ThermoFisher SCIENTIFIC

(U)HPLC columns

Acclaim mixed-mode WCX-1 HPLC columns

Product manual

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Safety and special notices

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

Safety and special notices defined

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 devices.

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 devices, 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

Introduction

Introduction

The Thermo Scientific™ Acclaim™ Mixed-Mode WCX-1 Column is based on a new mixed-mode silica-based packing material that incorporates both hydrophobic and weak cation-exchange properties. Unlike traditional reversed-phase stationary phases, the new packing features an alkyl long chain with an ionizable terminus, and demonstrates great potentials for separating a wide range of cationic compounds containing mixtures, including pharmaceuticals, food & beverage, chemical, and more.

Comparison of mixed-mode chromatography and reversed-phase, ion-exchange, and ion-paring chromatography

Reversed-phase (RP) silica columns (e.g. C18) are the most widely used stationary phases for a wide range of liquid chromatography (LC) separations. However, hydrophilic ionic compounds such as catecholamines, small organic acids or inorganic ions are poorly retained and separated on these columns.

Ion exchange columns are used to separate ionic or ionizable compounds such as proteins, nucleic acids, inorganic ions, small organic acids, etc. Because most conventional ion-exchange stationary phases provide inadequate hydrophobic retention for neutral molecules, they have limited applications in small molecules separations.

Ion pairing chromatography is a method for separating ionic or ionizable compounds on a conventional RP medium, which requires hydrophobic ionic compounds, typically comprised of an alkyl chain with an ionizable terminus, are added to the mobile phase. Generally, retention of neutral analytes is nearly unaffected, while analytes with charges complementary to the ion pairing reagent are retained for a longer period of time and analytes with the same charge as the ion pairing reagent are retained for a shorter period of time. Limitations of ion pairing chromatography include long column equilibration times and the quantity of solvent and time needed to elute the ion pairing reagent from the column.

Mixed-mode chromatography combines aspects of ion exchange chromatography and conventional reversed-phase chromatography. A mixed-mode stationary phase has both hydrophobic and ion-exchange properties. These two strong interactions of the phase with analytes allow for the independent control of the retention of ionizable and neutral molecules. As a result, many application challenges involving hydrophilic ionizable compounds that are difficult for C18 columns, can be easily tackled on a mixed-mode column.

Table 1. Specifications and operating conditions

Description	Parameters
Shipping solution	100% acetonitrile
Storage solution (recommended)	100% acetonitrile
pH range	2.5 – 7.0
Recommended operating pH	3 – 6.5
Temperature range	< 50 °C
Recommended operating temperature	< 40 °C
Recommended operating pressure	< 3500 psi

Table 2. Columns recommended parameters

Column	Maximum pressure (psi)	Typical flow rate (mL/min)
3 µm, 3.0 x 50 mm	4500	0.2 – 1.2
3 µm, 3.0 x 150 mm	5800	0.2 – 1.2
3 µm, 2.1 x 150 mm	5800	0.1 – 0.60
5 µm, 2.1 x 150 mm	5800	0.1 – 0.60
5 µm, 4.6 x 150 mm	5800	0.5 – 3.0
5 µm, 4.6 x 250 mm	5800	0.5 – 3.0

Table 3. Physical characteristics

Description	Parameters
Bonding chemistry	Proprietary alkyl carboxylic group
Silica substrate	Spherical, porous, high-purity
Particle size	5 µm
Surface are	300 m^2/g
Pore size	120 Å

Installation: step-by-step-user guide

Step-by-step-user guide

Thermo Fisher recommends that you perform an efficiency test on your Acclaim Mixed-Mode WCX-1 column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Steps 1–5 below outlinethe 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 that slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 1 - Visually inspect the column

Report any visual damage to Thermo Fisher Scientific.

Step 2 - Mobile phase preparation

Obtaining reliable, consistent and accurate results require mobile phases that are free of ionic and spectrophotometric impurities. Chemicals, solvents, and deionized water used to prepare mobile phase should be of the highest purity available. Maintaining low trace impurities and low particle levels in mobile phases helps to protect your columns and system components. Optimal column performance cannot be guaranteed if the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

Deionized water

The deionized water used to prepare the mobile phase should be Type 1 Reagent Grade water, or HPLC Grade Water. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

Note

Degas the aqueous component of the mobile phase and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents, if possible, since the volatile solvent can be 'boiled' off from the solution.

Solvents

The solvents used must be free from ionic and UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent.

Mobile phase for column performance test

Depending on specific application, the mobile phase system consists of an organic modifier (e.g. acetonitrile or methanol) and a buffer (e.g. phosphate buffer). Both pre-mixed and proportioning valve generated mobile phases give satisfactory results. The use of proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides less baseline noise.

Example A. Preparation of 50 mM, pH 6 phosphate buffer

1. Weigh 13.6 g potassium monobasic phosphate.
2. Completely dissolve above salt in 2000 g of DI water.
3. Carefully adjust the solution to pH 6 with HCl or NaOH.

