IonPac NS2 Columns

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Thermo SCIENTIFIC

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Product Manual

for

IonPac NS2 Columns

IonPac NS2, 5µm, 4x250mm Analytical Column (P/N 088786)
IonPac NS2, 5µm, 4x150mm Analytical Column (P/N 088787)
IonPac NG2, 5µm, 4x35mm Guard Column (P/N 088788)

Revision History:

Revision 01, April, 2014, Original Publication;

Revision 02, October, 2015, Converted special part numbers to standard 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

IonPac™ NS2 column is a silica-based reversed-phase column designed for Mobile-Phase Ion Chromatography (MPIC) and compatibility with suppressed conductivity detectors. MPIC is a method where an ion-pairing agent in the eluent provides retention for oppositely-charged analyte ions on a hydrophobic column, and then is removed by the suppressor to provide selective, sensitive detection of the analytes. The typical uses for MPIC include the separation of moderately to strongly hydrophobic organic ions such as ionic surfactants, quaternary amines and sulfonic acids, or the separation of multiply-charged ions.

1.1 Main features

The IonPac NS2 column:

- Mobile-Phase Ion Chromatography (MPIC)
- Anion analysis
- Cation analysis
- Compatible with suppressed conductivity detection

1.2 Physical data

Column Chemistry:	Polar-embedded C18
Silica Substrate:	Spherical, high-purity, porous
Particle size:	5 µm
Surface area:	300 m^2/g
Pore size:	120 Å

1.3 Specifications and Operational Parameters

Column Dimension (mm)	P/N	Maximum Pressure (psi)	Recommended pH Range	Recommended Temperature (°C)	Solvent/Aqueous Compatibility	Recommended Flow Rate (mL/min)	Maximum Flow Rate (mL/min)
4.0 x 250	088786	4,000	2.0 – 9.5	20 – 35	• Compatible with 0 – 100% aqueous eluents. • Compatible with most HPLC solvents, except THF 20% max.	0.5 – 1.0	1.2
4.0 x 150	088787	4,000	2.0 – 9.5	20 – 35		0.5 – 1.0	1.2
4.0 x 35	088788	4,000	2.0 – 9.5	20 – 35		0.5 – 1.0	1.2

1.4 Operational Guidelines

- Read and understand this Column Manual before using this product.
- Use the column following the direction of flow marked on the column.
- Operate this column according to "Operational Parameters" described above.
- Avoid sudden pressure surge.
- Always use guard column to protect the analytical column and extend its useful lifetime.
- Be sure to choose the correct suppression system, and follow the instructions in the manual for the suppressor.

Ion polarity	Cation		Anion	
MPIC agents	• 0.5 – 10 mM TFA • 0.5 – 10 mM HFBA • 0.5 – 10 mM hexanesulfonic acid • 0.5 – 10 mM octanesulfonic acid		• 5 – 10 mM sodium borate • 0.5 – 5 mM tetrabutylammonium borate • 0.5 – 5 mM tetrapropylammonium borate (pH 8 – 8.5)	
% Organic solvent	0 – 40%	0 – 100%	0 – 40%	0 – 100%
Compatible solvents	acetonitrile	most common water-miscible HPLC solvents (except acetone or THF)	acetonitrile	most common water-miscible HPLC solvents (except acetone or THF)
Recommended suppressor	CERS-500 4mm	CMMS-300 4mm	AERS-500 4mm	AMMS-300 4mm
Suppression mode	external water	chemical	external water	chemical
Regenerant	water; 2 – 5 mL/min	0.02 – 0.1 M tetrabutylammonium hydroxide; 2 – 10 mL/min	water; 2 – 5 mL/min	0.005 – 0.1 N sulfuric acid; 2 – 10 mL/min

1.5 Ordering Information

	Column Dimension	P/N
IonPac NS2 Analytical Column	4.0 x 250	088786
	4.0 x 150	088787
IonPac NG2 Guard Column	4.0 x 35	088788

2. Getting Started with Cation Analysis

It is recommended that you run the column performance test upon receiving your new IonPac NS2 column. The purpose of such test is to ensure no damage has occurred during shipping. Steps 1 - 5 below outline the necessary steps to perform this validation test. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time.

Note: Slight variations may be observed on two different HPLC systems due to differences in system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 1 – Visually inspect the column

Report any damage to Thermo Fisher Scientific. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement.

Step 2 – Decide on suppression mode

Since MPIC typically uses organic solvents in the eluent, only CMMS-300 or CERS-500 suppressors should be used. If the organic solvent is acetonitrile and it will not exceed 40% (v/v) then electrolytic suppression in external water mode may be used with the CERS-500. Chemical-mode suppression using the CMMS-300 is compatible with most water-miscible solvents at any concentration (except 20% maximum THF due to the PEEK construction).

