IonPac NS1 Columns

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Thermo SCIENTIFIC

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Product Manual

for

IonPac NG1 10µm Guard Column
(4 × 50 mm, P/N 039567)
(2 × 50 mm, P/N 088763)

IonPac NS1 Analytical Column
(4 × 250 mm, P/N 035321)
(2 × 250 mm, P/N 088762)

IonPac NS1-5µm Analytical Column
(4 × 150 mm, P/N 039568)

Revision History:

Revision 07, November, 2015, Rebranded for Thermo Scientific. Added new part numbers.

Safety and Special Notices

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

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

The IonPac™ NS1 (10-micron packing) and the IonPac™ NS1-5µm (5-micron packing) analytical columns are polymer-based reversed-phase columns for the analysis of ionic and nonpolar organic compounds. The packing material is a highly cross-linked, macroporous copolymer with a very high hydrophobic surface area. The 5-micron version provides higher efficiencies than the 10-micron version. A great advantage of polymer-based packings is their chemical inertness not only to commonly used HPLC solvents but also to the full pH range from 0 to 14. Often organic analytes of interest to the chromatographer are ionized at neutral pH. Ion suppression chromatography can often be used to great advantage to control the adsorption of ionizable molecules to the column packing through eluent pH adjustments and therefore control their resulting retention times. This translates to more resolving power and greater sensitivity. The Anion ICE II MicroMembrane Suppressor (AMMS® -ICE II) is a high-capacity, low void volume dynamic eluent suppressor designed for use with ion exclusion and ion suppression separation modes of ion chromatography.

Many analytes of interest are neither ionizable nor UV detectable. Organic and inorganic anions and cations which are not UV detectable can be analyzed using Mobile Phase Ion Chromatography (MPIC®) coupled with suppressed conductivity detection. In these analyses, ionic analytes are complexed in the mobile phase with an ion pair reagent. Separations are achieved through a two fold mechanism. The first consideration is the degree of adsorption that takes place between the hydrophobic portion of the ion pair reagent and the column packing. This varies with the length of the hydrophobic portion of the ion pair reagent. The second consideration is the stability of the ion pair complex between the ion pair reagent and the analyte ion. This can be varied by the addition of salts to the eluent.

The suppressor for Anion-MPIC is the Anion Self-Regenerating Suppressor ® ULTRA II (ASRS ULTRA II), which is designed to suppress tetraalkylammonium pairing reagents. Note: the Anion MicroMembrane Suppressor (AMMS III) cannot be used with MPIC. The suppressor for Cation-MPIC is the Cation Self-Regenerating Suppressor® ULTRA II (CSRS ULTRA II), which can also be used to suppress conventional cation-exchange eluents. Both the ASRS ULTRA II and CSRS ULTRA II are compatible with typical organic solvents up to 40% by volume used in reversed-phase ion-pair chromatography. The external water mode must be used for eluents containing organic solvent.

This manual assumes that you are familiar with the installation and operation of the ThermoFisher Scientific 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.

Table 1 IonPac NS1/NG1 Packing Specifications

Column	Particle Diameter µm	Substrate X-linking %
IonPac NS1 analytical column 4 x 250 mm, , 2 x 250mm	10	55
IonPac NG1 guard column 4 x 35 mm, , 2 x 50mm	10	55
IonPac NS1-5 µm analytical column 4 x 150 mm	5	55

Table 2 NS1/NG1 Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
NS1-10 µm Analytical	900 (6.21) - 1,400 (9.66)	1.0	3.0
NG1 Guard	200 (1.38) - 300 (2.07)	1.0	3.0
NS1 + NG1 columns	**1,100 (7.59) - 1,700 (11.73)**	**1.0**	3.0
NS1-5 µm analytical	1,700 (11.73) - 2,500 (17.24)	1.0	1.5
NG1 Guard	200 (1.38) - 300 (2.07)	1.0	1.5
NS1-5 µm + NG1 columns	**1,900 (13.11) - 2,800 (19.31)**	**1.0**	1.5

2. Installation

2.1 System Requirements

The IonPac NS1 Guard and analytical columns can be run on any PEEK Chromatograph. Depending upon the application, the system should be equipped with either a conductivity detector and an ASRS ULTRA II for MPIC applications or a UV/Vis detector for reversed-phase and ion pair applications. Gradient or isocratic methods should be performed on a system having a gradient pump configured for standard bore operation. For isocratic analyses at flow rates below 0.5 mL/min and gradient analyses, a gradient pump (1/16" pistons) must be employed.

2.2 The System Injection Loop, 10 - 15 µL

For most applications on a 4-mm analytical system, a 10–50 µL injection loop will be sufficient. Thermo Fisher Scientific recommends that a 10 µL injection loop be used to avoid overloading the IonPac NS1 4-mm analytical column. Generally, do not inject more than 10 nanomoles (100–200 ppm) of any one analyte onto the 4-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. This phenomenon will be more prevalent at higher concentrations of the analytes of interest.

2.3 The IonPac Anion Trap Column

Gradient or step change applications for anion MPIC require an IonPac Anion Trap Column (ATC-3 (4-mm), P/N 037151); cation MPIC applications require an IonPac Cation Trap Column (CTC-1 (4-mm), P/N 040192). The IonPac Trap Column should be installed in place of the high pressure gradient mixer between the gradient pump and the injection valve. The IonPac Trap Column is filled with high capacity ion exchange resin which helps to minimize the baseline shift caused by increasing ionic contaminant levels in the eluent as the ionic concentration of the eluent is increased over the course of the gradient analysis.

To install the IonPac Trap Column, complete the following steps:

A. Remove the gradient mixer. It is installed between the gradient pump pressure transducer and the injection valve.
B. Connect the gradient pump directly to the IonPac Trap Column. Connect a waste line to the trap column outlet and direct the line to a waste container.
C. If you are doing anion MPIC regenerate the ATC-3. Use 200 mL of 200 mM NaOH at a flow rate of 2.0 mL/min. Note that the guard and analytical column are out of line.
D. Rinse the IonPac Trap Column with 30 mL of the strongest eluent that will be used during the gradient analysis.
E. Connect the IonPac Trap Column, after flushing it with eluent, to the eluent line that is connected to the injection valve.

At the end of each operating day, the IonPac Trap Column should be regenerated to remove any impurities that may have accumulated on it.

A. Disconnect the ATC-3 or the CTC-1. It should be installed before the injection valve.
B. Direct the outlet of the ATC-3 or the CTC-1 to a separate waste container.
C. Regenerate the ATC-3 or the CTC-1. For detail information on the operation on the ATC-3 or the CTC-1, see Document No. 032697 for the ATC-3 and Document No. 034536 for the CTC-1.

On the next day, prior to the use of the chromatographic system, rinse the IonPac Trap Column. This will help with equilibration times.

A. Rinse the TC-1 with 30 mL of the strongest eluent used in the gradient analysis.
B. Reconnect the IonPac Trap Column, after flushing it with eluent, to the eluent line that is connected to the injection valve.

2.4 The IonPac NG1 Guard Column

An IonPac NG1 Guard Column is normally used with the IonPac NS1 analytical column. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than it is an analytical column. Replacing the NG1 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the NS1 analytical column.

2.5 Eluent Storage

IonPac NS1 columns are designed to be used with Tetrabutylammonium hydroxide eluent systems. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

2.6 Anion Self-Regenerating Suppressor Requirements

The Anion Self-Regenerating Suppressor (ASRS ULTRA II) is used for eluent suppression of MPIC (ion-pairing) eluents by using the MPIC Suppression Mode of operation. This suppression mode is a combination of the AutoSuppression External Water Mode augmented with a chemical regenerant such as sulfuric acid (H_2SO_4). The MPIC Suppression Mode uses an applied current and a constant source of dilute sulfuric acid solution delivered from an AutoRegen Accessory or a pressurized bottle system. The MPIC Suppression Mode reliably provides suppression of typical eluents for MPIC applications using suppressed conductivity detection. The ion pair reagents, such as tetrabutylammonium hydroxide (TBAOH), are used in concentrations ranging typically from 1.0 to 5.0 mM. For detailed information on the operation of the ASRS ULTRA II, see Document No. 031956.

The Cation Self-Regenerating Suppressor (CSRS ULTRA II) can be used for suppression of MPIC (ion-pairing) eluents by using the AutoSuppression External Water Mode of operation or the MPIC Suppression Mode depending on the specific MPIC application. The MPIC Suppression Mode is a combination of the AutoSuppression External Water Mode augmented with a chemical regenerant if necessary such as boric acid (H3BO3). When the CSRS ULTRA II is operating in this mode, it uses an applied current and a constant source of dilute boric acid solution delivered from a pressurized bottle system. For detailed information on the operation of the CSRS ULTRA II, see Document No. 031956.

An Anion or Cation Self-Regenerating Suppressor should be used for ion pairing applications that require suppressed conductivity detection. The ASRS ULTRA II in the MPIC Suppression Mode of operation and the CSRS ULTRA II in the AutoSuppression External Water or MPIC Suppression Mode of operation are compatible with aqueous ionic eluents of all concentrations with which the column and system are compatible and with solvent containing eluents up to 40% by volume. For applications requiring solvent above 40% by volume, the Chemical Suppression Mode of operation must be used. Aqueous ionic eluents can be used in all modes of operation.

