



Dionex IonPac CS21-Fast-4 μ m

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Product Manual for

Dionex IonPac CS21-Fast-4 μ m Analytical Column

303348 IP CS21-Fast-4 μ m 2 $\times$ 150 mm

Dionex IonPac CG21-Fast-4 μ m Guard Column

303349 IP CG21-Fast-4 μ m 2 $\times$ 30 mm

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Safety and Special Notices

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

Safety and special notices include the following:



SAFETY

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



WARNING

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



CAUTION

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



NOTE

Indicates information of general interest.

IMPORTANT

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

Tip

Highlights helpful information that can make a task easier.

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1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4 μ m column is used with suppressed conductivity coupled to triple-quadrupole mass spectrometric detection for the analysis of quaternary amine cationic polar pesticides in environmental waters, and food and beverage matrices. Due to its smaller particle size, the Dionex IonPac CS21-Fast-4 μ m column offers high peak efficiencies. It is offered in a single 2 mm i.d. and 150 mm length format. Its selectivity is particularly useful in the analysis of diquat and paraquat, a challenging peak pair for many cation separator columns. The Dionex IonPac CS21-Fast-4 μ m has a large separation window between monovalent and divalent cations in order to maximize the separation window of the quaternary amine cationic polar pesticides from the matrix cations.

The Dionex IonPac CS21-Fast-4 μ m 2 mm Analytical Column has been designed specifically for the analysis of four quaternary amine cationic polar pesticides in the presence of the six common cations at diverse concentration ratios. The Dionex IonPac CS21-Fast-4 μ m stationary phase is a high-capacity weak cation exchanger functionalized with carboxylic acid groups having a high selectivity for hydronium ion. It has both cation exchange and reverse phase properties. The Dionex IonPac CS21-Fast-4 μ m is compatible with 100% aqueous eluents and 100% acetonitrile without loss of performance.

An advantage of this single column format is that the operational flow rate is matched to the optimal flow of most triple-quadrupole mass spectrometers. Thus, direct flow into the MS interface is possible without the need for make-up solvents or flow splitting.



CAUTION

Do not use primary or secondary alcohols such as methanol or n-propanol in the eluent. Formation of esters will occur in the column packing that can irreversibly reduce the column capacity. Tertiary alcohols such as isopropyl alcohol (IPA) can be used up to 20%.

The Dionex IonPac CS21-Fast-4 μ m column can be operated up to 60°C without loss of performance.

The Dionex IonPac CS21-Fast-4 μ m column can be washed with up to 1 M acid concentration. The Dionex IonPac CS21-Fast-4 μ m column should not be used with basic eluents as this could result in excessive backpressure, irreversibly disrupting the packing.

The Thermo Scientific Dionex ICS-6000 Reagent-Free™ HPIC™ system and the Thermo Scientific Dionex Integrion™ HPIC system have a maximum total pressure rating of 5,000 psi. This includes the columns and all the different components in the system. When this pressure is reached, the system will shut down to avoid damaging the components. When creating a method, it is recommended that total system pressure is below 4,200 psi.

When developing a method, it should be taken into consideration that if column temperatures below 40°C are used, the total system pressure will be higher and therefore the ability of using higher flow rates will decrease. If column temperatures below 30°C are used, it is recommended to lower the flow rate to 0.25 mL/min.

IMPORTANT

Special attention should be paid to the column specifications during the method development process. Users of a new method should expect to receive columns that are close to the specification limits from time to time. As a good practice, it would be wise either to use such columns for the method development or, at least, model the separation behavior of a given application based on the column specification parameters.

Applications: Over the years, Thermo Fisher Scientific has developed numerous polymeric cation exchange columns specifically for the determination of amines, such as the Thermo Scientific Dionex IonPac CS17, CS18, CS19, CS19-4 μ m and CS20 columns. For most amine applications it is therefore highly recommended to investigate these columns first. The Dionex IonPac CS21-Fast-4 μ m column has been designed specifically for the analysis of quaternary amine cationic polar pesticides, in particular, chlormequat, mepiquat, diquat and paraquat.

The Dionex IonPac CS21-Fast-4 μ m, like most other carboxylic acid functionalized columns, can only tolerate up to 10 mM acid in the sample. Samples of lower pH can be pretreated before injection with a Dionex OnGuard™ II A cartridge in the bicarbonate form. For most environmental waters and food and beverage matrices this limit should not pose a problem.

Read the system manuals. This manual assumes that you are familiar with the installation and operation of your Thermo Scientific Dionex Ion Chromatograph (IC). If you do not understand the operation of the system, please take the time to familiarize yourself with the various system components before beginning an analysis. All instrument manuals are available on the Thermo Scientific website, <https://www.thermofisher.com/us/en/home/industrial/chromatography/ion-chromatography-ic/ion-chromatography-columns.html>.

Table 1. Dionex IonPac CS21-Fast-4µm/CG21-Fast-4µm Packing Specifications

Column	Nominal Particle Diameter µm	Substrate	Column Capacity µeq/column	Functional Group	Hydrophobicity
Dionex IonPac CS21-Fast-4µm Analytical Column 2 × 150 mm	4.0	Macroporous	110	Carboxylic acid	Low
Dionex IonPac CG21-Fast-4µm Guard Column 2 × 30 mm	4.0	Macroporous	22	Carboxylic acid	Low

Table 2 Dionex IonPac CS21-Fast-4µm/CG21-Fast-4µm Operating Parameters for 150 mm and 30 mm formats

Column	Typical Back Pressure at Standard Flow Rate and Temperature, psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate* mL/min	Standard Temperature (°C)
Dionex IonPac CS21-Fast-4µm 2 mm Analytical Column	~ 2,500 (17.2)	0.30	0.375	40
Dionex IonPac CG21-Fast-4µm 2 mm Guard Column	~ 500 (3.4)	0.30	0.375	40
Dionex IonPac CS21-Fast-4µm + CG21-Fast-4µm 2 mm column set	~ 3,000 (20.7)	0.30	0.375	40

***NOTE: Maximum Flow Rate (mL/min) defined in this context is the maximum flow rate that the column can withstand without loss of performance.**

**NOTE**

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

2. 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 CS21-Fast-4 μ m 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

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 running 2 mm applications (Item # 302965), and for Dionex Integrion RFIC systems with conductivity detectors

(Item # 088798). A separate Dionex IC PEEK Viper fittings kit that includes fittings for IC-MS interfaces is also available (Item # 22153-62049).

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 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.

2.3 Column Start-Up

The column is shipped using 4 mM methanesulfonic acid as the storage solution. Follow the Column Start-Up instructions (see section 4.1) to flush the columns prior to running QAR. After the column has gone through Quick Start flush steps, connect the column to the suppressor 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.

If peak efficiencies or resolution are poorer than the QAR, see Section 5, Troubleshooting for information regarding possible causes and solutions.

IMPORTANT

When making any tubing connections (e.g., column installation, replacing tubing, etc.), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions.

2.4 Column Storage

For storage of the column, use 4 mM methanesulfonic acid for the column storage solution. Flush the column at its standard flow rate for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

2.5 Installing the Dionex CR-CTC Trap Column for Use with Eluent Generation

For Dionex IonPac CS21-Fast-4 μ m column applications using eluent generation, a Thermo Scientific Dionex CR-CTC III Continuously Regenerated Cation Trap Column (Item # 104-60001) should be installed at the EGC eluent outlet to remove trace level cationic contaminants such as ammonium from the carrier deionized water. See the Dionex CR-TC Product Manual (Document No. 079684) for instructions on hydration and installation.

Before operating the system with the Dionex IonPac CS21-Fast-4 μ m installed, make sure the Dionex CR-CTC III has been flushed with 50 mM MSA for at least 30 minutes, rinsed, and equilibrated.



CAUTION

The Dionex CR-CTC III Cation Trap Column must be flushed with 50 mM MSA for 30 minutes prior to first use or after long-term storage. Failure to do so may affect the operational performance of the Dionex IonPac CS21-Fast-4 μ m.

IMPORTANT

The Dionex IonPac CTC-1 Cation Trap Column cannot be used as it has a maximum operating pressure of 3000 psi.

2.6 The Injection Loop

2.6.1 The 2 mm System Injection Loop, 2 - 10 μ L

For most applications on a 2 mm analytical system, a 2 - 10 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 number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. The Dionex IonPac CS21-Fast-4 μ m column has been optimized for 10 μ L injection loops.

2.7 Dionex IonPac CG21-Fast-4 μ m Guard Column

A Dionex CG21-Fast-4 μ m Guard Column is normally used with the Dionex IonPac CS21-Fast-4 μ m Analytical Column. The Dionex IonPac CG21-Fast-4 μ m guard column is packed with the same resin as the separator column. A guard column is placed prior to the analytical column to prevent sample contaminants from eluting onto 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. Cleaning or replacing a guard column is more economical than replacing an analytical column. For maximum life of the analytical column, the guard column should be changed or replaced as part of a regular maintenance schedule or at the first sign of performance deterioration. Use the test chromatogram that is shipped with the analytical column or the initial application run as a performance benchmark.

2.8 Eluent Storage

Dionex IonPac CS21-Fast-4 μ m columns are designed to be used with acid eluent systems. If manually prepared eluents are used (instead of Electrolytically Generated), it is recommended that storage of the eluent be under a nitrogen atmosphere to ensure contamination free operation and proper pump performance (helium should be used if eluents contain organic solvents). Eluent storage bottles made of glass should be avoided as sodium contamination will occur.

