameter | Specification | Results |
|-------------|----------------|---------------|---------|
| Perchlorate | Efficiency | >=7650 | Passed |
| Perchlorate | Asymmetry | 1.0-1.8 | Passed |
| Sulfate | Retention Time | 7.4-9.1 | Passed |
| | Pressure | <=3080 | 2586 |

Production Reference:

Datasource: Resin
 Directory: RTC\RTC_6
 Sequence: RQ_AS32_4x150_Validation_MJ_HG
 Sample No.: 27

6.80 SR16 Build 5387 (291597)

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8. Appendix C – 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 7 Tubing Back Pressures.

| Color | Part Number | I.D. inch | I.D. cm | Volume mL/ft | Back Pressure, Psi/ft. at 1mL/min | Back Pressure, Psi/ft. at 0.25mL/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.