NOTE

While acetonitrile, ester solvents and amide solvents are compatible with normal operation of the suppressor, they should be flushed out prior to shutting down the system. If left in the suppressor, they slowly hydrolyze, and cause excessive startup times when resuming work.

Step 3 – Mobile phase preparation

To obtain reliable, consistent and accurate results, mobile phases must be free of non-volatile, ionic or spectroscopic impurities. Therefore, maintaining trace impurities low and particulate matters low is essential to obtain good result, and protect the column and system components.

De-ionized Water

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade water, or HPLC Grade Water. The deionized water must be free of ionized impurities, organics, microorganisms and particulate matters. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE

Whenever applicable, degas the aqueous component and solvent component separately before mixing them together. Excessive purging or degassing of mobile phases should be avoided because it may change mobile phase composition.

Solvents

The solvent must be free from particulate, ionic or UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade ensures that your chromatography is not affected by impurities in the solvent.

Reagent preparation

Preparation of 10 mM, TFA eluent

Dilute 1.14 g (0.770 mL) of TFA with 999 g of deionized water to make 1.00 L.

Preparation of 10 mM HFBA eluent

Dilute 2.14 g (1.30 ml) of HFBA with 999 g of deionized water to make 1.00 L.

Preparation of 10 mM octanesulfonic acid eluent

Dilute 100 mL of 0.10 M OSA (P/N 035362) with 900 g of deionized water to make 1.00 L.

Preparation of 10 mM hexanesulfonic acid eluent

Dilute 100 mL of 0.10 M HSA (P/N 035361) with 900 g of deionized water to make 1.00 L

Preparation of 0.05 M tetrabutylammonium hydroxide (TBAOH) regenerant

Use a pressure-resistant, heavy-wall polyethylene 4-L carboy (included in kit P/N 038018)

Dilute 100 mL of 2.06 M TBAOH (P/N 057561) with 4 L of deionized water to make 4.1 L of regenerant.

Avoid contamination by atmospheric carbon dioxide by keeping the regenerant under inert gas in a closed container.

Step 4 – Set up the IC system

Use the IonPac NS2 column with a Thermo Scientific Dionex ion chromatography system, at minimum consisting of an eluent pump, injection valve, suppressor, conductivity detector, and regenerant delivery system. You may use either an isocratic pump and pre-mix the eluent, or a quaternary pump and proportion the solvent, water and MPIC-agent solution. See the recommendations in the suppressor manual for the regenerant delivery system.

Step 5 – Condition the column

When a new column is used for the first time, it should be washed thoroughly. Wash for 30 minutes with slightly higher concentrations of solvent and acid than you expect to use for the analysis, sending the out-flow to waste. Connect the column to the suppressor and detector, and equilibrate it until a stable baseline is obtained. If you will be using gradient elution, run repeated blank gradients until a consistent baseline is obtained.

Step 6 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column performance test using the conditions described in the Quality Assurance Report, and compare the result with the one in the report.

It is recommended that you run the column performance test upon receiving your new IonPac NS2 column. Repeat the test periodically to track the column performance over time.

NOTE

Slight variations may be observed on two different HPLC systems due to differences in system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 7 – Real sample analysis

Once the column performance is satisfactorily confirmed in Step 5, the column is ready for real sample analysis.

NOTE

It is recommended that the column performance test be performed periodically to monitor the condition of the column.

3. Getting Started with Anion Analysis

Step 1 – Visually inspect the column

Report any damage to Thermo Fisher Scientific. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement.

Step 2 – Decide on suppression mode

Since MPIC typically uses organic solvents in the eluent, only AMMS-300 or AERS-500 suppressors should be used. If the organic solvent is acetonitrile and it will not exceed 40% (v/v) then electrolytic suppression in external water mode may be used with the AERS-500. Chemical-mode suppression using the AMMS-300 is compatible with most water-miscible solvents at any concentration (except 20% maximum THF due to the PEEK construction).

NOTE

While acetonitrile, ester solvents, and amide solvents are compatible with normal operation of the suppressor, they should be flushed out prior to shutting down the system. If left in the suppressor, they slowly hydrolyze, and cause excessive startup times when resuming work.

Step 3 – Mobile phase preparation

To obtain reliable, consistent and accurate results, mobile phases must be free of non-volatile, ionic or spectroscopic impurities. Therefore, maintaining trace impurities low and particulate matters low is essential to obtain good result, and protect the column and system components.

De-ionized Water

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade water, or HPLC Grade Water. The deionized water must be free of ionized impurities, organics, microorganisms and particulate matters. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE

Whenever applicable, degas the aqueous component and solvent component separately before mixing them together. Excessive purging or degassing of mobile phases should be avoided because it may change mobile phase composition.

Solvents

The solvent must be free from particulate, ionic or UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade ensures that your chromatography is not affected by impurities in the solvent.