2.9 Dionex Cation Dynamically Regenerated Suppressor Requirements

A Dionex Cation Dynamically Regenerated Suppressor (Dionex CDRS 600, 2 mm) should be used for all applications that require suppressed conductivity detection, including IC-MS and IC-MS/MS applications. The Dionex CDRS 600 is compatible with solvent containing eluents and aqueous ionic eluents of all concentrations with which the systems and columns are compatible. Aqueous ionic eluents can be used in all Dionex CDRS 600 modes of operation.

Depending on the % level of organic solvent present in the eluent, solvent containing eluents must be used in the External Water Mode using the Dionex CDRS 600, or Chemical Suppression Mode using the Dionex Cation Chemically Regenerated Suppressor (Dionex CCRS 500).

IMPORTANT

The Chemical Suppression Mode cannot be used with IC-MS and IC-MS/MS applications.

For detailed information on the operation of the Dionex Cation Dynamically Regenerated Suppressor see Document No. 031956, "Product Manual for the Thermo Scientific Dionex DRS

600 Suppressor”. For detailed information on the operation of the Dionex Cation Chemically Regenerated Suppressor see Document No. 031727, “Product Manual for the Thermo Scientific Dionex CRS 500 Suppressor.”

For Dionex IonPac CS21-Fast-4 μ m Analytical Column use the Dionex CDRS 600 (2 mm), P/N 088670.

3. Operation

3.1 General Operating Conditions

Column:	Dionex IonPac CS21-Fast-4 μ m Analytical Column + Dionex IonPac CG21-Fast-4 μ m Guard Column
Sample Volume:	10 μ L Loop + 0.8 μ L Injection valve dead volume
Eluent:	4 mM Methanesulfonic acid (MSA)
Eluent Flow Rate:	0.30 mL/min
Temperature:	40 °C
Dionex Electrolytic Suppressors:	Dionex Cation Dynamically Regenerated Suppressor, Dionex CDRS 600 (2 mm) AutoSuppression Recycle Mode or External Water Mode Legacy (Constant Current) Mode, 4 mA
Expected Background Conductivity:	< 0.3 μ S/cm in the suppressed mode
Storage Solution:	Eluent

3.2 Dionex IonPac CS21-Fast-4 μ m Column Operation Precautions



- *Operate below 5,000 psi (34.5 MPa).*
- *Filter and Degas Eluents.*
- *Filter Samples.*
- *Eluent pH range: 0 to 7.*
- *Organic Solvent: 100% for column clean up (acetonitrile, do not use alcohols).*
- *Maximum Flow Rate ($\geq 30^{\circ}\text{C}$): 0.375 mL/min, 0.30 mL/min recommended.*
- *Maximum Flow Rate ($< 30^{\circ}\text{C}$): 0.300 mL/min, 0.25 mL/min recommended.*
- *Maximum Flow Rate (when using organic solvents): 0.25 mL/min.*
- *Column Temperature Range: 20 to 60 °C.*

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. The QAR supplied with the column should be used as a guide to ensure system performance.

3.4 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.

3.4.1 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 μ m. Filter water with a 0.2 μ m

filter. 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.

3.4.2 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. The following chemicals will perform reliably:

- A. Use Fluka or Sigma Aldrich Methanesulfonic Acid (MSA) (>99% pure), Thermo Scientific Dionex Methanesulfonic Acid Concentrate (0.4 M) Item # 057562, or Thermo Scientific Dionex Methanesulfonic Acid (15.4 M) Item # 080388.
- B. Use deionized water with a specific resistance of 18.2 megohm-cm to make all standards and eluents.

3.4.3 Solvents

Organic solvents can be added to the ionic eluents used with Dionex IonPac CS21-Fast-4 μ m column to modify the analytes retention in the column, to improve sample solubility, or to clear the column of hydrophobic contaminants. The organic 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 organic solvents available be used. Currently, several manufacturers make ultrahigh purity organic solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity organic solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at Thermo Fisher Scientific, we have obtained consistent results using Optima® solvents by Fisher Scientific.

When using an organic solvent in an ionic eluent, column back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent, the column, the temperature, and the flow rate used. It is recommended to first add 5% solvent to the eluent and rinse the column with it at half the standard flow rate for 15 minutes. The column back pressure will vary as the composition of water-solvent mixture varies. The maximum back pressure limit for the Dionex IonPac CS21-Fast-4 μ m columns is 5,000 psi (34.47 MPa). The Dionex IonPac CS21-Fast-4 μ m column is compatible with the HPLC solvents listed in Table 4, “HPLC Solvents for Use with the Dionex CS21-Fast-4 μ m Columns.” Solvents and water should be premixed in concentrations which allow proper mixing by the pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.



NOTE

At a characteristic concentration range of organic solvent in the eluent, the column back pressure may more than double. If this is the case, you should decrease the eluent flow rate to allow use of the eluent containing solvent in this concentration range. It is recommended to reduce by half the standard flow rate for the format when the eluent contains an organic solvent.

Table 3. HPLC Solvents for Use with Dionex IonPac CS21-Fast-4 μ m Columns

Solvent	Maximum Operating Concentration
Acetonitrile	100%
Isopropyl Alcohol (IPA)	20%
Alcohols (except IPA)	0%



CAUTION

Do NOT use primary or secondary alcohols such as methanol or n-propanol as an eluent component with the Dionex IonPac CS21-Fast-4 μ m column. Formation of esters will occur in the column packing that can irreversibly reduce the column capacity. Tertiary alcohols such as Isopropyl Alcohol (IPA) can be used up to 20%. Alcohols can be present in the sample matrix.



CAUTION

The Maximum Flow Rate when using organic solvents is 0.25 mL/min.

3.5 Preparation of Eluent Stock Solution Concentrates

Methanesulfonic acid (MSA) is very corrosive and a strong irritant.



WARNING

*Avoid breathing the vapors.
Always use these reagents in a fume hood. Wear gloves and goggles.*

3.5.1 1.0 N Methanesulfonic Acid (MSA) Stock Solution

- A. 1.0 N Methanesulfonic acid stock solution can be prepared as follows:
- B. Weigh out 96.1 g of Methanesulfonic acid (MSA, > 99%, Item # 033478).
- C. Carefully add this amount to a 1L volumetric flask containing about 500 mL of deionized water.
- D. Dilute to the mark and mix thoroughly.



WARNING

Never add water to neat Methanesulfonic acid. Always add neat Methanesulfonic acid to water.

3.5.2 0.4 N Dionex Methanesulfonic Acid (MSA) Eluent Concentrate

0.4 N Dionex Methanesulfonic Acid Eluent Concentrate (Item # 057562 or package of 4, Item # 057558) is available from Thermo Scientific.

3.5.3 Sulfuric Acid Stock Solution

It is not recommended to use Sulfuric acid as an alternative to Methanesulfonic acid with the Dionex IonPac CS21-Fast-4 μ m.

3.5.4 Eluent Preparation

Eluent: X mN Methanesulfonic acid (MSA)

Using the table below, pipet X mL of the 1.0 N MSA eluent concentrate (see Section 3.5, “Preparation of Eluent Stock Solution Concentrates”) into a 1 L volumetric flask. Dilute to 1 L using deionized water with a specific resistance of 18.2 megohm-cm. Degas the eluent.

Table 4. mN Eluent from Stock Solutions

[MSA] (mN)	V (mL)
4	4.0
6	6.0
10	10.0
15	15.0
20	20.0
30	30.0
50	50.0
100	100.0

3.6 Making and Using Eluents that Contain Solvents



NOTE

When purging or degassing eluents containing organic solvents, do not purge or degas the eluent excessively since it is possible that a volatile solvent can be "boiled" off from the solution. Always degas and store all eluents in 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.

When mixing solvents with water, remember to mix solvent with water on a volume to volume basis. If a procedure requires an eluent of 10% acetonitrile, prepare the eluent by adding 100 mL of acetonitrile to an eluent reservoir. Then add 100 mL of deionized water at a time to the acetonitrile in the reservoir and fill it up to the 1 L mark. 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.

If measuring solvents gravimetrically, always remember to take the density of the solvent into consideration. i.e., weigh 78.6 g of acetonitrile to measure 100 mL.

Avoid creating high viscosity pressure fronts that may disrupt the column packing when the eluent solvent component is added or changed. To do this, equilibrate the column at **half its standard flow rate** for approximately 10 minutes with an eluent containing only 5% of the current solvent type. Exchange this eluent for an eluent with 5% of the new solvent type and then equilibrate the column and allow the system to stabilize (approximately 10 minutes). Next run a 15-minute gradient from 5% of the new solvent type to the highest percentage that will be used during the new analysis protocol. Adjust the flow rate so that it does not exceed the maximum pressure limit at any point.

Properly equilibrate the column when changing to a solvent-free eluent system after using eluents containing organic solvent. First, equilibrate the column with 5 percent of the current solvent for approximately 5 minutes. Next, run a 10-minute gradient from the eluent with 5 percent of the current solvent to the new solvent free aqueous eluent.

Depending on the % level of organic solvent present in the eluent, solvent containing eluents must be used in the External Water Mode using the Dionex CDRS 600 or Chemical Suppression Mode using the Dionex Cation Chemically Regenerated Suppressor (Dionex CCRS 500).

Consult the appropriate suppressor manual for the suppressor operation when organic solvent is present in the eluent.

4. Example Applications

4.1 Column Start-Up Procedure

4.1.1 Overview

The Thermo Scientific™ Dionex™ IonPac™ CS21-Fast-4μm column is used with suppressed conductivity coupled to triple-quadrupole mass spectrometric detection for the analysis of quaternary amine cationic polar pesticides in environmental waters, and food and beverage matrices. Conditioning of the guard and separator column bed by following Start-Up Procedure is **recommended prior to initial use and after long-term storage (>7 days)**. This Start-Up Procedure will ensure extended column lifetime and reproducibility. This procedure also prevents any storage solution or column residuals from flowing to the suppressor, extending its lifetime and expediting the system equilibration time.