NOTE

When using eluents containing solvent above 40% by volume, the ASRS ULTRA II or CSRS ULTRA II should be used in the Chemical Suppression Mode.

If you are installing an IonPac NS1 4-mm analytical column for ion pairing chromatography with suppressed conductivity detection, use an ASRS ULTRA II, 4-mm, (P/N 061561) for Anion-MPIC or the CSRS ULTRA II, 4-mm, (P/N 061563) for Cation-MPIC.

The AMMS-ICE II (P/N 037107) is used with Ion Exclusion and Ion Suppression Modes of Ion Chromatography. For detailed information on the operation of the AMMS-ICE II, see Document No. 032661.

2.7 Detector Requirements

See Section 2, “Ion Chromatography System Operation Summary,” for 4-mm system detector, cell and thermal stabilizer requirements.

3. Operation

3.1 General Operating Conditions

Sample Volume:	4-mm: 10 µL Loop + 0.8 µL Injection valve dead volume
Column:	4-mm: IonPac NS1 4-mm analytical column + IonPac NG1 4-mm guard column
Eluent:	1–5 mM ion-pairing reagent with 1–100% solvent
Eluent Flow Rate:	1.0 mL/min
Detector:	UV or Suppressed Conductivity
Storage Solution:	Eluent

3.2 IonPac NS1 Operation Precautions

CAUTION

- ***Filter and Degas Eluents***
- ***Filter Samples***
- ***Eluent pH between 0 and 14***
- ***Sample pH between 0 and 14***
- ***3.0 mL/min Maximum Flow Rate for 4-mm Columns***
- ***Maximum Operating Pressure = 4,000 psi (27.57 MPa)***

3.3 Chemical Purity Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free from 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 reversed-phase 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.

3.3.1 Inorganic Chemicals

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

3.3.2 Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The deionized water should be free of 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.

3.3.3 Solvents

CAUTION

The IonPac NS1 and NS1-5µm column packings are spherical, highly cross-linked polymeric materials having very large hydrophobic surface areas. It is essential that these columns are operated so that the eluent being pumped over the column contains minimally 1% solvent to ensure that the hydrophobic surfaces are "wetted" and maximum column performance is maintained.

The IonPac NS1 and NS1-5µm analytical columns can withstand all common HPLC solvents in a concentration range of 1% to 100%. However, solvents and degassed water should be premixed in concentrations that allow proper mixing by the gradient pump and minimize outgassing. It is therefore more practical to say that these columns have an operational organic solvent concentration range of 1 to 95% to ensure proper chromatographic system performance.

Avoid creating high viscosity pressure fronts that may disrupt the column packing when the eluent solvent is changed. To do this, equilibrate the column for approximately 10 minutes with an eluent containing only 5% of the current solvent type (e.g., methanol). Exchange this eluent for an eluent with 5% of the new solvent type (e.g., acetonitrile) 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.

Solvents can be added to the ionic eluents used with IonPac NS1 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, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the IonPac NS1 columns is 4,000 psi (27.57 MPa).

The IonPac NS1 can withstand common HPLC solvents in a concentration range of 1–100%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

Table 3 HPLC Solvents for Use with IonPac NS1 Columns

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

3.3.4 Acid Modifiers

Mineral acids such as HCl, H_2SO_4, and HNO_3 can be used at concentrations as high as 1.0 N to acidify eluents.

3.3.5 Base Modifiers

Bases such as NaOH, KOH, and NH_4OH can be used up to 1.0 N to alkalify eluents.

3.4 Preparing Eluents that Contain Solvents

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

NOTE

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

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

Acetonitrile (ACN) hydrolyzes to ammonia and acetate when left exposed to basic solutions. To prevent eluent contamination from acetonitrile hydrolysis, always add acetonitrile to basic aqueous eluents by proportioning the acetonitrile into the basic eluent with the gradient pump. Keep the acetonitrile in a separate eluent bottle containing only acetonitrile and water. Never add the acetonitrile directly to the basic carbonate or hydroxide eluent bottle.

3.4.1 Eluents for Reversed-Phase and Ion Suppression

A typical eluent for a reversed-phase application will contain 10% to 70% solvent. Where ion suppression is advantageous, 0.02 to 10.0 mM mineral or organic acid or base is used. If ion pair reagents are used in specific reversed-phase applications, they typically are used at concentrations ranging from 1.0 to 10.0 mM. The chromatographic benefits of ion pair reagent concentrations above 10 mM are generally not significant.

3.4.2 Eluents for Gradients

Gradient applications are straightforward as long as solvents and water are premixed in concentrations that allow mixing by the gradient pump to give the required gradient ramp for your chromatography. For example, if you want to build a solvent gradient from 10% solvent to 90% solvent, make the following eluents:

Eluent A: 10% solvent/90% water

Eluent B: 90% solvent/10% water

Then, by programming the gradient pump properly, you can go from 100% Eluent A to 100% Eluent B in a prescribed time. This will avoid outgassing and refractive index problems associated with mixing neat solvents with water.

3.4.3 Eluents for Mobile Phase Ion Chromatography (MPIC)

All of the quaternary ammonium and sulfonic acid ion pair reagents can be used at any concentration for practical chromatographic purposes. Buffering these reagents for column stability is not necessary.

Typical eluents for MPIC applications using suppressed conductivity detection are very similar to those used for reversed-phase separations with respect to eluent enhancements with acids and bases, solvents and ion pair reagents. The ion pair reagents are used in concentrations ranging from 1.0 to 5.0 mM. At higher concentrations, they may be difficult to suppress during conductivity measurements.

The following Ion Pair Reagents are available from Thermo Fisher Scientific:

P/N 035360	Tetrabutylammonium hydroxide, 0.1 M TBAOH (MPIC-AR1)
P/N 035363	Tetrapropylammonium hydroxide, 0.1 M TPAOH (MPIC-AR2)
P/N 035361	Hexanesulfonic acid, 0.1 M HSA (MPIC-CR1)
P/N 035362	Octanesulfonic acid, 0.1 M OSA (MPIC-CR2)

3.5 Regenerant Preparation for the Self-Regenerating Suppressors

The ASRS ULTRA II when operated in the MPIC Suppression Mode requires a dilute sulfuric acid solution as the regenerant. For detailed information on the operation of the ASRS ULTRA II, see Document No. 031956.

The CSRS ULTRA II when operated in the AutoSuppression External Water Mode requires the use of water with a specific resistance of 10 megohm-cm or greater as the regenerant. For detailed information on the operation of the CSRS ULTRA II, see Document No. 031956.

3.6 Regenerant Preparation for the AMMS-ICE II

5 mM tetrabutylammonium hydroxide (TBAOH) is the recommended regenerant for use with the Anion-ICE II MicroMembrane Suppressor (AMMS-ICE II). Tetramethylammonium hydroxide or potassium hydroxide may be used as alternate regenerants, but cause higher background conductivity and therefore compromise total system performance. For ease of preparation and guaranteed purity, use Thermo Fisher Scientific Cation Regenerant Solution (P/N 039602). For detailed information on the operation of the AMMS-ICE II, see Document No. 032661.

3.7 Using AutoRegen and Eluents Containing Solvents

When performing Mobile Phase Ion Chromatography (MPIC) using an Anion MicroMembrane Suppressor for Mobile Phase Ion Chromatography in the analysis of anions with ion pair reagents (AR1 or AR2) or a Cation MicroMembrane Suppressor in the analysis of cations with ion pair reagents (CR1 or CR2) and a Conductivity Detector Module, Thermo Fisher Scientific recommends using an AutoRegen Accessory (P/N 039594). Typical regenerant flow rates required in MPIC analyses varies from 5 to 15 mL/min.

The use of an AutoRegen Accessory saves regenerant preparation time and reduces regenerant consumption and waste. However, when using an AutoRegen Accessory to continuously remove contaminants from the regenerant and restore the regenerant to the correct state, it is necessary to replace the regenerant on a regular basis. How often the regenerant is replaced will depend on the application and the concentration of the solvent in the eluent. Minimally, the regenerant should be replaced once a week. It is not necessary to change the AutoRegen Regenerant Cartridge until it is completely expended.

Solvent, much like the sodium ions, passes through the membrane of the MicroMembrane Suppressor from the eluent to the regenerant stream. The solvent and the sodium ions are carried from the suppressor to the regenerant reservoir by means of the regenerant stream. As the recycled regenerant is pumped through the AutoRegen Regenerant Cartridge, the sodium ions are exchanged for hydrogen ions. However, the solvent is not removed from the recycled regenerant and continues to accumulate in the recycled regenerant stream. Eventually the concentration of solvent in the recycled regenerant can cause the background conductivity to increase which can result in a noisy background. Most solvents have no effect on the cartridge lifetime.