Reagent preparation

Preparation of 20 mM sodium borate buffer

Dissolve 6.18 g (100 mmole) of boric acid in 998 g of deionized water

Add 1.60 g (20 mmole) of 50% sodium hydroxide solution

Check that the pH is 8.5

Filter through a 0.45 µm (or smaller) membrane filter

Preparation of 10 mM tetrabutylammonium borate MPIC buffer

Dissolve 3.09 g (50 mmole) of boric acid in 900 g of deionized water

Add 100 mL (10 mmole) of 0.10 M TBAOH (P/N 035360)

Check that the pH is 8.5

Filter through a 0.45 µm (or smaller) membrane filter

Preparation of 10 mM tetrapropylammonium borate MPIC buffer

Dissolve 3.09 g (50 mmole) of boric acid in 900 g of deionized water

Add 100 mL (10 mmole) of 0.10 M TPAOH (P/N 035363)

Check that the pH is 8.5

Filter through a 0.45 µm (or smaller) membrane filter

Preparation of 0.025 N sulfuric acid regenerant

Use a pressure-resistant, heavy-wall polyethylene 4-L carboy (included in kit P/N 038018)

Dilute 1.25 g (2.3 mL) of 98% sulfuric acid in 4.0 L of deionized water

Step 4 – Set up the IC system

Use the IonPac NS2 column with a Thermo Scientific Dionex ion chromatography system, at minimum consisting of an eluent pump, injection valve, suppressor, conductivity detector, and regenerant delivery system. You may use either an isocratic pump and pre-mix the eluent, or a quaternary pump and proportion the solvent, water and buffer solution. See the recommendations in the suppressor manual for the regenerant delivery system.

Step 5 – Condition the column

When a new column is used for the first time, it should be washed thoroughly. Wash for 30 minutes with slightly higher concentrations of solvent and buffer than you expect to use for the analysis, sending the out-flow to waste. Connect the column to the suppressor and detector, and equilibrate it until a stable baseline is obtained. If you will be using gradient elution, run repeated blank gradients until a consistent baseline is obtained.

4. Considerations In Method Development

4.1 Before you start

Read this Product Manual. The conditions listed in section 1.4 and the example methods in section 7 can give you good starting points for the development of your method.

4.2 MPIC (Ion-Pairing) Agent

There are a few main factors to consider. The first factor is the hydrophobicity of the agent. More hydrophobic agents partition more strongly into the stationary phase, and increase the dynamic ion-exchange capacity (See example 7.3). The second factor is concentration. Higher concentrations in the eluent raise the concentration partitioned into the stationary phase, and likewise increase the dynamic ion-exchange capacity (see examples 7.1 and 7.2). For example 1 mM HFBA provides roughly the same retention as 10 mM TFA. The third factor is compatibility with suppressed conductivity. The counter-ion to the agent should give low conductivity in the background after suppression. For example TFA suppresses to water, and TBA borate suppresses to boric acid; both products have low background conductivity. The fourth factor is pH. The eluent should not exceed the recommended pH range of 2.0 – 9.5 for the NS2 column. The final factor is purity of the reagent. Otherwise attractive agents may not be available in the proper form or grade for conductivity detection.

The advantages of reducing the concentration of ion-pairing agent include: lower background, lower noise, lower concentration of regenerant for suppression, less retention for hydrophobic ions, less retention for multi-valent ions. The advantages of increasing the concentration of ion-pairing agent include: more retention of monovalent ions, more retention of hydrophilic ions, more resistance to sample overload, more resistance to matrix effects, faster equilibration time.

4.3 Organic Solvent

The IonPac NS2 column is compatible with most water-miscible HPLC solvents; however, because of the PEEK column hardware, do not exceed 20% THF. The main solvent limitation is from the suppressor. Electrolytic suppressors (AERS-500 and CERS-500) are compatible with up to 40% acetonitrile when used in external water mode. Avoid leaving acetonitrile, amide solvents, or ester solvents in the suppressor with no flow for more than an hour; these can slowly hydrolyze and result in excessive start-up time when resuming work.

The concentration of solvent in the eluent affects the concentration of MPIC agent partitioned into the stationary phase. This effect can be quite sensitive, especially for the more hydrophobic agents (see example 7.2). Increasing the solvent concentration reduces the MPIC concentration in the stationary phase, and lowers the dynamic ion-exchange capacity. This also increases the mobility of hydrophobic analytes. The interplay between the concentrations of solvent and ion-pairing agent offers a means of controlling selectivity. A rapid change in solvent concentration can cause the column to “dump” the MPIC agent into the eluent, leading to a disturbed baseline; curved gradients may be helpful in minimizing this problem.

Organic solvents are less conductive than water. This leads to a sloping baseline with gradient elution. It also reduces peak height.