WARNING

If your mobile phase composition generates back pressure in excess of the maximum operation pressure, reduce the flow rate to ensure the upstream back pressure of the column is less than the maximum operation pressure.

The maximum pressure limit for the Dionex IonPac CS21-Fast-4μm column is 5000 psi (34.5 MPa).

4.1.2 Gradient Program for Dionex IonPac CS21-Fast-4μm

- After connecting the inlet of the column to the IC system in the correct flow direction, direct the outlet of the column to waste.
- Pump eluent through the column using the conditioning flow rates and gradient program below.
- Proceed to test the column under Quality Assurance Report conditions (see Appendix B).



NOTE

If using an EGC to generate eluent, be sure the CR-CTC III is turned off during this step. If the IC system was not used for more than one week, be sure to hydrate the CR-CTC III eluent channel with DI water first and then with 50mM MSA for 30 minutes by adding a back-pressure tubing at injection valve outlet

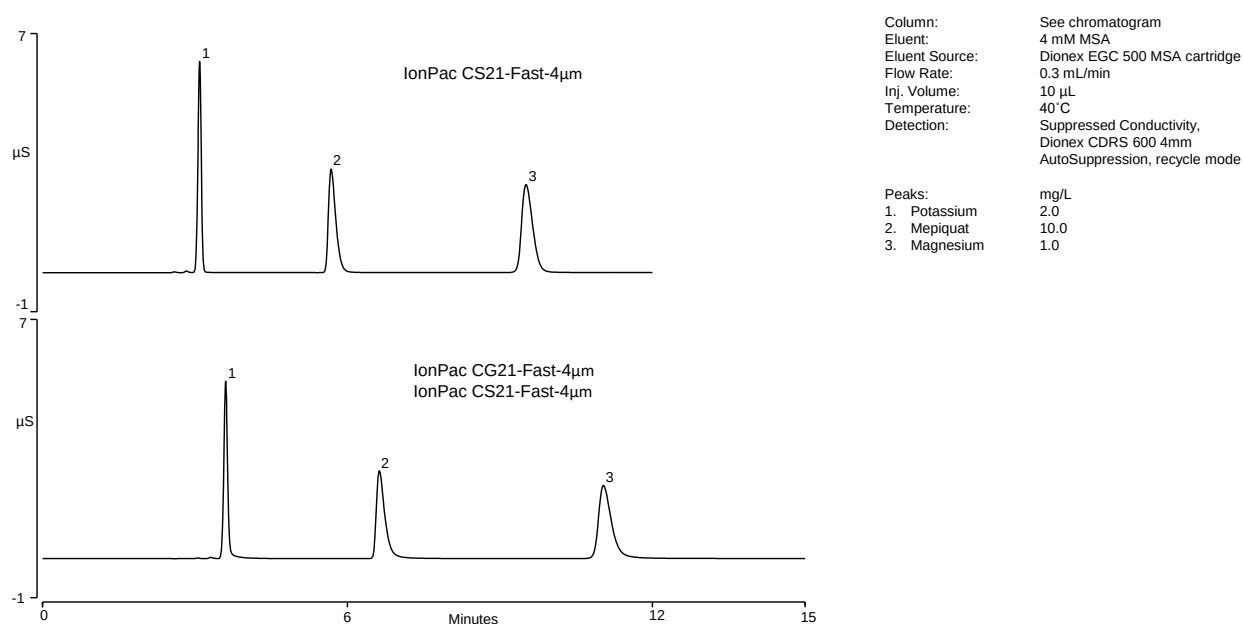
Conditioning flow rate is 0.25 mL/min.

Time	Conc. Methanesulfonic acid
0	4
5	4
10	25
30	25
35	4
40	4

4.2 Isocratic Elution of Potassium, Mepiquat, and Magnesium using the Dionex IonPac CS21-Fast-4 μ m Column with and without Dionex IonPac CG21-Fast-4 μ m Guard Column

The chromatograms below show the separation of the potassium, mepiquat, and magnesium ions using the Dionex IonPac CS21-Fast-4 μ m column with and without a Dionex IonPac CG21-Fast-4 μ m guard column. The Dionex IonPac CS21-Fast-4 μ m analytical column should always be used with the Dionex IonPac CG21-Fast-4 μ m guard column. An operating temperature of 40°C is used to ensure reproducible resolution and retention. Note that the Dionex IonPac CG21-Fast-4 μ m guard column is packed with the same resin as the Dionex IonPac CS21-Fast-4 μ m analytical column and contributes to an approximately 20% increase in retention time when placed in-line prior to the analytical column under isocratic test conditions.

Figure 1 Dionex IonPac CS21-Fast-4 μ m: Without and With Guard Column

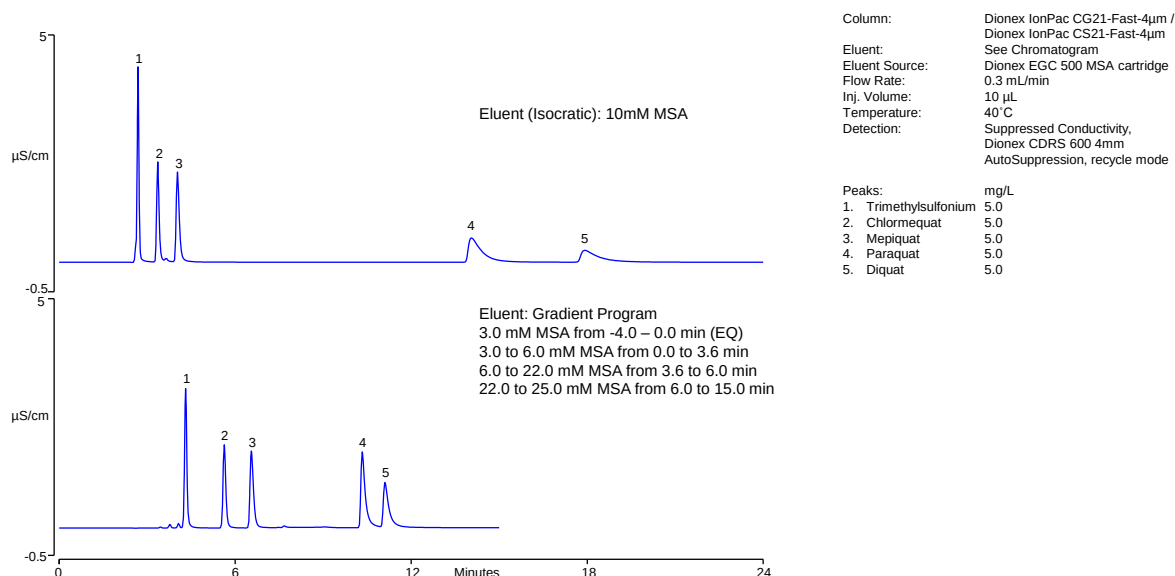


4.3 Isocratic vs. Gradient Elution of Four Quaternary Amine Cationic Polar Pesticides and Trimethylsulfonium using the Dionex IonPac CS21-Fast-4 μ m Column with Dionex IonPac CG21-Fast-4 μ m Guard Column

Figure 2 shows isocratic and gradient separations of four quaternary amine cationic polar pesticides and trimethylsulfonium using a Dionex IonPac CS21-Fast-4 μ m column with a Dionex IonPac CG21-Fast-4 μ m guard column. Note that an eluent concentration of 10 mM methanesulfonic acid is used in order to elute the paraquat and diquat ions isocratically in a reasonable amount of time as shown in top chromatogram of Figure 2.

In order to improve the analysis speed further, gradient elution is highly recommended. The bottom chromatogram of Figure 2 shows the effect of a gradient eluent profile on the four quaternary amine cationic polar pesticides and trimethylsulfonium using a Dionex IonPac CS21-Fast-4 μ m column set.

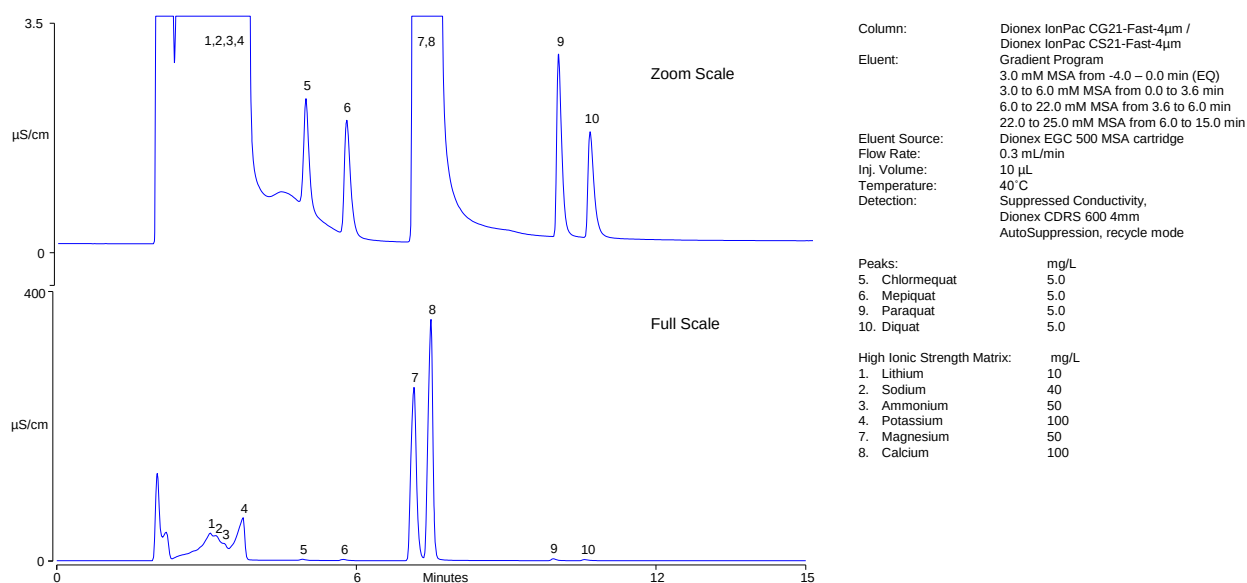
Figure 2 Isocratic vs. Gradient Elution of Four Quaternary Amine Cationic Polar Pesticides and Trimethylsulfonium Using the Dionex IonPac CS21-Fast-4 μ m Column Set



4.4 Gradient Elution of Four Quaternary Amine Cationic Polar Pesticides in a High Ionic Strength Matrix using the Dionex IonPac CS21-Fast-4 μ m Column with Dionex IonPac CG21-Fast-4 μ m Guard Column

Using gradient elution can improve the resolution between the matrix ions and ions of interest. Figure 3 shows the effect of a gradient eluent profile on the four quaternary amine cationic polar pesticides in the presence of a high ionic strength matrix using a Dionex IonPac CS21-Fast-4 μ m column set.