In all cases, the ionic strength of the eluent determines the lifetime of the AutoRegen Regenerant Cartridge. However, if the eluent contains acetonitrile and is used with a cation AutoRegen Regenerant Cartridge in the AutoRegen Accessory, the acetonitrile will decompose to acetate and ammonium in the strong basic environment of the cartridge. Acetate ions will exchange onto the Cation AutoRegen Regenerant Cartridge and severely limit its lifetime. Solvents other than acetonitrile have no effect on the cartridge lifetime.

CAUTION

Acetonitrile decomposes to ammonium acetate when subjected to basic solutions such as the 0.1 M tetrabutylammonium hydroxide (TBAOH) used as the regenerant solution in the AutoRegen Cation Regenerant Cartridge. The acetate ion exchanges onto the AutoRegen Cation Regenerant Cartridge. If high levels of acetonitrile are used in the eluents, the cartridge can become expended within a few hours. A pressurized regenerant delivery system should be used as an alternative.

When replacing the recycled regenerant, the first 200 mL of the regenerant should be pumped to waste before recycling of the regenerant is started. Utilizing AutoRegen in this manner will allow the use of high regenerant flow rates with the minimum of consumption and waste.

3.7.1 ASRS ULTRA II in MPIC Suppression or Chemical Suppression Mode

To save regenerant preparation time, consumption, and waste, it is recommended that the AutoRegen Accessory (115 V ac version, P/N 039594; 230 V ac version, P/N 039608) be purchased. The AutoRegen Accessory should be equipped with an Anion AutoRegen Regenerant Cartridge (P/N 039564). For detailed information on the operation of the AutoRegen Accessory, see Document No. 032853. For detailed information on the use of the AutoRegen Regenerant Cartridge, see Document No. 032852.

However, when using an AutoRegen Accessory, it is necessary to replace the regenerant on a regular basis. How often the regenerant is replaced will depend on the application and the concentration of solvent in the eluent. Minimally, the regenerant should be replaced once a week. It is not necessary to change the AutoRegen Regenerant Cartridge until it is completely expended.

Solvent, much like the TBA + ions, passes through the membranes of the ASRS ULTRA II suppressor from the eluent into the regenerant stream. The solvent and the TBA + ions are carried from the suppressor to the regenerant reservoir by means of the

Solvent, much like the TBA + ions, passes through the membranes of the ASRS ULTRA II suppressor from the eluent into the regenerant stream. The solvent and the TBA + ions are carried from the suppressor to the regenerant reservoir by means of the regenerant stream. As the recycled regenerant is pumped through the AutoRegen Regenerant Cartridge, the TBA + ions are exchanged for hydronium ions. However, the solvent is not removed from the recycled regenerant and continues to accumulate in the recycled regenerant stream. Eventually the concentration of solvent in the recycled regenerant can cause the background conductivity to increase which can result in a noisy background. Most solvents have no affect on the cartridge lifetime. In all cases the ionic strength of the eluent determines the lifetime of the AutoRegen Regenerant Cartridge.

For ease of use and guaranteed high purity, use Thermo Fisher Scientific Anion Regenerant Concentrate (P/N 037164, 039601) to make regenerant for the ASRS ULTRA II.

3.7.2 CSRS ULTRA II in Chemical Suppression

For ease of use and guaranteed high purity, use Thermo Fisher Scientific Cation Regenerant Concentrate (P/N 039602) to make regenerant for the CSRS ULTRA II.

CAUTION

Acetonitrile is not compatible with the CSRS ULTRA II in the Chemical Suppression Mode when using an AutoRegen Accessory unit. The acetonitrile diffuses into the TBAOH regenerant, concentrates during recirculation and eventually hydrolyzes to acetate and ammonia, rapidly depleting the capacity of the AutoRegen Cation Regenerant Cartridge. If acetonitrile is used as an eluent component, a pressurized regenerant delivery bottle must be used instead of the AutoRegen Accessory.

3.8 Using Pressurized Regenerant Reservoir with the AMMS-ICE II

The operation of the AMMS-ICE II requires a constant flow of the regenerant over the membrane, in a direction that is countercurrent to the flow of the eluent.

For the best signal to noise ratio and overall performance, Thermo Fisher Scientific recommends that the Cation Regenerant Solution (P/N 039602) be pressurized with nitrogen or helium. Do not use air.

NOTE

Use nitrogen or helium to pressurize the Regenerant Reservoir.

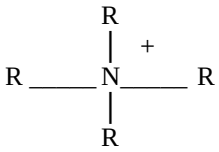
4. Example Applications

Before attempting any of the following example applications, take the time to ensure that your system is properly configured. Ensure 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. For chemical purity requirements, see Section 3.3, "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 has been determined that the guard or the analytical column has been fouled, refer to the column cleanup protocols in "Column Care."

4.1 Ion Pair Chromatography

There are a number of mechanistic hypotheses for ion pair chromatography. In all cases they involve an eluent reagent that contains a hydrophobic portion and an ionic portion on one molecule. These ion pair reagents can be anionic or cationic. Here is a partial list of typical ion pair reagents:

A. Cationic: Tetramethyl, ethyl, propyl and butyl ammonium salts of the general form:



Counter anions for these quaternary amines are chloride, bromide, phosphate and hydroxide.

B. Anionic: Alkyl sulfonates of varying chain length such as pentane, hexane, heptane and octane sulfonates of the general form:

$CH_3(CH2)_nSO_3$

Counter cations can be hydrogen ion, sodium ion and potassium ion.

From an ion exchange point of view, it may be said that the hydrophobic part of the ion pair molecule associates with the hydrophobic surface of the stationary phase. This creates an ion exchange surface that is in dynamic equilibrium with the mobile phase. The ion exchange capacity can be increased with a higher concentration of ion pair reagent in the eluent.

Inorganic and organic ions can have remarkable selectivity with this technique. The quaternary amine salt reagents make a loosely defined anion exchange stationary phase. The sulfonates create a similar cation exchange stationary phase. In both cases, the ion pair reagent itself can act as a mobile phase "pusher" for the analytes.

The following chromatograms and conditions illustrate the versatility of this method.

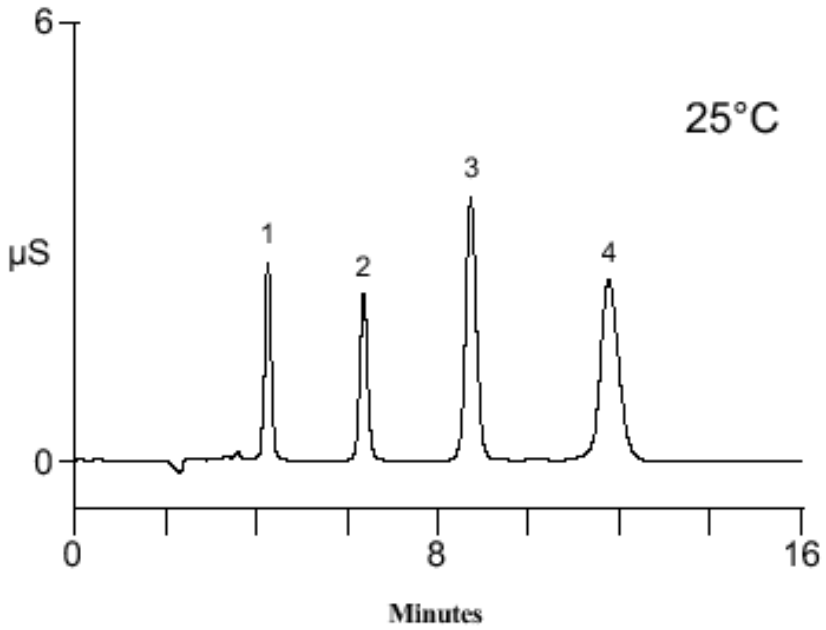
4.1.1 Production Test Chromatogram

Isocratic elution of anions on the IonPac NS1 analytical column has been optimized utilizing tetrabutylammonium ion as the ion-pairing agent and acetonitrile as the organic modifier. Using these eluent conditions, highly retained anions can be eluted from the hydrophobic packing of the IonPac NS1. To guarantee that all IonPac NS1 analytical columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

Column:	IonPac NS1 (10 µm)
Eluent:	3 mM Tetrabutylammonium hydroxide / 28% Acetonitrile
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	Suppressed conductivity, ASRS ULTRA II AutoSuppression® MPIC Mode
Regenerant:	10 mN Sulfuric acid

	Peaks	**mg/L**
1.	Propanesulfonate	15
2.	Iodide	15
3.	Thiocyanate	15
4.	Hexanesulfonate	50
	here 1 mg/L= 1 ppm	