Example B. Preparation of 100 mM, pH 5 ammonium acetate buffer

1. Weigh 50 g ammonium acetate buffer (2M, pH 5.4)
2. Add 950 g of DI water to above solution.

Installation: step-by-step-user guide (continued)

Step 3 - Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a UV detector, and an injector (or an autosampler). The system should be thoroughly primed before use.

Step 4 - Condition the column

The column is shipped in an acetonitrile-ammonium acetate mixture. When a new column is used for the first time, it should be washed thoroughly with the mobile phase (e.g., for at least 30 min at 1 mL/min) before any injection is made. When switching to a new mobile phase, make sure that the new mobile phase is compatible with the previous mobile phase in the column to avoid column clogging due to precipitation. The column should be fully conditioned before any injection is made (e.g. 30 min at 1 mL/min).

Step 5 - 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. After the column is fully equilibrated, multiple injections should be made until the reproducible retention is obtained. Keep a record of the column performance for future reference.

Note

Due to various reasons, such as difference of LC systems, mobile phases, oven temperature control, etc, you may observe slightly different retention time for the iodide peak from that in the report.

Step 6 - Real sample analysis

Once the satisfactory result is obtained, the column is ready for use.

Installation: step-by-step-user guide (continued)

Quality assurance report examples

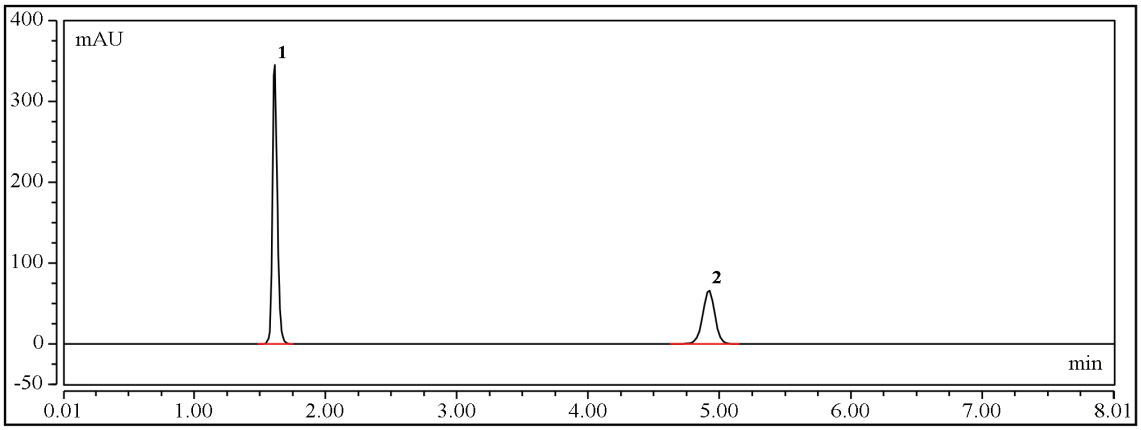
QAR example 1 - 4.6 x 150 mm

Acclaim Mixed-Mode WCX-1
5µm 120Å (4.6 x 150 mm)
Product No. 068353

Date: 08-May-25 07:32
Serial No. : 009682
Lot No. : 02324160

Mobile Phase: 50:50 v/v Acetonitrile:0.10 M NH4OAc, pH 5.4
Flow Rate: 1.00 mL/min **Temperature:** 30 °C
Detection: UV, 254 nm **Injection Volume:** 5.0 µL

Storage Solution: Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (1) (EP)	Concentration
1	Iodide	1.6	1.17	9437	100
2	Naphthalene	4.9	0.96	14001	100

QA Results:

Analyte	Parameter	Specification	Results
Naphthalene	Efficiency	>=10,800	Passed
Naphthalene	Asymmetry	0.95-1.32	Passed
Naphthalene	Retention Time	4.8-5.8	Passed
	Pressure	<=1320	694

Production Reference:
Datasource: Acclaim7
Directory: Silica4\Silica4_1
Sequence: 2029621_068353_NL
Sample No: 2 7.2.10.24543

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Installation: step-by-step-user guide (continued)