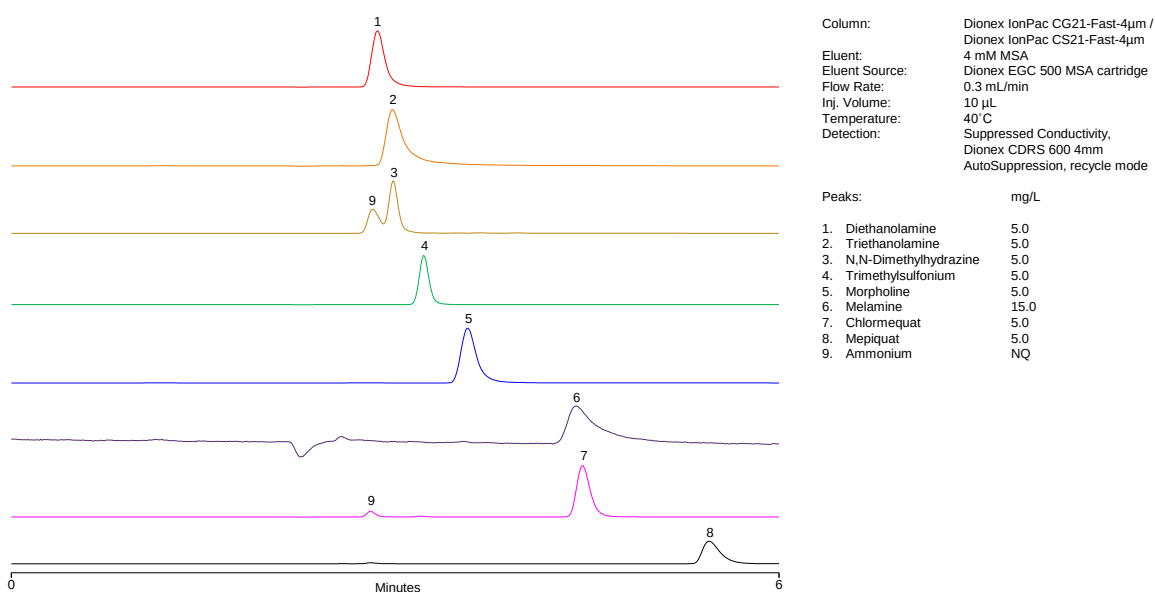
Figure 3 Separation of Four Quaternary Amine Cationic Polar Pesticides in the Presence of a High Ionic Strength Matrix Using CS21-Fast-4 μ m Column Set and Gradient Elution



4.5 Isocratic Elution of Eight Cationic Polar Pesticide Compounds of Interest using a Dionex IonPac CS21-Fast-4 μ m Column with a Dionex IonPac CG21-Fast-4 μ m Guard Column

Figure 4 shows isocratic separations of eight cationic polar pesticide compounds of interest using a Dionex IonPac CS21-Fast-4 μ m column with a Dionex IonPac CG21-Fast-4 μ m guard column. Under isocratic conditions di- and poly-valent cations of interest are not eluted from the Dionex IonPac CS21-Fast-4 μ m column in a reasonable time. The chromatogram below shows the cations of interest that can be eluted in a reasonable amount of time under isocratic conditions. Note that potassium elutes immediately before trimethylsulfonium, making analysis of diethanolamine, triethanolamine, and N,N-dimethylhydrazine in the presence of potassium challenging with this column.

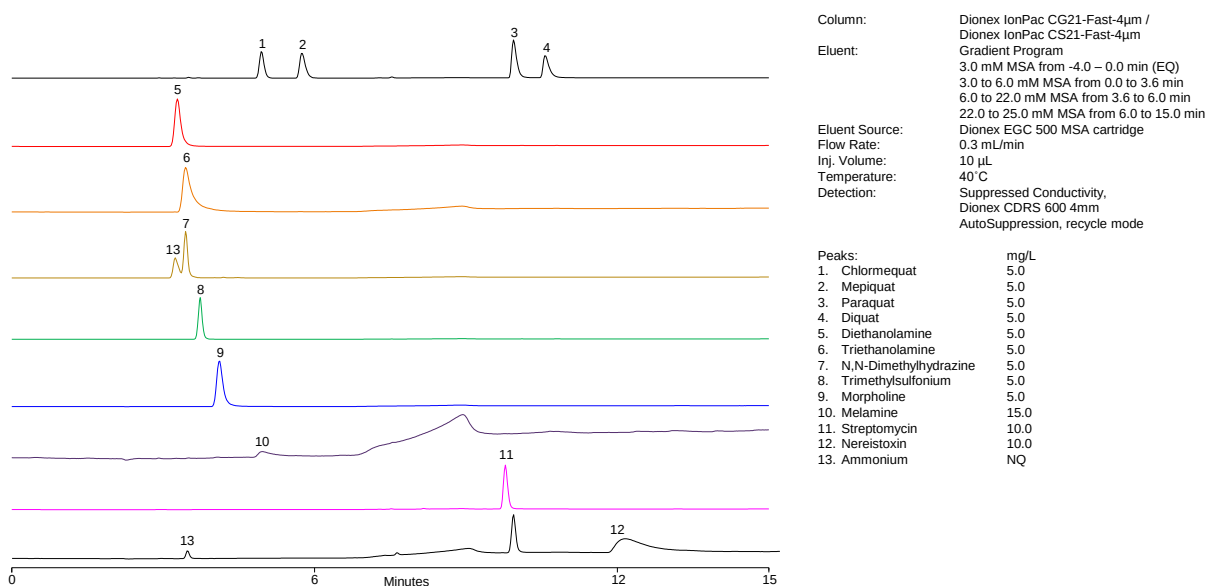
Figure 4 Isocratic Elution of Eight Cationic Polar Pesticide Compounds of Interest Using a Dionex IonPac CS21-Fast-4 μ m Column Set



4.6 Gradient Elution of Four Quaternary Amine Cationic Polar Pesticides and Eight Cationic Polar Pesticide Compounds of Interest using a Dionex IonPac CS21-Fast-4 μ m Column with a Dionex IonPac CG21-Fast-4 μ m Guard Column

Figure 5 shows gradient separations of four quaternary amine cationic polar pesticides along with eight cationic polar pesticide compounds of interest using a Dionex IonPac CS21-Fast-4 μ m column with a Dionex IonPac CG21-Fast-4 μ m guard column. By using a gradient, a longer list of target compounds can be eluted from the Dionex IonPac CS21-Fast-4 μ m in a reasonable amount of time. Potassium elutes immediately before trimethylsulfonium, making the analysis of diethanolamine, triethanolamine, and N,N-dimethylhydrazine in the presence of potassium a challenge.

Figure 5 Gradient Elution of Four Quaternary Amine Cationic Polar Pesticides and Eight Cationic Polar Pesticide Compounds of Interest Using a Dionex IonPac CS21-Fast-4 μ m Column Set



4.7 Separation and Detection of Four Quaternary Amine Cationic Polar Pesticides in a High Ionic Strength Matrix using IC-MS/MS

The Dionex IonPac CS21-Fast-4 μ m provides good separation of the four quaternary amine cationic polar pesticides including chlormequat, mepiquat, paraquat, and diquat from common inorganic cations using a methanesulfonic acid eluent and triple quadrupole mass spectrometric detection. Please note that the 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 four quaternary amine cationic polar pesticides in the high ionic strength matrix is shown in Figure 6.

For this analysis, positive ion, Selected Reaction Monitoring (SRM) Mode was used with a 10 μ L injection volume. Good extracted ion chromatograms were produced from SRM Mode for all four target analytes down to single digit parts per billion levels. Two diversion windows were used, one to divert monovalent matrix cations (lithium, sodium, ammonium, and potassium) to waste, the second to divert divalent matrix cations (magnesium and calcium) to waste.

The following table can provide initial guidance on setting up time windows for matrix diversion based on the retention times of potassium and magnesium. The first diversion window begins at time $t = 0$ and ends 0.90 min after the potassium peak. The second diversion window begins 0.70 min before the magnesium peak and ends 2.10 min after the magnesium peak. These values are recommendations and should be adjusted based on the gradient delay volume of the HPIC system and the retention times of the Dionex IonPac CG21/CS21-Fast-4 μ m columns to ensure analytes of interest are not diverted to waste and/or matrix ions are not allowed into the mass spectrometer.