Figure 1 Production Test Chromatogram



4.1.2 Separation of Anions by MPIC with Suppressed Conductivity Detection and Solvent

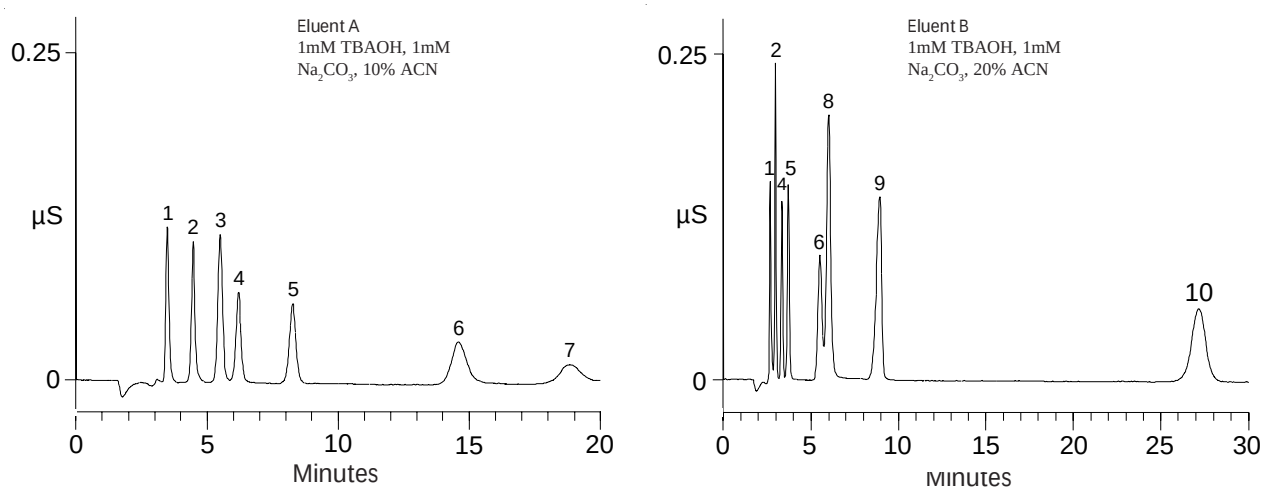
Ion-pairing can separate monovalent anions, such as chlorate and nitrate, which differ in hydration energy. Because ion pairing has low selectivity for higher valency ions, selected monovalent and higher valency ions can be eluted isocratically on the IonPac NS1 analytical column. Example B illustrates the use of a higher solvent concentration to elute hydrophobic anions such as thiosulfate and perchlorate.

Column:	IonPac NS1 (10 µm
Eluent:	See Chromatogram
Flow Rate:	1.0 mL/min.
Injection Loop:	50 µL
Detection:	Suppressed conductivity, ASRS ULTRA II AutoSuppression MPIC Mode
Regenerant:	10 mN H_2SO_4

	Peaks	mg/L
1.	Fluoride	5
2.	Chloride	1
3.	Nitrite	2
4.	Bromide	2
5.	Nitrate	2
6.	Sulfate	2
7.	Phosphate	3
8.	Thiosulfate	5
9.	Thiocyanate	5
10.	Perchlorate	10

where 1 mg/L= 1 ppm

Figure 2 Separation of Anions by Ion-Pairing (MPIC) with Suppressed Conductivity and the Effect of Solvent



4.1.3 Separation of Aliphatic Sulfonic Acids by Ion-Pairing with Suppressed Conductivity (MPIC)

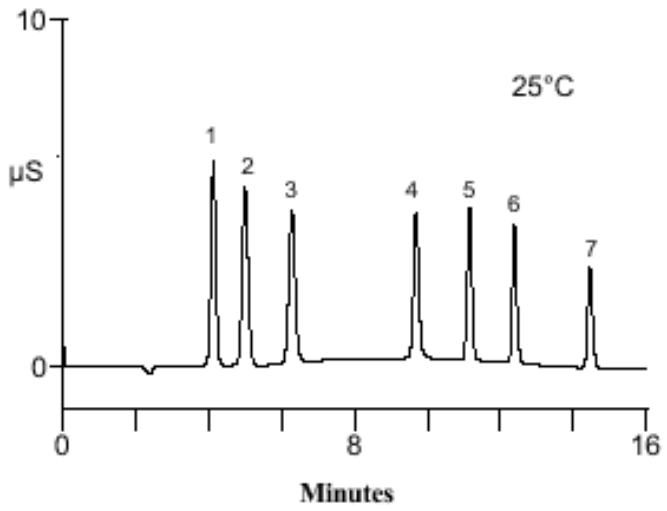
Ion-pairing can provide excellent selectivity for surface active analytes such as surfactants. The separation of aliphatic sulfonic acids on the IonPac NS1 analytical column can be achieved using tetrabutylammonium ion as the ion-pairing agent with conductivity detection. The eluent is suppressed with an ASRS ULTRA II augmented with 10 mN sulfuric acid regenerant. Aliphatic sulfonic acids are UV transparent, but well detected by suppressed conductivity.

Column:	IonPac NS1 (10 µm)
Eluent:	2 mM Tetrabutylammoniumhydroxide, 24% to 48% Acetonitrile in 10 min
Flow Rate:	1.0 mL/min
Injection Loop:	50 µL
Detection:	Suppressed conductivity, ASRS ULTRA II AutoSuppression MPIC Mode
Regenerant:	10 mN Sulfuric acid

	Peaks: **(as the acid forms)**	**mg/L**
1.	Methanesulfonic acid	5.0
2.	1-Propanesulfonic acid	8.6
3.	1-Butanesulfonicacid	8.7
4.	1-Hexanesulfonicacid	8.8
5.	1-Heptanesulfonic acid	8.9
6.	1-Octanesulfonicacid	8.9
7.	1-Decanesulfonicacid	9.1

where 1 mg/L= 1 ppm

Figure 3 Separation of Aliphatic Sulfonic Acids by Ion-Pairing with Suppressed Conductivity (MPIC)



4.1.4 Separation of Aromatic Sulfonic Acids Using Ion-Pairing with Suppressed Conductivity

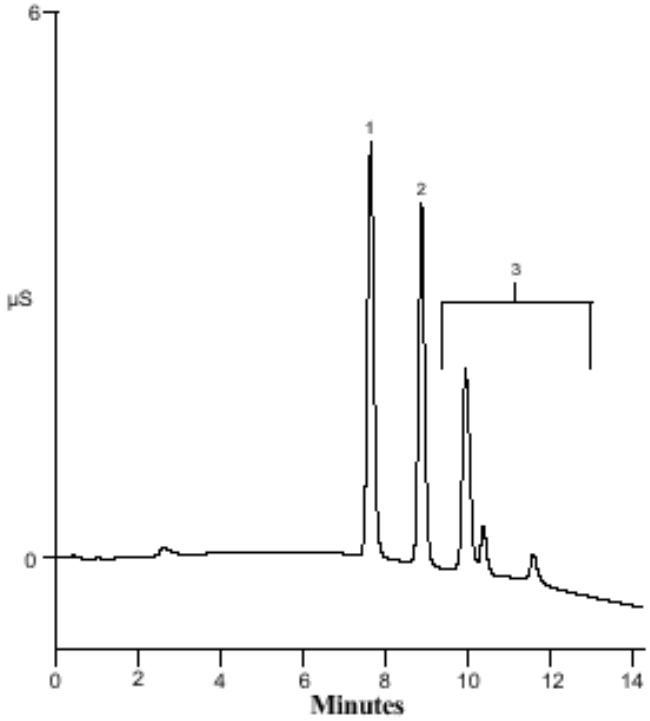
The separation of aromatic sulfonic acids is easily obtained by ion-pairing. The eluent system is identical to that used for the separation of aliphatic sulfonic acids.

Column:	IonPac NS1 (10 µm)
Eluent:	2 mM Tetrabutylammonium hydroxide, 24% to 48% Acetonitrile in 10 min
Flow Rate:	1.0 mL/min
Injection Loop:	50 µL
Detection:	Suppressed conductivity, ASRS ULTRA II AutoSuppression MPIC Mode
Regenerant:	10 mN Sulfuric acid

	Peaks (as the acid forms)	**mg/L**
1.	Benzenesulfonic acid	10.0
2.	Toluenesulfonic acid	8.0
3.	o-, m-, p-Xylenesulfonic acids	9.0

where 1 mg/L= 1 ppm

Figure 4 Separation of Aromatic Sulfonic Acids Using Ion-Pairing with Suppressed Conductivity



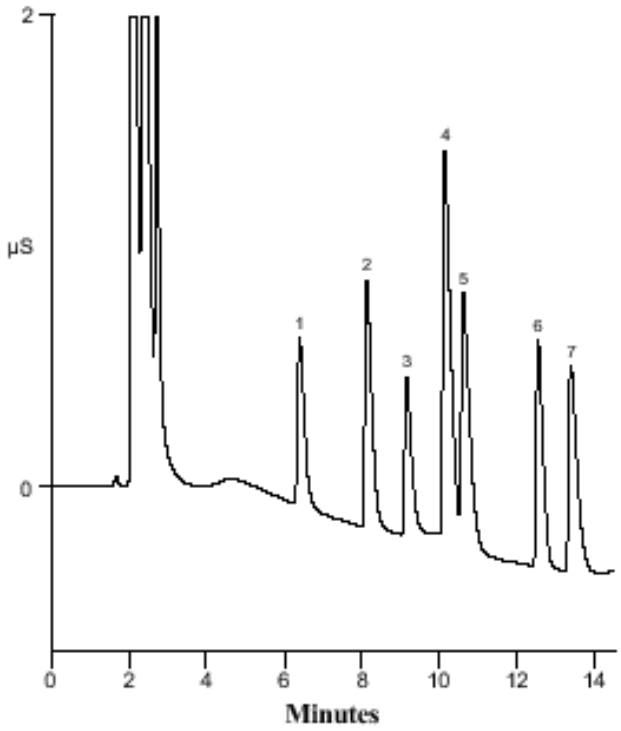
4.1.5 Separation of Aliphatic Quaternary Ammonium Ions Using Ion-Pairing with Suppressed Conductivity (MPIC)

The separation of a series of aliphatic quaternary ammonium ions can be obtained by using an anionic ion-pair reagent such as nonafluoropentanoic acid. The eluent is suppressed with a CSRS ULTRA II operated in the external water mode. The separation uses an acetonitrile gradient from 20% to 80%* in 10 minutes. As the concentration of acetonitrile increases, a decrease in the background conductivity is observed.