4.4 Eluent pH

Due to the silica substrate of the IonPac NS2 column, the eluent pH should stay in the range of 2.0 – 9.5. When using ion-pairing acids for cation analysis (pH < 3), the silanol activity of the column is suppressed, and plays a very minor role is the retention process. When using borate buffer systems (pH 8 – 9) for anion analysis, there is significant silanol activity in the column, and ionic repulsion plays a significant role in the retention process. Raising the ionic strength will increase retention, and increase efficiency. For sodium-containing eluents, use 5 – 10 mM sodium for best results (see example 7.6).

4.5 Ionic strength of Eluent

The ionic strength may be raised by the addition of a compatible acid or salt that can be suppressed. This provides competition for the ion-exchange capacity of the column, and causes the ionic analytes to elute earlier. Hydrophobic analytes will tend to elute slightly later. Silanol interactions will be reduced. This technique can be used to adjust selectivity in the separation. For cation-MPIC, addition of methanesulfonic acid contributes competing H^+ cations, but the methansulfonate anions do not contribute to the retention of cations. For anion-MPIC, addition of sodium borate contributes competing borate anions, but the Na^+ cations do not contribute to the retention of anions (see example 7.2). The effect of ionic strength increases as the charge on the analyte increases.

4.6 Suppression

Electrolytic suppression using external water mode is compatible with up to 40% acetonitrile in the eluent. The flow rate of the water should be 2 – 5 mL/min. For cation analysis, set the current according to the total acid concentration. For anion analysis, set the current according to the total sodium, TPA and/or TBA concentration (not the borate concentration). When using gradient elution, it is good practice to maintain a constant concentration of electrolytes and vary the organic solvent; this will extend the service life of the suppressor.

When using chemical mode suppression, the ion flux (concentration times flow rate) of the regenerant should be 2 – 20 times the ion flux of the eluent. The regenerant flux needs to be sufficient to suppress both the eluent and any ions introduced by the sample matrix. If the regenerant concentration is either too high or too low the background conductivity and noise will increase. Find the concentration for the minimum background, and then increase the concentration and/or flow rate enough to make the method robust against effects of the sample matrix, against expected variability in the method, and against common minor disruptions.

Protect TBAOH regenerant from carbon dioxide in the atmosphere or silica in glass by storing it in a polyethylene carboy under nitrogen. Contamination can cause high background, loss of sensitivity, and distorted peaks.

4.7 Isocratic vs. Gradient

Isocratic separations have the advantages of simplicity, low noise, and a flat baseline. Pre-mixed isocratic eluent will give the best baseline performance. When needing to analyze complex

samples, or analytes with very different retention times, the power of gradient elution may be required.

When using gradient elution with electrolytic suppression (CERS-500 or AERS-500 in external water mode) it is recommended that the MPIC agent be maintained at constant concentration, and the organic solvent composition be varied. This allows the suppressor current to be well-matched to the ion flux, and so prolong the service life of the suppressor.

4.8 Sample Matrix

The IonPac NS2 column is tolerant of samples in the range of pH 2 – 9.5, at almost any ionic strength or solvent composition. For low-volume injections (≤ 10 µL) the composition of the sample matrix has little effect on the chromatography.

As the injection volume increases, the sample matrix can affect peak shape or retention time, or introduce artifacts in the baseline. In this case, it is good practice to reduce the organic solvent concentration in the matrix below the concentration in the eluent. If it is practical to do so, add the ion-pairing agent to the sample matrix, and reduce the difference in pH between matrix and eluent. These suggestions are especially helpful for early-eluting peaks.

For complex matrices, match the calibration standards to the expected matrix as much as practical.

5. Column Care

5.1 Eluent (mobile phase)

All eluents should be freshly prepared. All chemicals and solvents should be of high purity quality. In-line filters are recommended.

5.2 Guard cartridge

When analyzing real-life samples, a guard cartridge must be used with the analytical column, and replaced periodically depending on the nature of the sample. Failing to do so will result in rapid column deterioration and premature column failure.

5.3 Column storage

The column can be stored in mobile phase for short period of time, such as overnight. For long-term storage, use 90:10 acetonitrile:water as the storage solution.

5.4 Operating pH range: pH 2.0 to 9.5

The column is compatible with eluents with pH between 2.0 and 9.5, but longer service life can be expected for pH between 3.0 and 8.5.

5.5 Operating temperature: 5 to 50 ºC

The typical temperature for routine analysis is between 20 to 35 °C. To extend the column lifetime, elevated temperature is not recommended and should be avoided.

5.6 Flow rate and pressure

The columns can withstand up to 4000 psi (276 bar), and flow rates up to 1.2 mL/min.

5.7 Column washing procedure

With use, the column may accumulate contaminating ions, organic substances, or particulate matter. As a result, it may exhibit poor peak shape, changes in retention time or excessive back pressure. The following column washing steps may be attempted to restore normal function. Use a 5-minute gradient between eluent conditions.