Table 5. Matrix Diversion Window Guidance

	Window Begin (min)	Window End (min)
Matrix Diversion Window 1	0.00	RT (Potassium) + 0.90
Analysis Window 1	RT (Potassium) + 0.90	RT (Magnesium) – 0.70
Matrix Diversion Window 2	RT (Magnesium) – 0.70	RT (Magnesium) + 2.10
Analysis Window 2	RT (Magnesium) + 2.10	15.00

Example Dionex IonPac CG21/CS21-Fast-4μm set RT (Potassium) = 3.40 RT (Magnesium) = 7.30	Window Begin (min)	Window End (min)
Matrix Diversion Window 1	0.00	3.40 + 0.90 = 4.30
Analysis Window 1	4.30	7.30 – 0.70 = 6.60
Matrix Diversion Window 2	6.60	7.30 + 2.10 = 9.40
Analysis Window 2	9.40	15.00

4.7.1 System, Conditions, and Consumables

A Thermo Scientific™ Dionex ICS-5000⁺ HPIC System with Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer was used for this example. A Thermo Scientific™ Dionex ICS-6000 or Thermo Scientific™ Dionex Integrion HPIC system can be used as the HPIC system. A Thermo Scientific™ TSQ Fortis™ or Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer can be used as the mass selective detector.

Table 6. HPIC System Conditions

Mobile Phase:	MSA (Gradient Conditions in Error! Reference source not found.)
Eluent Source:	Dionex EGC 500 MSA Dionex CR-CTC III
Analytical Column:	Dionex IonPac CS21-Fast-4µm
Guard Column:	Dionex IonPac CG21-Fast-4µm
Suppressor:	Dionex CDRS 600 (2 mm), 22 mA
External Flow Pump:	0.3 mL/min
Eluent Flow Rate:	0.3 mL/min
Injection Volume:	10 µL
Column Temperature:	40°C

Table 7. Gradient Conditions

Time (min)	Concentration of MSA (mM)
-4.0	3.0
0.0	3.0
3.6	6.0
6.0	22.0
15.0	25.0

Table 8. Mass Spectrometer Global Parameters

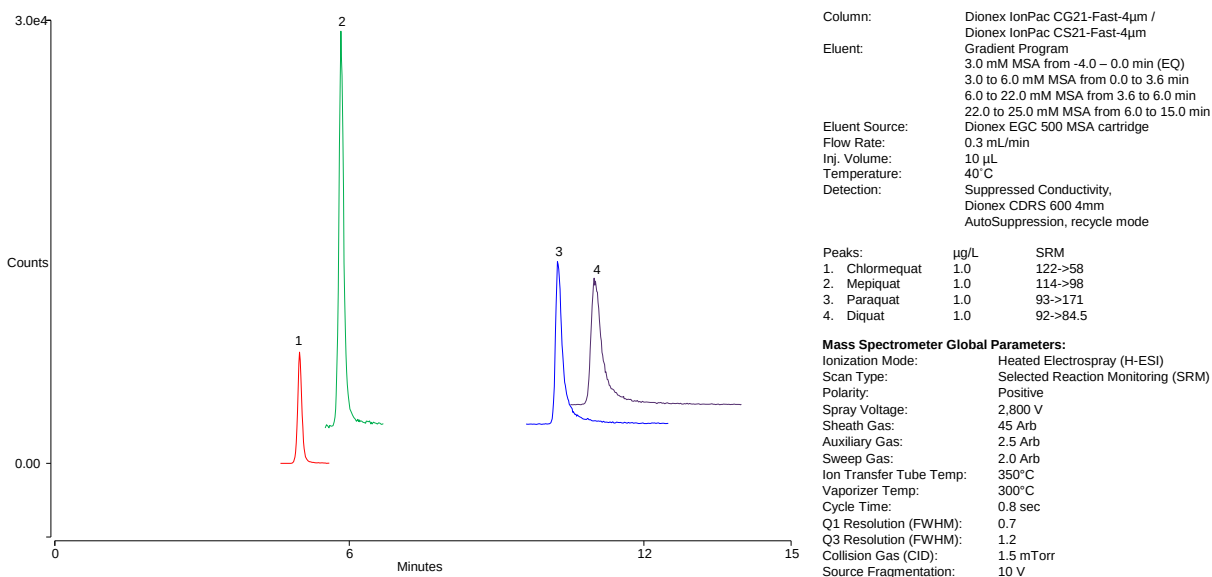
Ionization Mode:	Heated Electrospray (H-ESI)
Scan Type:	Selected Reaction Monitoring (SRM)
Polarity:	Positive
Spray Voltage:	2,800 V
Sheath Gas:	45 Arb
Auxiliary Gas:	2.5 Arb
Sweep Gas:	2.0 Arb
Ion Transfer Tube Temp:	350°C
Vaporizer Temp:	300°C
Cycle Time:	0.8 sec
Q1 Resolution (FWHM):	0.7
Q3 Resolution (FWHM):	1.2
Collision Gas (CID):	1.5 mTorr
Source Fragmentation:	10 V

Table 9. IC-MS/MS Parameters for Selected Reaction Monitoring Transitions

Compound	Analyze Time (min)	Transition Type	Precursor (m/z)	Product (m/z)	Collision Energy (V)
Chlormequat	4.3 – 5.5	Quantifier	122.1	57.9	30
		Qualifier	122.1	62.9	30
Mepiquat	5.0 – 6.1	Quantifier	114.1	98.1	30
		Qualifier	114.1	58.0	30
Paraquat	9.4 – 11.0	Quantifier	93.0	171.0	19
		Qualifier	93.0	85.0	19
Diquat	10.0 – 12.0	Quantifier	92.0	84.5	19
		Qualifier	92.0	157.1	19

4.7.2 Example Chromatogram

Figure 6 Analysis of Four Quaternary Amine Cationic Polar Pesticides in the Presence of a High Ionic Strength Matrix Using the Dionex IonPac CS21-Fast-4µm Column Set and IC-MS/MS



4.7.3 System Setup

The setup described in this section provides general guidance on 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) or Dionex IC PEEK Viper tubing (0.007" or 0.005" ID) should be used for all chromatographic connections. The Dionex CR-CTC III is installed between the eluent generator and the injection valve as described in the Dionex CR-CTC 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 Figures 7 and 8 below, 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, see Figure 7. For diversion, the suppressor is operated in the recycled eluent mode, see Figure 8. 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 7 IC-MS/MS System in Analyze Mode (Flow from IC enters MS; Flow from Auxiliary Pump enters Suppressor and CR-TC Regen path)

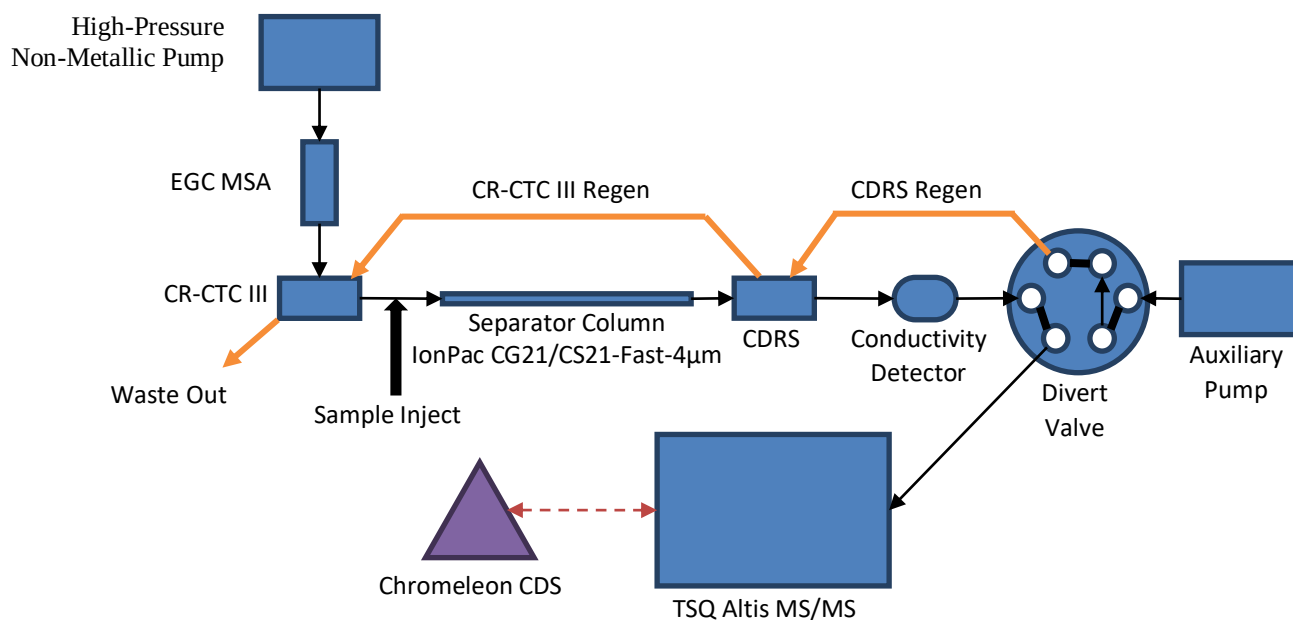
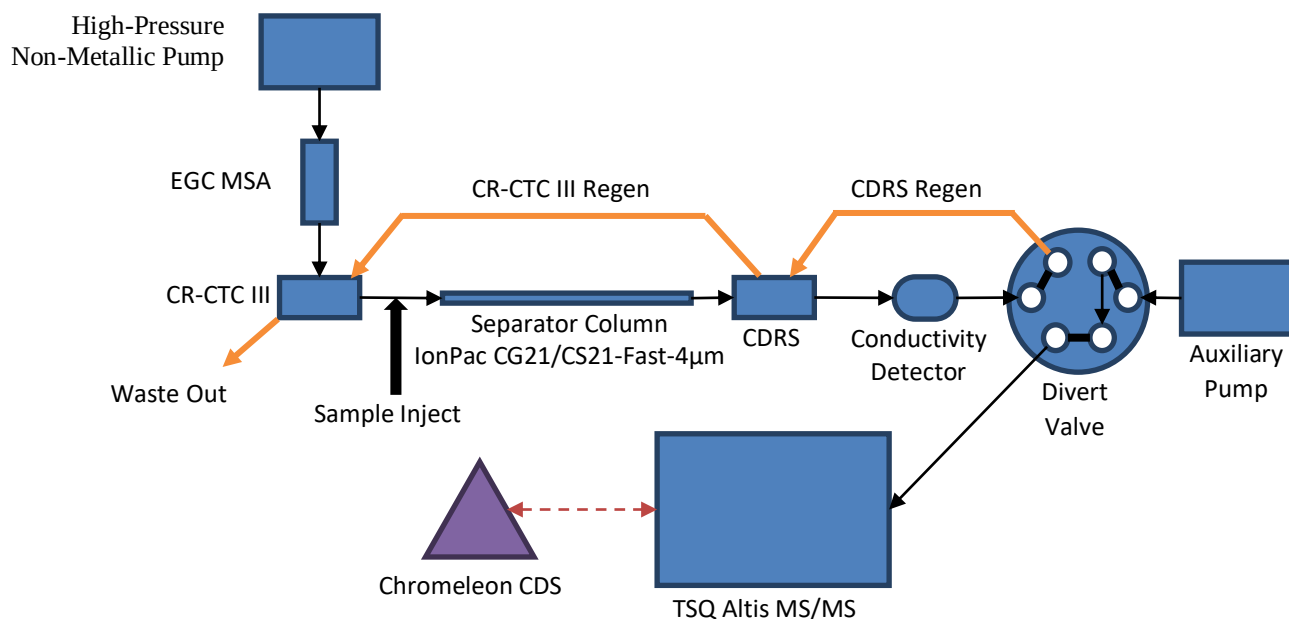