Column:	IonPac NS1 (10 µm)
Eluent:	2 mM Nonafluoropentanoic acid, 20% to 80%* Acetonitrile in 10 min
Flow Rate:	1.0 mL/min
Injection Loop:	25 µL
Detection:	Suppressed conductivity, CSRS ULTRA II

* Chemical Suppression is recommended for eluents containing solvents above 40% by volume

Figure 5 Separation of Aliphatic Quaternary Ammonium Ions Using Ion-Pairing with Suppressed Conductivity (MPIC)



	Peaks (as the chloride salts)	mg/L
1.	Tetrapropylammonium	25
2.	Tributylmethylammonium	50
3.	Decyltrimethylammonium	25
4.	Tetrabutylammonium	50
5.	Dodecyltrimethylammonium	50
6.	Tetrapentylammonium	100
7.	Hexadecyltrimethylammonium	100

where 1 mg/L= 1 ppm

4.1.6 Separation of Alkanolamines Using Ion-Pairing with Suppressed Conductivity (MPIC)

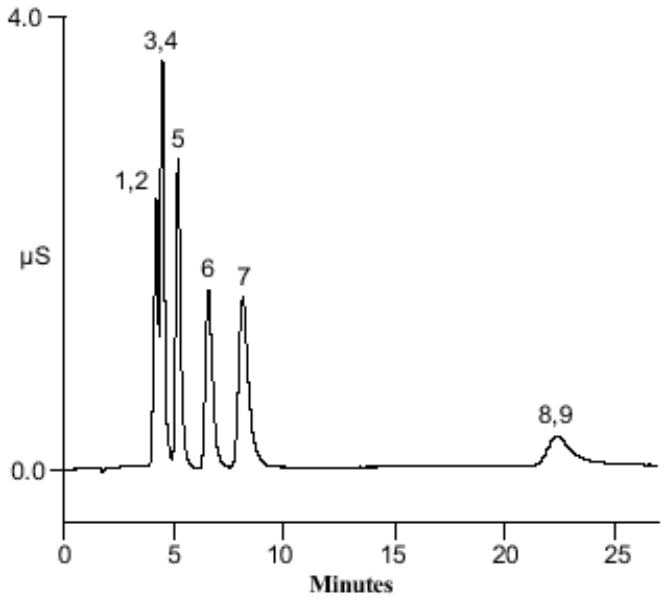
The separation of alkanolamines is also obtained with the use of nonafluoropentanoate ion as the ion-pairing agent. The eluent is suppressed with a CSRS ULTRA II, using 10 mN boric acid as regenerant. The boric acid is added to increase the ionization of the ethanolamines, thereby increasing the conductivity. The borate counter ion displaces the hydroxide counter ion from the fully ionized alkali and alkaline earth metals which reduces the observed conductance for these ions.

Column:	IonPac NS1 (10 µm)
Eluent:	2 mM Nonafluoropentanoicacid / 2% Acetonitrile
Flow Rate:	1.0 mL/min
Inj.Volume:	25 µL
Detection:	Suppressed conductivity, CSRS ULTRA II AutoSuppression MPIC Mode
Regenerant:	10 mN Boric acid

	Peaks:	**mg/L**
1.	Lithium	0.5
2.	Sodium	2.0
3.	Ammonium	5.0
4.	Potassium	5.0
5.	Monoethanolamine	25.0
6.	Diethanolamine	50.0
7.	Triethanolamine	100.0
8.	Calcium	5.0
9.	Magnesium	2.5

where 1 mg/L= 1 ppm

Figure 6 Separation of Alkanolamines Using Ion-Pairing with Suppressed Conductivity (MPIC)

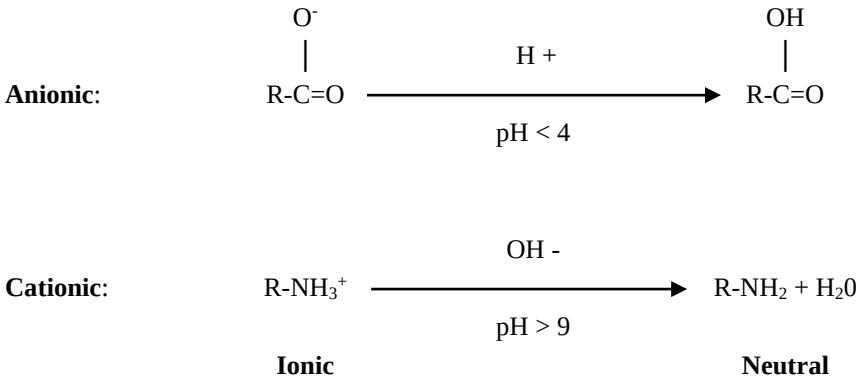


4.2 Ion Suppression Chromatography

This chromatographic technique takes advantage of the effect of pH on the dissociation constants for the acidic and basic organic species. Depending upon the pKa of an organic ionic molecule, the extent of ionization can be controlled by pH.

Because the IonPac NS1 10-µm and NS1-5µm column packings have hydrophobic surfaces, a non-ionic or neutral organic molecule will have a greater affinity for it. Therefore, retention and selectivity can be enhanced by suppressing ionization at either end of the pH scale depending upon the acidic and/or basic nature of the molecule. (see figures 8 and 9).

Acidic molecules such as alkyl or aryl carboxylic acids can be protonated at low pH's (<4). Basic molecules can be deprotonated at high pH's (>9).



Additionally, selectivity can be effected by working within one pH unit of the pKa of a particular organic species such as substituted benzoic acids, (see Figure 10).

The difference in the pKa's of o-nitrobenzoic acid and benzoic acid was utilized to increase resolution of the compounds.

At the higher pH of 2.52 in the “after” chromatogram compared to pH 2.30 in the “before” chromatogram, the resolution of o-nitrobenzoic acid and benzoic acid is almost complete. The pKa of o-nitrobenzoic acid is 2.18. As the pH is raised to pH 2.52, the o-nitrobenzoic acid becomes more ionic and moves to shorter retention times compared to the benzoic acid which has a much higher pKa of 4.19. The benzoic acid is completely protonated in both eluents and therefore its affinity for the column does not change as rapidly as the o-nitrobenzoic acid affinity.

4.2.1 Water Soluble Vitamins by Ion Pairing / Ion Suppression Chromatography

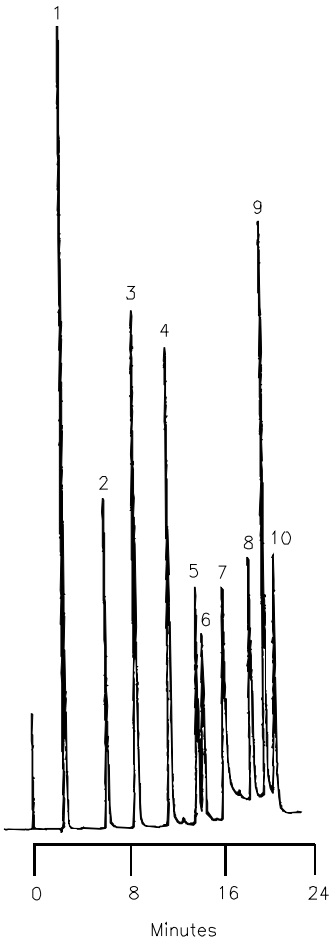
Column:	IonPac NS1-5µm
Eluent Conditions:	12 to 48% Acetonitile in 35 minutes 5 mM Octane Sulfonic Acid
Flow Rate:	1.0 mL/min
Detection:	UV 254 nm

Peaks

1. Ascorbic acid
2. Nicotinic acid
3. Nicotinamide
4. Riboflavin
5. Pyridoxine
6. PABA
7. Folic acid
8. Pyridoxamine
9. Thiamine
10. Cyanocobalamin