Cleaning Conditions	To remove contaminants	Time (min)	Flow rate (mL/min)
50:50 acetonitrile : water (reverse the column to flush out to waste)	particulate	8 – 10	0.4
1 mM methanesulfonic acid (cation MPIC) or 1 mM sodium borate buffer (anion MPIC)	ionic	8 – 10	0.8
100 % acetonitrile	hydrophobic	8 – 10	0.8
Eluent (re-equilibration)		>20	0.8

NOTE

If above treatments fail to improve the column performance, replace it with a new one.

6. Frequently Asked Questions

6.1 What is the IonPac NS2 Column?

The IonPac NS2 column is a silica-based, reversed-phase column designed for Mobile-Phase Ion Chromatography, and compatibility with suppressed conductivity detectors.

6.2 What are the differences between IonPac NS1 and IonPac NS2 Columns?

The IonPac NS1 and IonPac NS2 columns are both intended for MPIC, but there are some differences. The IonPac NS1 column uses a polymeric stationary phase, and the IonPac NS2 column uses a bonded silica stationary phase. The IonPac NS1 column has a pH range of 0 – 14; the IonPac NS2 column has a pH range of 2.0 – 9.5. The IonPac NS1 column is strongly hydrophobic; the IonPac NS2 column is moderately hydrophobic. The IonPac NS1 column is available in 10 or 5 µm particles; the IonPac NS2 column is available in 5 µm particles. The IonPac NS2 column usually offers better efficiency than IonPac NS1column, especially for aromatic analytes. The IonPac NS2 column can be used for neutral analytes with other detectors such as UV or charged aerosol.

6.3 How does the IonPac NS2 Column work?

MPIC is a technique where a hydrophobic ion-pairing agent in the eluent provides retention of oppositely-charged analyte ions on the hydrophobic stationary phase. The MPIC agent partitions into the stationary phase, giving it enough charge to retain the analyte ions. This "dynamic" ion-exchange capacity is determined by the type and concentration of MPIC agent, and the type and concentration of solvent. Increased concentration of MPIC agent or decreased concentration of solvent will increase the ion-exchange capacity. The MPIC agent is chosen for compatibility with suppressed conductivity detection; typically a hydrophobic, strong acid is used for cation analysis; typically a borate buffer is used for anion analysis.

6.4 When do I need the IonPac NS2 Column?

When the analytical problem involves hydrophobic ions or polyvalent ions where conventional ion chromatography shows excessive retention or unacceptable peak shape, the MPIC technique should be considered.

6.5 What factors should I consider for method development using the IonPac NS2 Column?

Read section 1.4 and look at the example application in section 7, then read section 4.

6.6 What mobile phases should I use with the IonPac NS2 Column?

The IonPac NS2 column is compatible with eluents in the range of pH 2.0 – 9.5, and with most water-miscible solvents. Solvents that attack PEEK (such as THF) should be kept below 20%.

6.7 What should I do before starting using the IonPac NS2 Column?

Read section 1 for general advice. Read section 2 for startup advice with cation analysis or read section 3 for startup advice with anion analysis.

6.8 How to store the IonPac NS2 Column?

Eluent is acceptable for overnight storage. Long term storage should be in ≥70% acetonitrile.

6.9 What detectors can be used with the IonPac NS2 Column?

The IonPac NS2 column is intended for suppressed conductivity detection, but it may be used with other IC or HPLC detectors.

6.10 What detection limit I should expect from the IonPac NS2 Column?

The detection limits will vary substantially depending on the maximum injection volume, the concentration of organic solvent in the eluent, the mobility of the organic analyte, and whether the analyte is weakly or strongly dissociated. Quantification limits as low as 0.1 µg/mL have been achieved in favorable cases.

6.11 Must I use a guard column with an IonPac NS2 analytical column?

It is not strictly required, but it is highly recommended to prolong the service life of the analytical column. It is much cheaper to replace a fouled guard column than a fouled analytical column.

6.12 What should I do if the column shows deteriorated performance?

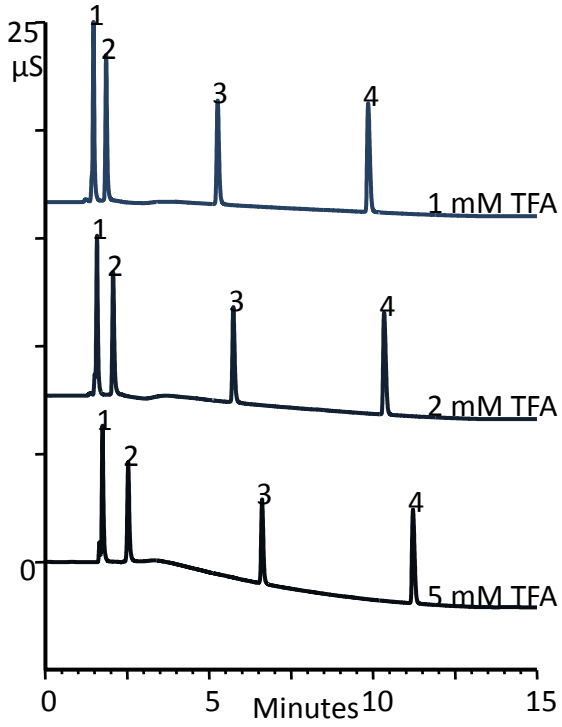
Attempt to clean the column using the recommendations in section 5.7. If the column cannot be cleaned, replace it.