Figure 8 IC-MS/MS System in Divert Mode (Flow from IC is diverted away from MS to Suppressor and CR-CTC Regen path; Flow from Auxiliary Pump enters MS)



During target analyte elution, the 6-port valve is set to Analyze Mode (Figure 7), where flow from the conductivity cell is directed to the Mass Spectrometer for gas-phase ionization and analysis. When matrix ions such as sodium, potassium, magnesium, and calcium elute, the valve is switched to Divert Mode (Figure 8) 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 many samples). Poor sensitivity and poor recovery may result due to ion-suppression in the mass spectrometer 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 performance if excessive contamination occurs. Note that the divert valve should not be switched to Analyze Mode (Figure 7) until the background conductivity is below 3 $\mu\text{S}/\text{cm}$.



NOTE

If a Dionex ICS-5000⁺ or Dionex ICS-6000 is used, a high pressure 6-port valve in the Automation Manager (AM) module can be used for matrix diversion. The stainless steel 6-port valve on the mass spectrometer should not be used.

Auxiliary DI water is directed to the mass spectrometer during matrix diversion to keep the mass spectrometer equilibrated. During analysis, the auxiliary DI water provides flow to the suppressor and CR-CTC III. If a Dionex ICS-5000⁺ or Dionex ICS-6000 with a DP pump module is used, the second pump can be used as the auxiliary pump to provide the auxiliary flow. Otherwise, a dedicated pump such as a Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump is used for this purpose. The flow of auxiliary DI water is 0.3 mL/min in the IC-MS/MS example above.



CAUTION

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 a second ground.

5. Troubleshooting

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex IonPac CS21-Fast-4 μ m and Dionex IonPac CG21-Fast-4 μ m columns. For more information on problems that originate with the Ion Chromatograph (IC) or suppressor, refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact technical support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

Table 10. Dionex IonPac CS21-4 μ m/CG21-4 μ m Column Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown component	Isolate blockage	6.1.1
	Plugged column bed supports	Replace bed supports	6.1.2
	Plugged system hardware	Unplug, Replace	Component manual
High Background Conductivity and/or High Noise	Dionex CDRS 600 or Dionex CERS 500 not suppressing	Check current	6.5, Component manual
	Improper Suppressor Operation	Check REGEN OUT flow	6.5 D, Component manual
		Check for leaks	6.5 B, Component manual
Contamination	Air bubble trapped in suppressor	Remove bubble by loosening fittings	6.4
	Bad eluents	Remake eluents	6.2, 6.4, 6.7 A
	Contaminated column	Clean column	6.3.2, Appendix A
Hardware Operation Poor Efficiency	Contaminated suppressor	Clean suppressor	6.3.1, Component manual
	Proportioning valve	Service valve	Component manual
	Large system void volumes	Replumb system	6.6.1 A, Component manual
	Sluggish injection valve	Service valve	6.6.3 B, Component manual
	Column dehydrated	Hydrate column	Appendix A
	Contaminated or deformed bed support	Replace bed support	6.1.2
	Column headspace	Replace column	6.6.1 B
	Column overloading	Reduce sample size	34
Fronting Peaks	Low sample pH	Reduce sample size, Dilute Sample, Use Dionex OnGuard II A	3.4, 1.0, Dionex OnGuard II A manual
	Column overload	Reduce sample size	3.4
	Contaminated or deformed bed support	Replace bed support	6.1.2
	Column headspace	Replace column	6.6.1 B
Tailing Peaks	Contaminated suppressor	Clean suppressor	6.3.1, Component Manual
	Column overloading	Reduce sample size	3.4
	Sluggish injection valve	Service valve	6.6.3 B, Component Manual
	Contaminated sample loop	Replace loop	6.3.3
Short Retention Times	Flow rate too fast	Recalibrate pump	6.6.2 A, Component Manual
	First peaks elute too fast	Equilibrate to first eluent	6.6.3 A
	Bad eluents	Remake eluents	6.6.2 B
	Column contamination	Clean column	6.6.2 C, 6.3.2, Appendix A
Spurious Peaks	Eluent contamination	Remake eluents	6.7 A, 6.2, 6.4
	Column contamination	Clean Column	6.3.2, 6.7 B, Appendix A
	Sluggish injection valve	Service valve	6.7 C, Component Manual
Poor Quantification of Divalents	Sample loop contamination	Flush, replace	6.3.3
	Suppressor Contamination	Clean Suppressor	6.3.1, Component Manual
	Column Contamination	Clean, replace	5.6.4

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex IonPac CG21-Fast-4 μ m Guard Column plus the Dionex IonPac CS21-Fast-4 μ m Analytical Column when using the test chromatogram conditions should be as indicated in Table 2. If the system pressure is approximately 700 psi higher than this, it is advisable to determine the cause of the high system pressure.

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

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the EGC Cartridge and turn the pump on at its standard flow rate (0.30 mL/min). Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding system components (EGC cartridge, CR-CTC III, RFIC Eluent Degasser, 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 (see Table 2 and Table 3 for the Typical Operating Back Pressures).

The Dionex EGC 500 cartridge may add up to 200 psi (1.38 MPa) and Cation Dynamically Regenerated Suppressor 600 (CDRS 600) may add up to 100 psi (0.69 MPa) of back pressure. 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.

- C. **Make sure your system does not have extra tubing** to increase the back pressure (as needed for the eluent generator to work properly with a low-pressure column), left over from a previous set up.



NOTE

Ensure that any backpressure coils added to a system for a preceding method or consumable conditioning are removed prior to operation of the Dionex IonPac CS21-Fast-4 μ m.

5.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. If the bed support is contaminated and/or deformed, it may be the cause of poor efficiency and/or poor peak shape. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

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



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.

Part

**Dionex IonPac
CG/CS21-Fast-4µm
(Item #)**

Bed Support Assembly
End Fitting

044689
043278

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



NOTE

Replace the outlet bed support ONLY if high pressure persists after replacement of the inlet fitting. If this has to be replaced, a Zitex membrane (Item # 063167) should be placed between the resin bed and the outlet bed support.

5.2 Preparation of Eluents

- A. Make sure that the eluents and 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.

5.3 Contamination

5.3.1 Suppressor Contamination

A contaminated suppressor could be a cause for high background conductivity due to inadequate eluent suppression, as well as a cause for poor divalent peak efficiencies and high divalent peak asymmetries (i.e., tailing peaks). If tailing peaks are observed, test and clean the suppressor.

- A. Testing if the suppressor has been contaminated and is the source of poor divalent peaks chromatography:
 - 1. Modify the QAR test chromatogram stated conditions for the Dionex IonPac CS21-Fast-4 μ m column so that the suppressor current applied is half of what is stated in the QAR.
 - 2. Without much delay, inject the QAR standard for the Dionex IonPac CS21-Fast-4 μ m.
 - 3. As soon as the three cations have eluted, repeat the injection under these new conditions. Save the data. If you leave the suppressor too long with this reduced current, the background conductivity will start increasing as the lower current is insufficient to regenerate the suppressor.
 - 4. Increase the suppressor current to what is stated in the QAR test chromatogram.
 - 5. Inject the QAR standard for the Dionex IonPac CS21-Fast-4 μ m.
 - 6. If the peak efficiencies and asymmetries for magnesium are worse in step 5 than in step 3, this is an indication that the source is a contaminated suppressor.
- B. Cleaning the suppressor:
 - 1. Remove the suspected suppressor from the system.
 - 2. With a piece of tubing, connect the Eluent In port of the suppressor to its Regen OUT port.
 - 3. Connect the Regen In port to a waste line.
 - 4. Connect the Eluent OUT port to a pump with 0.5 M NaOH eluent. It is highly recommended to use a different pump than the analytical pump you are using for the cation analysis.
 - 5. For 2 mm suppressors, pump 0.5 M NaOH eluent for at least one hour at 0.25 mL/min. After cleaning the suppressor with 0.5 M NaOH, be sure to prime and rinse the pump with deionized (DI) water for 10-15 minutes in order to avoid issues with pump seals when using 0.5 M NaOH as a cleaning eluent.
 - 6. Rinse the suppressor by pumping DI water for 30 minutes at the same flow rate.
 - 7. The suppressor is now ready to be re-installed and used.