Injection: 5 nmoL each

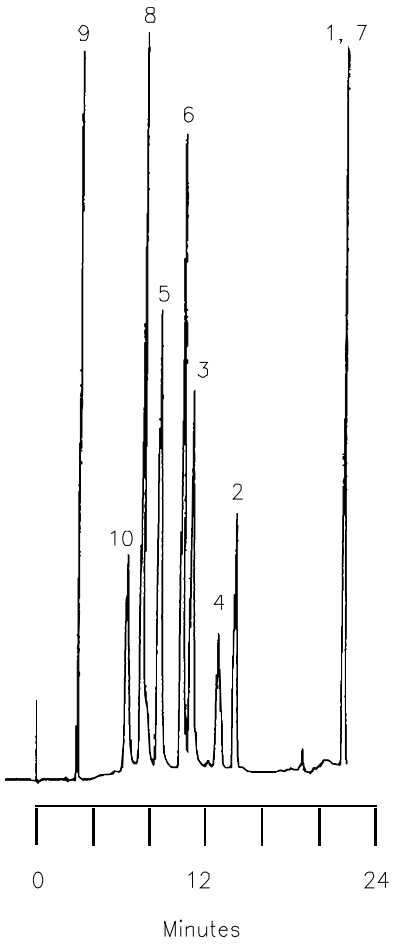
Figure 7 Water Soluble Vitamins by Ion Pairing / Ion Suppression Chromatography



4.2.2 Water Soluble Vitamins by Ion Suppression / Ion Pair Chromatography

Column:	IonPac NS1-5µm
Eluent Conditions:	12 to 56% Acetonitile
	0 to 35 minutes
	5 mM TBAOH
Flow Rate:	1.0 mL/min
Detection:	Suppressed Conductivity

Figure 8 Water Soluble Vitamins by Ion Suppression / Ion Pair Chromatography



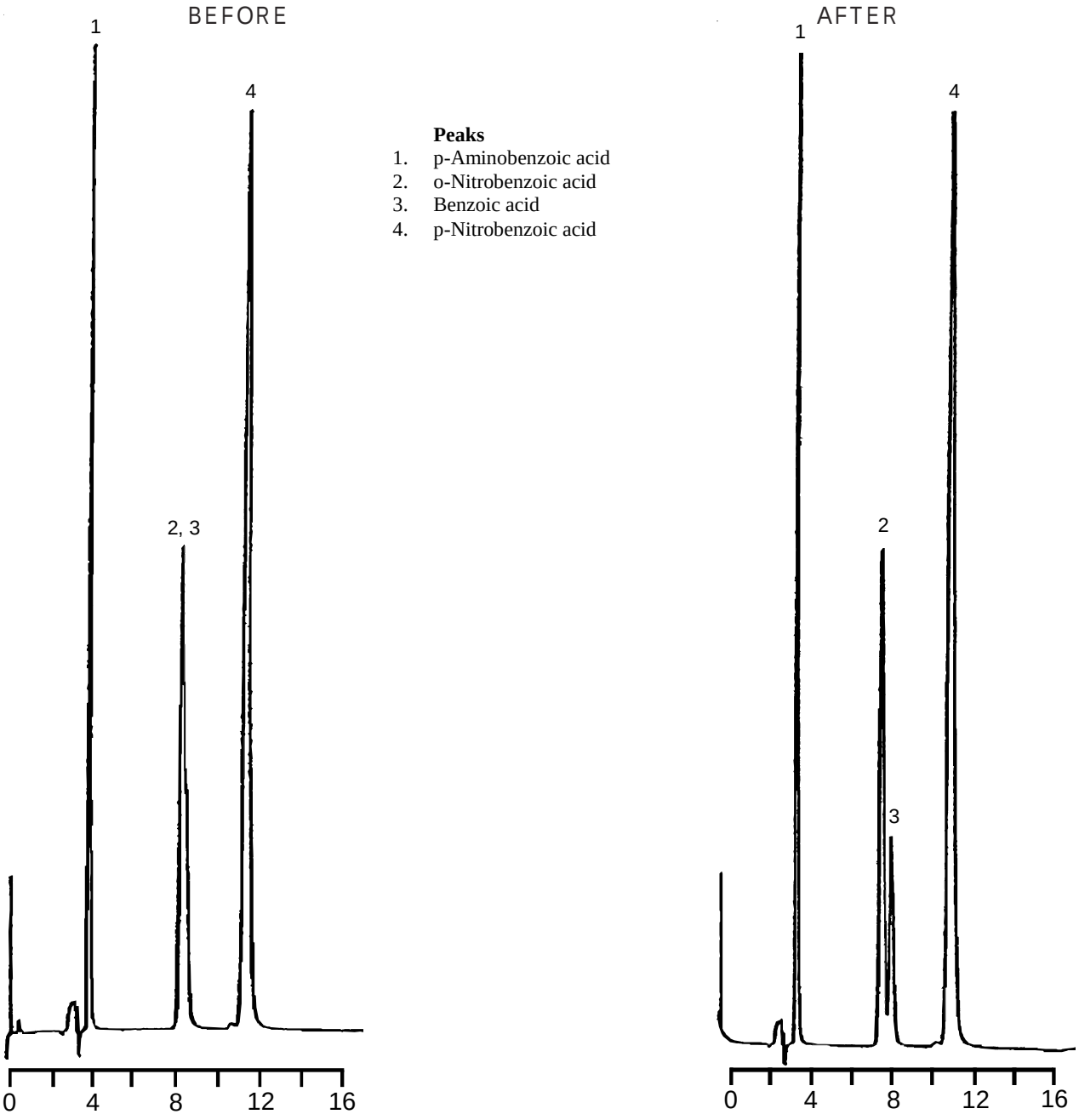
Peaks

1. Ascorbic acid
2. Nicotinic acid
3. Nicotinamide
4. Riboflavin
5. Pyridoxine
6. PABA
7. Folic acid
8. Pyridoxamine
9. Thiamine
10. Cyanocobalamin

Injection: 2 nmoL each

4.2.3 Separation of Benzoic Acid and o-nitrobenzoic Acid

Figure 9 Separation of Benzoic Acid and o-Nitrobenzoic Acid Using Ion Suppression Chromatography



4.2.4 Gradient Separation of Aliphatic Carboxylic Acids

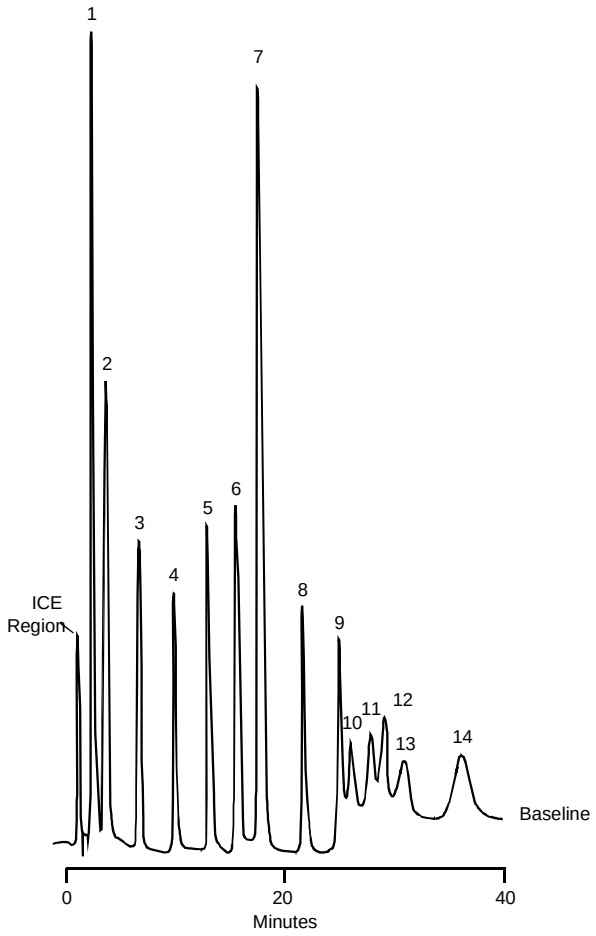
Ion-suppression chromatography uses an acidic eluent that suppresses ionization of the analytes, allowing separation of weak acids by using a reversed-phase column such as the IonPac NS1. The AMMS-ICE II suppressor regenerant for this ion suppression application is potassium hydroxide.

Column:	IonPac NS1
Flow Rate:	1.0 mL/min
	Eluent 1: 24% ACN, 6% MeOH, 0.03 mM HCl
	Eluent 2: 60% ACN, 24% MeOH, 0.05 mM HCl
	Gradient: 0-100% E2 in 20 min.
Temperature:	42 °C
Detection:	Suppressed Conductivity
	AMMS-ICE II Suppressor
Regenerant:	2.5 mM Potassium hydroxide

Peaks

1. Butyric
2. Pentanoic
3. Hexanoic
4. Heptanoic
5. Octanoic
6. Nonanoic
7. Decanoic
8. Dodecanoic
9. Tetradecanoic
10. Linolenic
11. Linoleic
12. Hexadecanoic
13. Oleic
14. Octadecanoic

Figure 10 Gradient Separation of Aliphatic Carboxylic Acids



5. Troubleshooting

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac NS1 columns. For more information on problems that originate with the Ion Chromatograph (IC) or the suppressor, refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the Thermo Fisher Scientific North America Technical Support.