6.13 What should I do if the column exhibits excessively high backpressure?

Attempt to clean the column using the recommendations in section 5.7. If the column cannot be cleaned, replace it. Take appropriate preventive measures.

7. Example Applications

7.1 Effect of Concentration of MPIC-Agent on Cation MPIC



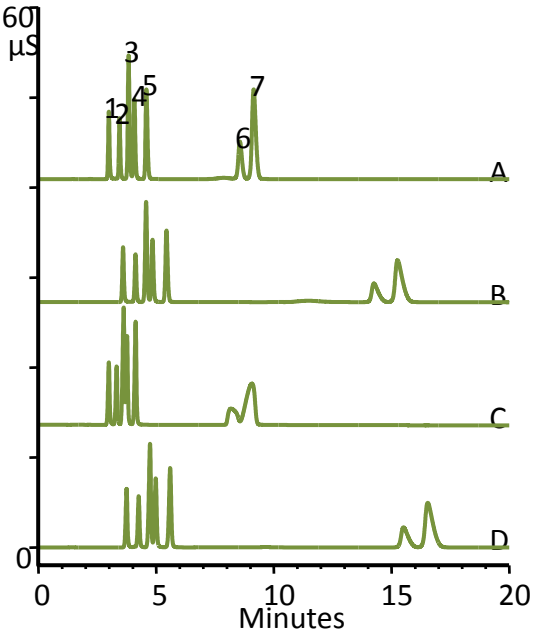
HPLC Conditions

Column:	IonPac NS2			
Dimensions: (PEEK)	5 µm, 4.0 x 150 mm			
System:	ICS 3000			
Mobile Phases:	A: Acetonitrile B: 25mM Trifluoroacetic acid C: Water			
Gradient 1:	-7.0	0.0	11.0	16.0 min.
%A	5	5	60	60
%B	4	4	4	4
%C	91	91	36	36
Gradient 2:	-7.0	0.0	11.0	16.0 min.
%A	5	5	60	60
%B	8	8	8	8
%C	87	87	32	32
Gradient 3:	-7.0	0.0	11.0	16.0 min.
%A	5	5	60	60
%B	20	20	20	20
%C	75	75	20	20
Flow rate:	0.800 mL/min			
Injection:	5 µL			
Temperature:	25 °C			
Detection:	Conductivity			
Suppressor: TBAOH at 3 mL/min	CMMS-4mm; 0.05M			

Peaks:

1. $(CH_3)_4N^+$
2. $(C_2H_5)_4N^+$
3. $(C_3H_7)_4N^+$
4. $(C_4H_9)_4N^+$

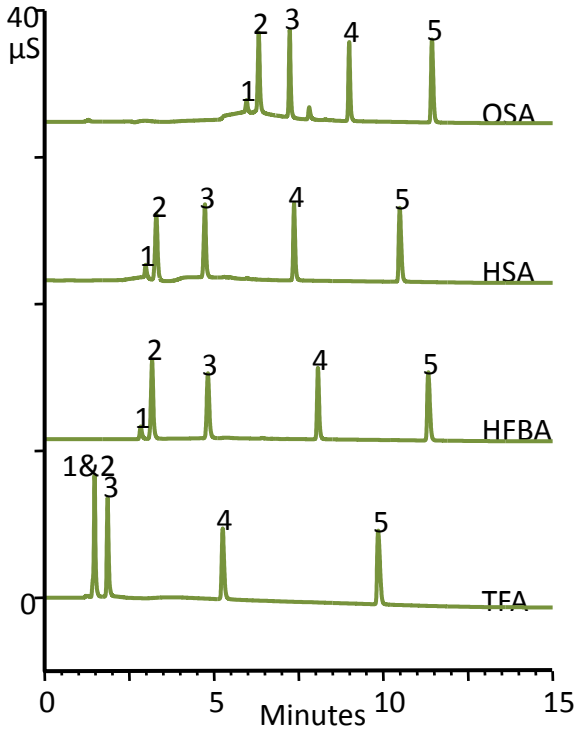
7.2 Effects of MPIC Concentration, Organic Solvent Concentration, and Ionic Strength on Anion-MPIC