5.3.2 A Contaminated Guard or Analytical Column

Determine if the column is contaminated. Column contamination can lead to a loss of column capacity since all of the cation exchange sites will no longer be available for the sample ions. Polyvalent cations may be concentrating on the column over a series of runs. Remove the Dionex IonPac CG21-Fast-4 μ m and Dionex IonPac CS21-Fast-4 μ m columns from the system. If the background conductivity decreases, the columns are the cause of the high background conductivity. Clean or replace the guard column at the first sign of column performance degradation (compared to the original QAR) to eliminate downtime. Clean the column(s) as instructed in, “Column Cleanup” (See Appendix A, “Column Care”). To make sure that contaminated hardware is not causing the high background, use deionized water with a specific resistance of 18.2 megohm-cm as eluent. The background should be less than 0.5 μ S/cm. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

- A. **Check for a contaminated Gradient Mixer.** Gradient Mixers in the Gradient Pump Module should be flushed thoroughly to remove any contaminant.
- B. **Use chemicals and deionized water of the proper purity.** 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.
- C. **The system should be as metal-free as possible.** Use only IC systems with a chemically inert, metal-free flow path such as the Dionex ICS-6000 or Dionex Integrion HPIC systems. All high-pressure tubing connections should be made with either PEEK tubing or Dionex IC PEEK Viper fittings.
- D. **Glass eluent reservoirs can be a source of sodium contamination in the eluent.** Two-liter polyethylene eluent reservoirs (Item # 039163) are preferred.
- E. **For EG operation, use a Dionex CR-CTC III Trap Column.** Install a Dionex CR-CTC III Cation Trap Column (Item # 104-60001) if using an Eluent Generator with Dionex EGC 500 MSA cartridge.

5.3.3 Sample Loop and/or Tubing Contamination

Eluents made with deionized water that is contaminated with bacteria and samples such as humic acids and soil extracts can potentially contaminate eluent lines and sample loops. Weak cation exchange sites are created on (or attached to) the tubing. This can happen to either Tefzel or PEEK tubing. Thus, the sample loop itself can act as a concentrator and depending on the pH of the sample or the standard and the way it is introduced; inaccurate readings for divalent analytes on weak cation exchange resins may be observed.

A. Weak Cation Exchangers

Carboxylated resins (used in Thermo Scientific Dionex IonPac CS12, CS12A, CS14, CS15, CS21, CS17, CS18, CS19, CS19-4 μ m, and CS21-Fast-4 μ m columns) are weak acid cation exchangers. These resins have high selectivity for hydronium ion and are used with weak acid eluents. When the sample pH is high (pH 5), the weak cation exchange sites on the contaminated tubing are ionized and divalent cations are preferentially retained. When the sample pH is low (< pH 4), these sites are protonated by the sample and rendered inactive, so that the divalent quantification is not affected.

B. Testing for Loop Contamination when Using Carboxylated Cation Exchange Columns

A simple test can be performed when using a column such as the Dionex IonPac CS21-Fast-4 μ m which contains a carboxylated resin with Methanesulfonic acid to see if the sample loop has been contaminated:

1. Prepare a standard containing 0.5 ppm of calcium and add a small amount of 0.2 mM sodium hydroxide so that the final pH of the standard is between 6.5 and 7.5.
2. With the sample loop in the load position, flush the loop with just enough standard to rinse and fill the loop (e.g., if the loop is 10 μ L, flush it with 30 – 40 μ L).
3. Run the standard and record the peak area.
4. Repeat steps 2 and 3, but this time flush the loop with about 5 mL of standard.
5. If after repeating steps 2 through 4, the peak area for calcium recorded in 4 is significantly larger than that in 3, then the sample loop is contaminated and acting as a concentrator.
6. Replace the sample loop with new tubing and repeat this test.
7. If there is still a quantification problem, check other components of the system (tubing, injection valve, detector cell) or call your Dionex Products representative.

If you have a divalent quantification problem in your system but you neither have the time nor replacement parts, you can still get accurate results for divalent cations if any one of the following applies:

1. Your application involves high levels of divalent cations (e.g., > 5 ppm calcium); the “concentration error” is small, percentage-wise.
2. The pH of your samples and standards is < 4.
3. A constant volume of sample (and standard), only slightly larger than the sample loop, is flushed through the loop at a constant sampling flow rate.

5.4 High Background or Noise

In a properly working system, the background conductivity using the operating conditions described in Section 3, “Operation,” should be $< 0.3 \mu\text{S}/\text{cm}$ with a Dionex suppressor. If the background is low but the system is noisy, an air bubble may be trapped in the suppressor. With the system running, disconnect the **ELUENT OUT** line from the suppressor and apply pressure to the open port with your gloved finger to dislodge a suspected bubble. Reconnect the line. Do not take too long to do this, as the current is still being applied to the Dionex suppressor and the eluent flow is needed to produce regenerant.

- A. Check the conductivity flow cell for bubbles. See the conductivity detector manual for details.
A system with a high background ($> 0.5 \mu\text{S}/\text{cm}$) will probably also have high noise, resulting in increased detection limits.
- B. Make sure that the eluents and regenerant are prepared correctly (see Section 5.2, “Eluent Preparation”).
- C. Determine if the columns or system are contaminated (see Section 5.3, “Contamination”).
- D. Determine if the Suppressor is the cause of the high background and/or noise. If the above items have been checked and the problem persists, the suppression system is causing the problem. See Section 5.5, “Suppressor Not Suppressing Properly.”

Typical background conductivity levels, in a properly working system, are shown below:

<u>ELUENT</u>	<u>EXPECTED BACKGROUND CONDUCTIVITY</u>
4 mM Methanesulfonic acid	$< 0.3 \mu\text{S}/\text{cm}$
20 mM Methanesulfonic acid	$< 0.5 \mu\text{S}/\text{cm}$
50 mM Methanesulfonic acid	$< 1 \mu\text{S}/\text{cm}$

5.5 Suppressor Not Suppressing Properly

If the Dionex Cation Dynamically Regenerated Suppressor, or Dionex Cation Chemically Regenerated Suppressor is causing the problem, refer to the product manual for detailed troubleshooting assistance.

- A. **Check that the Dionex CDRS 600 suppressor is not in an alarm state.**
- B. **Check for Dionex CDRS 600 suppressor leaks.**
- C. **Make sure that the correct back pressure tubing is properly installed after the Dionex CDRS 600 suppressor.**
- D. **Check the regenerant flow rate at the REGEN OUT port of the Dionex CDRS 600 suppressor.** Turn the power to the Dionex CDRS 600 suppressor off. Measure the regenerant flow rate. If it is being used in the recycle mode, it should be the same flow rate as the eluent (0.30 mL/min for the Dionex IonPac CS21-Fast-4 μ m). For IC-MS applications, the Dionex CDRS 600 suppressor is typically used in the AutoSuppression External Water Mode, and the regenerant flow rate should be matched to the eluent flow rate.
- E. **Check the eluent flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol.** Measure the eluent flow rate after the column using a stopwatch and graduated cylinder. Refer to the appropriate suppressor product manual for assistance in determining if the eluent is within suppressible limits.



NOTE

Do not recycle the regenerant through the Dionex Cation Regenerant Cartridge if the eluent contains acetonitrile.

- F. Non-linear response or loss of sensitivity. Indications of carbonate contamination are:
 1. A higher ammonium peak than should be expected.
 2. Dips on either side of an analyte peak's base.

Non-linear response or loss of sensitivity may occur when the suppressor is contaminated with carbonate. This contamination is possibly from dissolved carbon dioxide in the DI water. Degassing will help minimize the presence of carbon dioxide in acidic eluents or in DI water. When pressurizing eluent reservoirs on the system, use inert gases such as nitrogen (aqueous applications) or helium.

When the Dionex CDRS 600 suppressor is contaminated with carbonate, the following treatment is recommended:

1. Push 5 mL of 2 M NaOH (freshly prepared) through the ELUENT IN port and divert a line from the ELUENT OUT port to waste.
2. Push 10 mL of 2 M NaOH (freshly prepared) through the REGEN IN port and divert a line out from the REGEN OUT port to waste.
3. Allow the suppressor to equilibrate for 20 minutes.
4. Repeat steps 1 and 2 with degassed DI water and reinstall the unit on the system.
5. If problem persists repeat steps 1–4.

5.6 Poor Peak Resolution

Poor peak resolution can be due any or all the following factors.

5.6.1 Loss of Peak Efficiency throughout the Chromatogram

- A. **Extra-column effects can result in sample band dispersion, causing loss of peak efficiencies.** Make sure you are using PEEK tubing with an i.d. of no greater than 0.005" (PEEK tubing) or 0.007" (Dionex IC PEEK Viper tubing) to make all eluent liquid line connections between the injection valve and the detector cell inlet. Cut the tubing lengths as short as possible. Check for leaks.
- B. **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 submitting it to high pressures, high flow rates, abrupt changes in eluent composition (such as high ionic strength or solvents), or improper storage such as storing it without column plugs inserted. Be careful not to disturb the resin bed, remove the column's top end fitting (see 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, and the column must be replaced.