Table 4 NS1/NG1 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	5.1.1
	Plugged Column Bed Supports	Replace Bed Supports	5.1.2
	Other System Modules	Disconnect, Replace	System Module Manual
High Background Conductivity	Bad Eluents	Remake Eluents	5.2, 5.2.1
	Contaminated Columns	Clean Column	5.2.2, 5.2.3
	Contaminated ASRS or AMMS	Clean Suppressor	5.2.5
	Contaminated Hardware	Clean Component	
Poor Resolution	Poor Efficiency Due to Large	Replumb System	5.3.1.A, 5.3.3.D
	System Void Volumes		
	Column Headspace	Replace Column	5.3.1.B
Short Retention Times	Unequilibrated System	Lengthen First Eluent Time before Inject	5.3.3.C
	Flow Rate Too fast	Recalibrate Pump	5.3.2.A
	Bad Eluents	Remake Eluents	5.3.2.B
	Column Contamination	Clean Column	5.3.2.C, 5.3.2.D
Poor Front End Resolution	Bad Eluents	Remake Eluents	5.3.3.A
	Column Overloading	Reduce Sample Size	5.3.3.B, 2.2
	Sluggish Injection Valve	Service Valve	5.3.3.C
	Large System Void Volumes	Replumb System	5.3.3.D
Spurious Peaks	Sample Contamination	Pretreat Samples	5.4.A, 5.4.B
	Sluggish Injection Valve	Service Valve	5.4.C

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Total system pressure when using the IonPac NG1 Guard and IonPac NS1 analytical columns at 1.0 mL/min should be less than 1,600 psi (11.03 MPa) when using the eluent used to generate the test chromatogram. Total system pressure when using the IonPac NG1 Guard and IonPac NS1 analytical columns at 2.0 mL/min should also be less than 1,600 psi (11.03 MPa) when using the eluent used to generate the test chromatogram. If the system pressure is higher than 1,600 psi (11.03 MPa), it is advisable to find out what is causing the high system pressure.

The system should be used with a High-Pressure In-Line Filter (P/N 035331) for eluents. The filter should be positioned between the gradient pump pressure transducer and the injection valve. Since the liquid lines on the gradient pump have 10-32 ferrule/bolt fittings and the High-Pressure In-Line Filter has one male 1/4-28 fitting and one female 1/4-28 port, it is necessary to install two adaptor assemblies on the filter. On the end of the filter with the 1/4-28 male fitting, place a 1/4-28 to 10-32 union (P/N 042806). On the end of the filter with the 1/4-28 female port place a 1/4-28 male to 10-32 female port adaptor assembly (P/N 043291). Make sure you have a High-Pressure In-Line Filter in place and that it is not contaminated.

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

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding the system's components (injection valve, column(s), suppressor, and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 1,500 psi (10.34 MPa) at a flow rate of 2.0 mL/min when the column(s) are connected. The suppressor may add up to 100 psi (0.69 MPa). No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

Table 5 Typical NS1/NG1 Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min
NS1 Analytical	900 (6.21) - 1,400 (9.66)	1.0
NG1 Guard	200 (1.38) - 300 (2.07)	1.0
NS1 + NG1 columns	**1,100 (7.59) - 1,700 (11.73)**	**1.0**
NS1-5 µm analytical	1,700 (11.73) - 2,500 (17.24)	1.0
NG1 Guard	200 (1.38) - 300 (2.07)	1.0
NS1-5 µm + NG1 columns	**1,900 (13.11) - 2,800 (19.31)**	**1.0**

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. 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 using two open end wrenches.
C. Remove the old 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.

Part	IonPac NS1 Column 4-mm (P/N)
Analytical Column (10µm)	035321
Analytical Column (5µm)	039568
Guard Column	039567
Bed Support Assembly	042955
End Fitting	052809

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.

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

5.2 High Background or Noise

5.2.1 Preparation of Eluents

A. Make sure that all eluents and regenerants are made correctly. Were the proper precautions taken to prepare the sodium hydroxide eluent? If carbonate was present in the eluent, the Anion Trap column will eventually be spent and the background level will increase.
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 Contaminated Anion Trap Column, the ATC-3 (4-mm)

When doing gradient analysis, ensure that the Anion Trap Column, the ATC-3 (2-mm) or the ATC-3 (4-mm) has been installed correctly. If it has not, install one as directed in Section 2.3, "The Anion Trap Column," and watch the background conductivity. If the background conductivity is now low, this means that the ATC is trapping contaminants from the eluent. The eluents probably have too many impurities (see items A–C above).

Determine if the ATC is the source of high background conductivity. Remove the ATC. If the background conductivity remains high, then the ATC is not the problem. If the background conductivity decreases, the ATC is the source of the high background conductivity.

A. Disconnect either the ATC-3 (4-mm) from the injection valve and direct the outlet to waste.
B. Flush the ATC with 200 mL of 35 mM NaOH or the highest NaOH concentration used in the application. Use a flow rate of 2.0 mL/min on a 4-mm system.
C. Equilibrate the ATC with the strongest eluent used during the gradient run. Use a flow rate of 2.0 mL/min on a 4-mm system.
D. If the problem persists, replace the ATC.

5.2.3 Contaminated Guard or Analytical Column

Remove the IonPac NG1 Guard and IonPac NS1 analytical columns from the system. If the background conductivity decreases, then one (or both) of these columns is (or are) the cause of the high background conductivity, clean the column as instructed in Appendix B - Column Care.

5.2.4 Contaminated Hardware

To eliminate the hardware as the source of the high background conductivity, bypass the SRS suppressor and pump deionized water with a specific resistance of 18.2 megohm-cm through the system. The background conductivity should be less than 2 µS. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

5.2.5 Contaminated Suppressor

Assume that the SRS or the AMMS-ICE is causing the problem if the above items have been checked and the problem persists.

A. Check the regenerant flow rate at the REGEN OUT port of the SRS or the AMMS-ICE. For the example isocratic applications, this flow rate should be 3–5 mL/min in the Chemical Suppression Mode of operation.
B. Check the eluent flow rate. For most applications, the eluent flow rate for 2-mm applications should be 0.50 mL/min and for 4-mm applications, it should be 2.0 mL/min. Refer to the Self-Regenerating Suppressor Product Manual (Document No. 031956) or the Anion-II MicroMembrane Suppressor-ICE Product Manual (Document No. 032661) to ensure that the eluent is within suppressible limits.
C. Test both the suppressor and the Anion AutoRegen Regenerant Cartridge for contamination if you are using an AutoRegen Accessory with the ASRS ULTRA II (in the MPIC or Chemical Suppression Mode) or the CSRS (in the Chemical Suppression Mode). Prepare fresh regenerant solution.

1. Clean or replace your SRS or AMMS-ICE if the background conductivity is high after preparing fresh regenerant and bypassing the Anion AutoRegen Regenerant Cartridge. Refer to the "Self-Regenerating Suppressor Product Manual" (Document No. 031956) or the "Anion-ICE MicroMembrane Suppressor II Product Manual" (Document No. 032661) for assistance.
2. Test the Anion AutoRegen Regenerant Cartridge to see if it is expended. If the background conductivity is low when freshly prepared regenerant is run through the ASRS or CSRS without an AutoRegen Accessory in-line then the cartridge is expended. Connect a freshly prepared regenerant to the Anion AutoRegen Regenerant Cartridge. Pump approximately 200 mL of regenerant through the Anion AutoRegen Regenerant Cartridge to waste before recycling the regenerant back to the regenerant reservoir.
   If the background conductivity is high after placing the AutoRegen Accessory in-line, you probably need to replace the Anion AutoRegen Regenerant Cartridge (P/N 039564). Refer to the "AutoRegen Regenerant Cartridge Refill Product Manual" (Document No. 032852) for assistance.

5.3 Poor Peak Resolution

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

5.3.1 Loss of Column Efficiency

A. Ensure that system void volumes have been minimized. Extra-column system effects can result in sample band dispersion and decreasing peak efficiencies. Make sure you are using PEEK tubing with an i.d. of no greater than 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4-mm systems. Make all tubing lengths are as short as possible. Check for leaks.

B. Check to see if headspace has developed in the guard or analytical column (e.g., due to improper use of the column such as submitting it to high pressures). 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. The column must be replaced.

5.3.2 Poor Resolution 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 eluent flow rate. If it is different than the flow rate specified by the analytical protocol, recalibrate the pump. 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.
   For isocratic analysis, an eluent that is too strong will cause the peaks to elute faster. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent 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%.
   For gradient analysis, remake the eluents or adjust the times in the gradient program to obtain the required peak resolutions.

C. Column contamination can lead to a loss of column capacity because fewer of the anion exchange sites will be available for the sample ions. Polyvalent anions or metal ions might be concentrating on the column. Refer to Appendix B - Column Care, for recommended column cleanup procedures.
Possible sources of column contamination are impurities in chemicals, in the deionized water or from the sample matrix being used. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 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 ("Appendix B - Column Care").
After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. 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 the nearest Thermo Fisher Scientific Office.

5.3.3 Loss of Front End Resolution

If poor resolutions and efficiencies are observed for the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

A. Improper 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.

B. 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. The column may not be equilibrated to the first eluent. Increase the amount of time that the first eluent runs through the columns before injection.