HPLC Conditions

Column:	IonPac NS2
Dimensions:	5 µm, 4.0 x 150 mm (PEEK)
System:	ICS 3000
MPIC buffer:	10mM tetrabutylammonium hydroxide + 50 mM boric acid (pH 8.5)
Borate buffer:	20mM sodium hydroxide + 100 mM boric acid (pH 8.5)
Isocratic (A):	10% acetonitrile; 40% MPIC buffer; 20% Borate buffer; 30% water
Isocratic (B):	10% acetonitrile; 40% MPIC buffer; 50% water
Isocratic (C):	10% acetonitrile; 20% MPIC buffer; 70% water
Isocratic (D):	7% acetonitrile; 20% MPIC buffer; 73% water
Flow rate:	0.800 mL/min
Injection:	2 µL
Temperature:	25 °C
Detection:	Conductivity
Suppressor:	AMMS-4mm; 0.05 N sulfuric acid at 2 mL/min
Peaks:	1. Fluoride 2. Chloride 3. Nitrite 4. Bromide 5. Nitrate 6. Phosphate 7. Sulfate

7.3 Effect of Hydrophobicity of Ion-Pairing Agent



HPLC Conditions

Column:	IonPac NS2
Dimensions:	5 µm, 4.0 x 150 mm (PEEK)
System:	ICS 3000
Mobile Phases:	A: Acetonitrile B: 10mM MPIC agent C: Water

Gradient:	-7 min.	0	11	16
%A	5	5	60	60
%B	10	10	10	10
%C	85	85	30	30

Flow rate:	0.800 mL/min
Injection:	5 µL
Temperature:	25 °C
Detection:	Conductivity
Suppressor:	CMMS-4mm; 0.05M TBAOH at 3 mL/min
Peaks:	1. Na^+ 2. $(CH_3)_4N^+$ 3. $(C_2H_5)_4N^+$ 4. $(C_3H_7)_4N^+$ 5. $(C_4H_9)_4N^+$

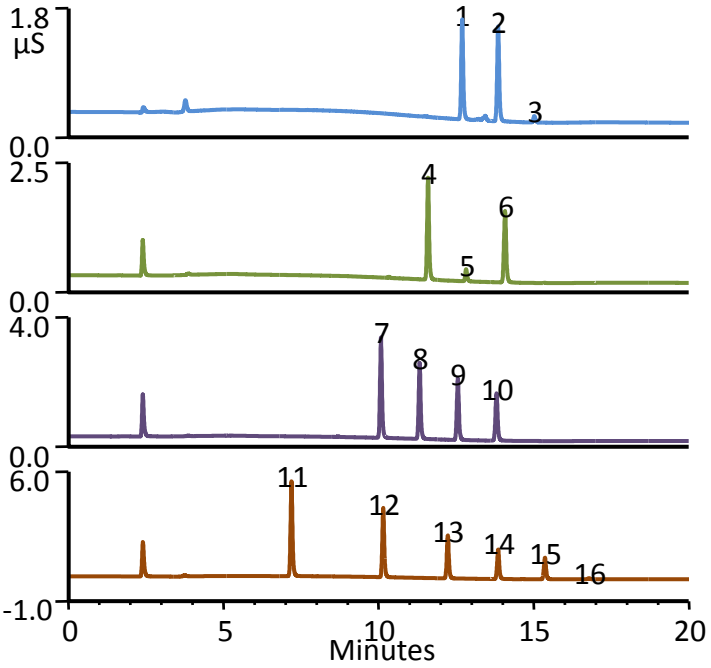
7.4 Cation-MPIC of Quaternary Surfactants

HPLC Conditions

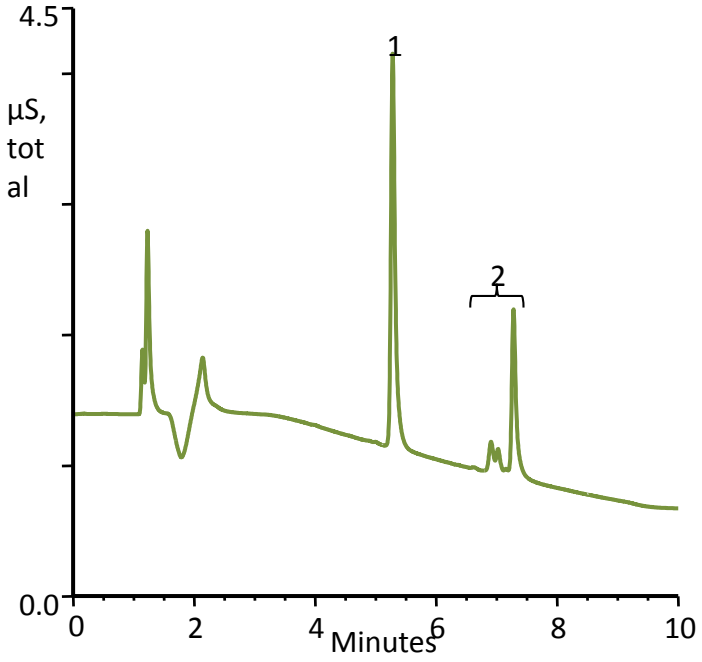
Column:	IonPac NS2			
Dimensions:	5 µm, 4.0 x 150 mm (PEEK)			
System:	ICS 3000			
Mobile Phases:	A: Acetonitrile			
	B: 10 mM Heptafluorobutyric acid			
	C: Water			
Gradient 1:	-8.0 min.	0.0	14.0	20.0
%A	5	5	80	80
%B	10	10	10	10
%C	85	85	10	10
Flow rate:	0.800 mL/min			
Injection:	5 µL			
Temperature:	25 °C			
Detection:	Conductivity			
Suppressor: TBAOH at 3 mL/min	CMMS-4mm; 0.05 M			