5.6.2 Loss of Resolution throughout the Chromatogram Due to Shortened Retention Times

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

- A. **Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol.** Measure the eluent flow rate after the column using a stopwatch and graduated cylinder.
- B. **Check to see if the eluent compositions and concentrations are correct.** An eluent with higher than intended concentration will cause the peaks to elute faster. Prepare fresh eluent. If you are using a gradient pump to proportion the eluent components from two or three different eluent reservoirs, the resulting eluent composition may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.
- C. **Column contamination can lead to a loss of column capacity.** This is because all the cation exchange sites will no longer be available for the sample ions. For example, polyvalent cations from the sample or metals may concentrate on the column. Refer to, “Column Cleanup” (see Appendix A, “Column Care”), for recommended column cleanup procedures.

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

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

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific Office.

5.6.3 Loss of Early Eluting Peak Resolution

Lack of equilibration to the initial eluent or improperly swept out void volumes are usually the cause of poor resolution or efficiency of peaks eluting near the system void volume compared to the later eluting peaks.

- A. **Be sure that the column is equilibrated to the initial eluent.** Typically, gradient applications require approximately 4 – 5 minutes (at standard flow rate conditions for the column format) to equilibrate to the initial eluent. The minimum equilibration time can be determined by making successive runs with increasing equilibration times. The column is equilibrated to the initial eluent when additional equilibration time does not increase the runtime of the first eluting peaks.
- B. **Sluggish operation of the injection valve may be the problem.** Refer to the valve manual for instructions.
- C. **Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem.** Swap components, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change.

5.6.4 Loss of Peak Efficiency or Excessive Asymmetry for Divalent Cations only

If only the divalent cations (i.e., magnesium, calcium, paraquat, and diquat) are displaying loss of efficiency while the monovalent cations (i.e., sodium, potassium, chlormequat, and mepiquat) are not, this may indicate contamination of the cation exchange resin with a negatively charged organic contaminant.

- A. **In this scenario it is possible that the column contamination can lead to a decrease in column performance for divalent cations.** This is because the negatively charged contaminant acts as a cation exchanger that is selective for divalent cations. Refer to “Column Cleanup” (see Appendix A, “Column Care”, “Hydrophobic Cations and Organic Contaminants”) for recommended column cleanup procedures.

5.7 Spurious Peaks

- A. **Eluents made with chemicals lacking the required purity will contaminate columns rapidly.** Remake all stock solutions and eluents using chemicals that meet the chemical requirements specified in Section 3.4, “Chemical Purity Requirements.” Clean the column as indicated in “Column Cleanup” (see Appendix A, “Column Care”).
- B. **Spurious peaks may be due to column contamination.** If the samples contain an appreciable level of polyvalent cations, these cations may contaminate the column. As a result, the retention times for the analytes will decrease, and spurious, inefficient peaks can show up at unexpected times. This problem may be solved by increasing the time between analyses or by adding a regeneration step between successive runs to elute polyvalent cationic contaminants off the column before the next sample injection takes place.
- C. **An injection valve that needs service may produce baseline upsets.** This baseline upset can show up as one or multiple peaks of varying size(s) and shape(s). Typically, this will occur when the valve needs to be cleaned. Refer to the operator’s manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked if they do not interfere with the quantification of the peaks of interest.

Appendix A – Column Care

A.1 Recommended Operating 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 CS21-Fast-4 μ m Analytical or Guard Column is 5,000 psi (34.46 MPa). **Maximum Operating Flow Rates for the column (see Table 2) should never be exceeded.**



CAUTION

***Do not use primary or secondary alcohols in the eluent.
Do not use the Dionex IonPac CS21-Fast-4 μ m column with basic eluents. This can significantly increase column back pressures and disrupt the packing.***

A.2 Column Start-Up

The column is shipped using 4 mM Methanesulfonic acid as the storage solution. Prepare the eluent shown on the Column Start-Up Procedure (see Section 4.1) and follow the Column Start-Up Procedure instructions to hydrate the columns prior to running the QAR. After the column has gone through the Column Start-Up Procedure steps, connect the column to the suppressor 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.

If peak efficiencies or resolution are poorer than the QAR, see Section 5, Troubleshooting, for information regarding possible causes and solutions.

A.3 Column Storage

For storage of the column, use 4 mM Methanesulfonic acid as the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends **tightly**, using the plugs supplied with the column.

A.4 Column Cleanup

The following column cleanup protocols have been divided into two general isocratic protocols:

- I. Polyvalent cations and acid soluble contaminants or transition metals
- II. Hydrophobic cations and organic contaminants.

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

When in doubt, always use low eluent flow rate (half of standard flow rate for the particular format), and include short column 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. This intermediate low concentration step will prevent precipitation or high viscosity zones. Avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

I. Column Cleanup Procedure for Polyvalent Cations and Acid-Soluble Contaminants or Transition Metals

- A. **Prepare 500 mL of 500 mM oxalic acid** to remove transition metals such as iron or aluminum contamination.
- B. **Disconnect the Suppressor from the Dionex IonPac CS21-Fast-4 μ m Column.** If your system is configured with both a guard column and separator column, place the guard after the separator column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels.



NOTE

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

- C. **Set the pump flow rate to 0.25 mL/min.**
- D. **Rinse the column for 15 minutes with eluent** (4 mM MSA) before pumping the cleanup solution through the column.
- E. **Pump the cleanup solution** (500 mM oxalic acid) through the column for at least 60 minutes.
- F. **Rinse and equilibrate the column(s)** with 4 mM MSA eluent for at least 60 minutes before resuming normal operation (send effluent to waste).
- G. **Reconnect the Suppressor** to the Dionex IonPac CS21-Fast-4 μ m Column and place the guard column in line between the injection valve and the separator column if your system was originally configured with a guard column.
- H. **Equilibrate the system with eluent** before resuming normal operation.

II. Hydrophobic Cations and Organic Contaminants

- A. **Disconnect the column** from the injection valve and the suppressor. Disconnect the Gradient Mixer or the Dionex Cation Trap from the pump. Connect the Dionex IonPac CS21-Fast-4 μ m Column directly to the pump. Direct the effluent from the column directly to a waste container.
- B. **Set the pump flow rate to 0.15 mL/min**
- C. **Use the following gradient program** to remove hydrophobic cations and organic contaminants.

Eluent 1 (E1): 4 mM MSA
 Eluent 2 (E2): 75% Acetonitrile in deionized water

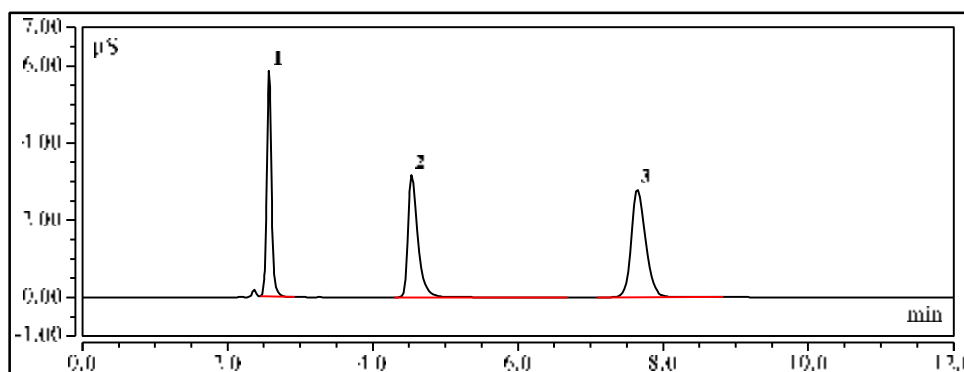
Time (min)	% E1	% E2
0.0	100	0
10.0	0	100
110.0	0	100
120.0	100	0

- D. **Rinse and equilibrate the column(s)** with 4 mM MSA eluent for at least 30 minutes before resuming normal operation.
- E. **Reconnect the Dionex IonPac CS21-Fast-4 μ m column.** Connect the column outlet to the Suppressor and the inlet to the Dionex IonPac CG21-Fast-4 μ m Guard Column or the Pump Module.
- F. **Equilibrate the column** with eluent before resuming normal operation.

Appendix B – Quality Assurance Report

Dionex IonPac™ CS21-Fast-4μm **Date:** 15-Sep-20 10:53
Analytical (2 x 150 mm) **Serial No. :** 201030654
Product No. 303348 **Lot No. :** 02005124

Eluent: 4 mM Methanesulfonic acid
Eluent Flow Rate: 0.30 mL/min
Temperature: 40 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Cation Dynamically Regenerated Suppressor (Dionex CDRS™ 600 2 mm)
 AutoSuppression™ Recycle Mode
Applied Current: 4 mA
Injection Volume: 10 μL
Storage Solution: Eluent



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration mg/L
1	Potassium	2.57	1.0	11.54	8282	2.0
2	Mepiquat	4.54	2.1	10.83	6337	10.0
3	Magnesium	7.64	1.5	n.a.	7750	1.0

QA Results:

Analyte	Parameter	Specification	Results
Potassium	Efficiency	>=6300	Passed
Magnesium	Efficiency	>=5400	Passed
Magnesium	Asymmetry	1.1-2.2	Passed
Mepiquat	Retention Time	4.16-5.15	Passed
	Pressure	<=3190	2443

Production Reference:

Datasource: QAR7
 Directory: Cation/CS21
 Sequence: CS21-Fast-4um_2X150mm
 Sample No.: 1

7.2.10.23925

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