D. Sluggish operation of the injection valve may be the problem. Check the air pressure and make sure there are no gas leaks or partially plugged port faces. Refer to the valve manual for instructions.

E. 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.

5.4 Spurious Peaks

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

B. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac NS1 columns, contact the nearest Thermo Fisher Scientific Office.

C. The injection valve may be creating a baseline disturbance. This baseline upset can show up as a peak of varying size and shape. It will happen when the injection valve

needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

If cleaning and retorquing the valve does not help, replace the valve. Use a Thermo Fisher Scientific High Pressure Injection Valve (P/N 037142) or a Thermo Fisher Scientific High Pressure Inert Valve (P/N 037143) as required.

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (Thermo Fisher Scientific P/N 044697), consult the accompanying manual for service instructions. See Section 2.2, “The Injection Loop,” for injection valve and loop requirements for 4-mm operation.

5.5 Small Analyte Peak Areas

Assuming that the suppressor is the cause of this problem, small analyte peak areas are a result of running eluent through the suppressor with the power off while using the ASRS ULTRA II in the MPIC Suppression Mode or the CSRS ULTRA II in the AutoSuppression External Water Mode. The problem may also occur in any suppressor while using it in the Chemical Suppression Mode by not running regenerant through the suppressor chambers.

A. Disconnect the eluent line from the analytical column attached to the ELUENT IN port of the Self-Regenerating Suppressor (SRS) at the analytical column end of the line. Direct this line to a separate waste beaker.
B. Disconnect the eluent line from the ELUENT OUT port of the Self-Regenerating Suppressor (SRS) to the detector conductivity cell at the suppressor end of the line.
C. Install a 10-32 Luer adaptor fitting with a plastic syringe in the ELUENT OUT port of the Self-Regenerating Suppressor (SRS) and inject 5 mL of 0.5 N H_2SO_4 through the ASRS ULTRA II or 5 mL of 0.5 M NaOH in the reverse direction to normal flow so that the waste comes out of the ELUENT IN port.
D. Reconnect the eluent lines from the ELUENT IN port of the Self-Regenerating Suppressor (SRS) to the analytical column and from the ELUENT OUT port of the Self-Regenerating Suppressor (SRS) to the conductivity detector cell line.
E. Establish the regenerant flow through the suppressor, turn on the power and begin pumping eluent.

NOTE

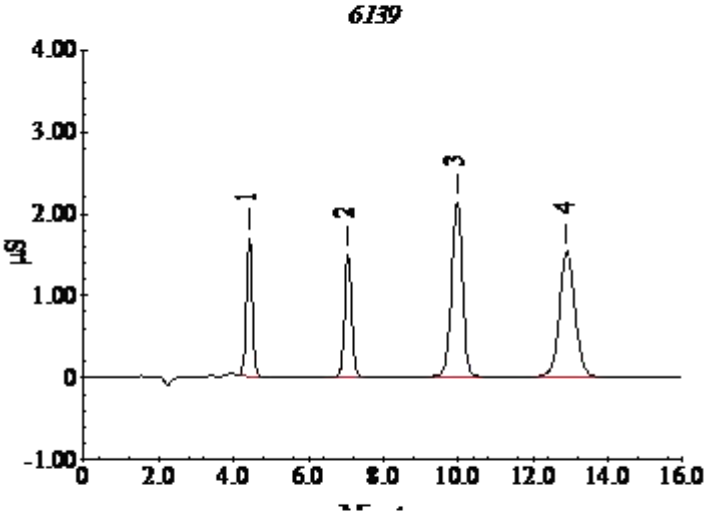
If you are operating in the Chemical Suppression Mode, all you have to do is reestablish acid or base regenerant flow through the suppressor and begin pumping eluent. Allow the system to equilibrate before beginning analysis. Power is not used in this mode of operation.

Appendix A – Quality Assurance Reports

A.1 IonPac NS1 10µm

IonPac™ NS1 10µ
Analytical (4 x 250 mm)
Product No. 035321

Serial No.: 6139 Pressure (PSI): 1250 Date: 8/1/00 7:09:11 AM



Eluent 28% Acetonitrile
3.0 mM Tetrabutylammonium hydroxide

Flow Rate 1.0 mL/min

Detection Suppressed Conductivity
ASRS ™-ULTRA
AutoSuppression®
External Water Mode
with 10mN Sulfuric Acid

Range 10 µSFS

Background Conductivity 3-15 µS

Injection Volume 10 µL

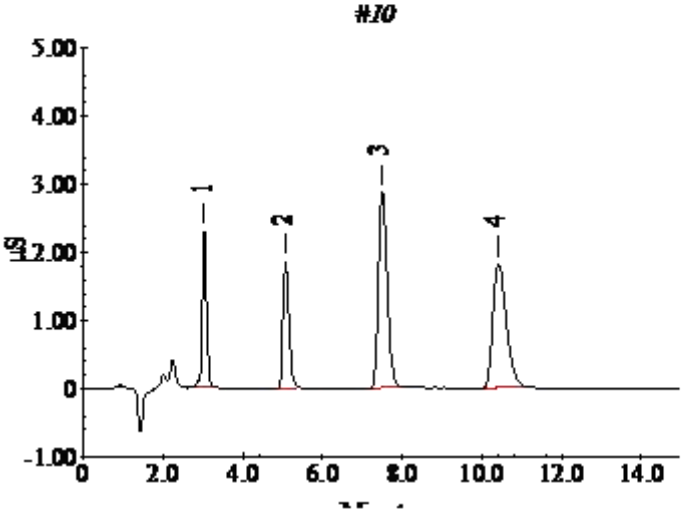
Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	4.42	Propanesulfonate	15.0	4057	1.0	8.99
2	7.05	Iodide	15.0	6447	1.2	6.52
3	9.98	Thiocyanate (SCN)	15.0	5278	0.9	4.59
4	12.90	Hexanesulfonate	50.0	4895	1.2	n/a

A.2 IonPac NS1 5µm

IonPac® NS1 5µ
Analytical (4 x 150 mm)
Product No. 039568

Serial No.: #10 Pressure (PSI): 1930 Date: 2/21/01 9:13:47 AM



Eluent 26% Acetonitrile
4.0 mM Tetrabutylammonium hydroxide

Flow Rate 1.0 mL/min

Detection Suppressed Conductivity
ASRS®-ULTRA
AutoSuppression®
External Water Mode
with 10 mN Sulfuric Acid

Range 10 µSFS

Background Conductivity 3-15 µS

Injection Volume 10 µL

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	5.02	Propanesulfonate	15.0	5704	1.6	8.44
2	5.07	Iodide	15.0	4921	1.5	6.96
3	7.48	Thiocyanate	15.0	5186	1.6	5.58
4	10.40	Hexanesulfonate	50.0	4223	1.8	n/a

Appendix B – Column Care

B.1 Recommended Operation Pressures

The maximum recommended operating pressure for IonPac NS1 columns is 4,000 psi (27.57 MPa). Operating a column above its recommended pressure limit can cause irreversible loss of column performance.

B.2 Column Start-Up

The IonPac NS1-5µm is tested with, and shipped in, 26% acetonitrile/4.0 mM tetrabutylammonium hydroxide. The IonPac NS1-10µm is tested with, and shipped in, 28% acetonitrile/3.0 mM tetrabutylammonium hydroxide. The column should be thoroughly washed with 80% acetonitrile/deionized water until the background is below 1 µS before switching to other eluents.

Prepare the eluent shown on the test chromatogram, install the column in the chromatography module and test the column performance under the conditions described in the test chromatogram. 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.

B.3 Column Storage

For short-term storage, the strongest eluent in use can be used as the storage solution.

For long-term storage, the strongest eluent in use should be used as the storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

B.4 Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble or organic contaminants. They can be combined into one gradient protocol if desired but the following precautions should be observed.

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

When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to 5% levels and the ionic strength of the eluent to 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

B.4.1 Choosing the Appropriate Cleanup Solution

A. 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 listed in Table 4, HPLC Solvents for Use with IonPac NS1 Columns.

B. Concentrated acid solutions such as 1 to 3 M HCl can be used with compatible organic solvents to remove contamination that is ionic and organic. The acid suppresses ionization and ion exchange interactions of the contamination with the resin. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. See Section B.4 above.

C. 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.

D. Regardless of the cleanup solution chosen, use the following cleanup procedure in Section B.6, "Column Cleanup Procedure," to clean the IonPac NG1 and IonPac NS1.

B.4.2 Column Cleanup Procedure

A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in Section B.4.1, "Choosing the Appropriate Cleanup Solution."

B. Disconnect the Suppressor from the IonPac NS1 analytical column. 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 direction designated on each of the column labels. Direct the effluent from the outlet line of the NG1 Guard Column to a separate waste container.

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. Set the pump flow rate to 0.50 mL/min (2-mm systems) or 2.0 mL/min (4-mm systems).

D. Pump the cleanup solution through the column for 60 minutes.

E. Reconnect the Suppressor to the IonPac NS1 analytical column.

F. Place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

G. Equilibrate the column(s) with eluent before resuming normal operation.