Peaks:

1. C_{12} Bz Me_2 N+
2. C_{14} Bz Me_2 N+
3. C_{16} Bz Me_2 N+
4. C_{12} pyridinium
5. C_{14} pyridinium
6. C_{16} pyridinium
7. C_{10} Me_3 N+
8. C_{12} Me_3 N+
9. C_{14} Me_3 N+
10. C_{16} Me_3 N+
11. $(C_3H_7)_4N$+
12. $(C_4H_9)_4N$+
13. $(C_5H_{11})_4N$+
14. $(C_6H_{13})_4N$+
15. $(C_7H_{15})_4N$+
16. $(C_8H_{17})_4N$+



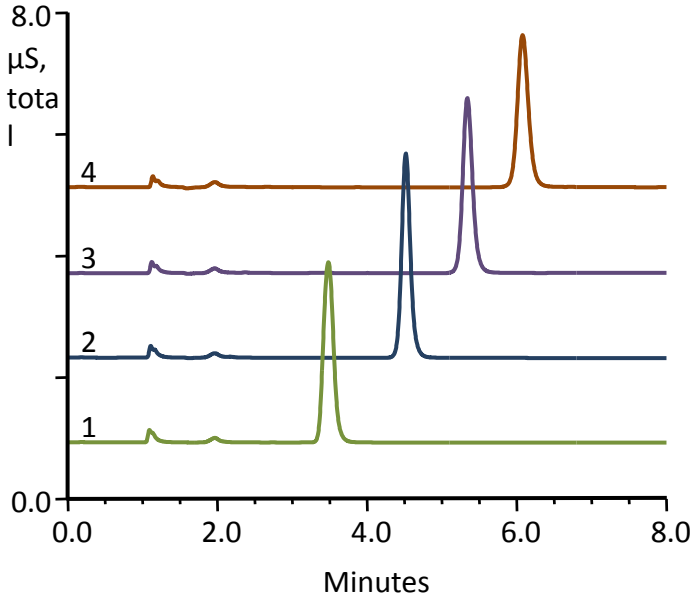
7.5 Separation of Perfluorinated Acids



HPLC Conditions

Column:	IonPac NS2			
Dimensions:	5 µm, 4.0 x 150 mm (PEEK)			
System:	ICS 3000			
Mobile Phases:	A: Acetonitrile			
	B: 20 mM sodium hydroxide + 100 mM boric acid (pH 8.5)			
	C: Water			
Gradient :	-8.0	0.0	7.0	10.0
%A	25	25	50	50
%B	50	50	50	50
%C	25	25	0	0
Flow rate:	0.800 mL/min			
Injection:	10 µL			
Temperature:	25 °C			
Detection:	Conductivity			
Suppressor: mL/min	AMMS-4mm; 0.02 N sulfuric acid at 2			
Sample:	Standards 50 µg/mL in 50% methanol			
Peaks:	1. Perfluorooctanoic acid			
	2. Perfluorooctanesulfonic acid			

7.6 Effect of Buffer Concentration on Performance for Anion Analysis



HPLC Conditions

Column:	IonPac NS2
Dimensions:	5 µm, 4.0 x 150 mm (PEEK)
System:	ICS 3000
Buffer:	20 mM sodium hydroxide + 100 mM boric acid (pH 8.5)
Isocratic	1: 35% acetonitrile, 20% buffer, 45% water
	2: 35% acetonitrile, 30% buffer, 35% water
	3: 35% acetonitrile, 40% buffer, 25% water
	4: 35% acetonitrile, 50% buffer, 15% water
Flow rate:	0.800 mL/min
Injection:	10 µL
Temperature:	25 °C
Detection:	Conductivity
Suppressor:	AMMS-4mm; 0.02 N sulfuric acid at 2 mL/min
Sample:	Sodium dodecyl sulfate, 50 µg/mL

Performance:

mM Na^+	Retention Background	Asym. Noise	Width	Efficiency
4	3.48 0.926	1.14 0.0012	0.24	3035
6	4.52 0.959	1.12 0.0014	0.21	7421
8	5.34 1.002	1.14 0.0014	0.24	7946
10	6.08 1.051	1.11 0.0026	0.27	8204