Quality assurance report examples

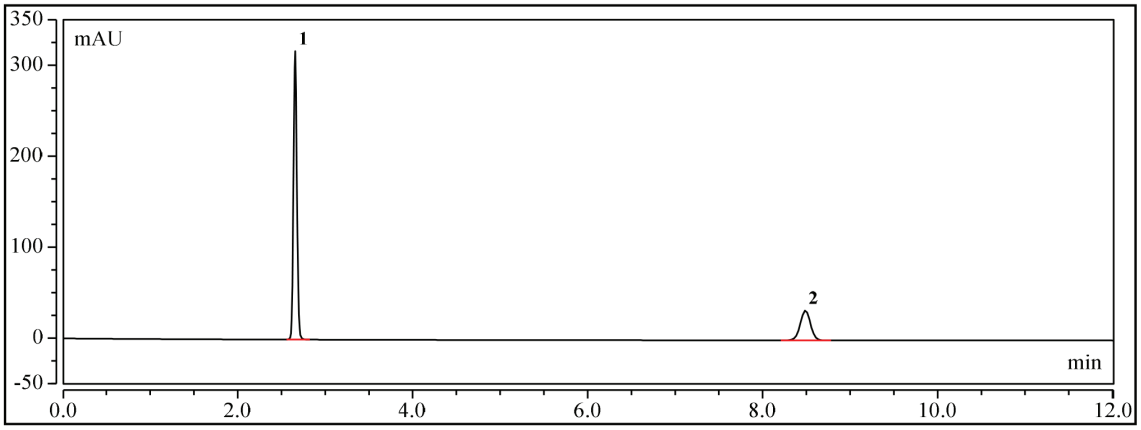
QAR example 2 - 4.6 x 250 mm

Acclaim Mixed-Mode WCX-1
5µm 120Å (4.6 x 250 mm)
Product No. 068352

Date: 17-May-24 08:38
Serial No. : 001718
Lot No. : 02324052

Mobile Phase: 50:50 v/v Acetonitrile:0.10 M NH4OAc, pH 5.4
Flow Rate: 1.00 mL/min **Temperature:** 30 °C
Detection: UV, 254 nm **Injection Volume:** 5.0 µL

Storage Solution: Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (1) (EP)	Concentration
1	Cytosine	2.7	1.03	21754	100
2	Naphthalene	8.5	0.98	24831	100

QA Results:

Analyte	Parameter	Specification	Results
Naphthalene	Efficiency	>=16,200	Passed
Naphthalene	Asymmetry	0.95-1.32	Passed
Naphthalene	Retention Time	8.0-9.6	Passed
	Pressure	<=1980	1159

Production Reference:
Datasource: Acclaim7
Directory: Silica3\Silica3_3
Sequence: 2020358_068352_OKA
Sample No: 25 7.2.10.25868

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Method development

To optimize chromatographic methods, mobile phase ionic strength, pH, and organic modifier are three key variables that can be adjusted either independently or concurrently.

Ionic strength

Ionic strength is crucial for changing retention of charged molecules. Increase in ionic strength results in retention decrease, very little increase, and virtually no effect for basic, acidic, and neutral molecules, respectively.

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (215 nm)		
Mobile phase	50/50 v/v MeCN/sodium phosphate, pH 6.5		
Peaks	1	Benzoic acid	200 µg/mL
	2	Naphthalene	50 µg/mL
	3	Benzyl amine	300 µg/mL

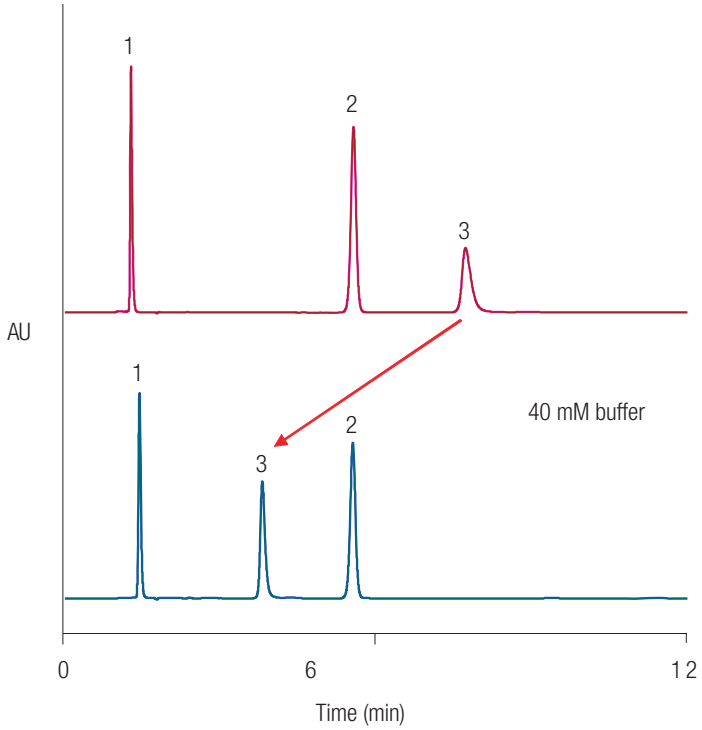

Figure 1. Adjustable selectivity - ionic strength effect

Organic modifier

Hydrophobic retention is markedly affected by organic modifier composition in the mobile phase. In general, all types of molecules (acids, bases, and neutrals) are less retained with increase in organic content in the mobile phase to different extents when keeping other conditions constant (e.g. ionic strength, pH, temperature, etc).

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (215 nm)		
Mobile phase	MeCN/30 mM sodium phosphate, pH 6.5		
Peaks	1	Benzoic acid	200 µg/mL
	2	Naphthalene	50 µg/mL
	3	Benzyl amine	300 µg/mL

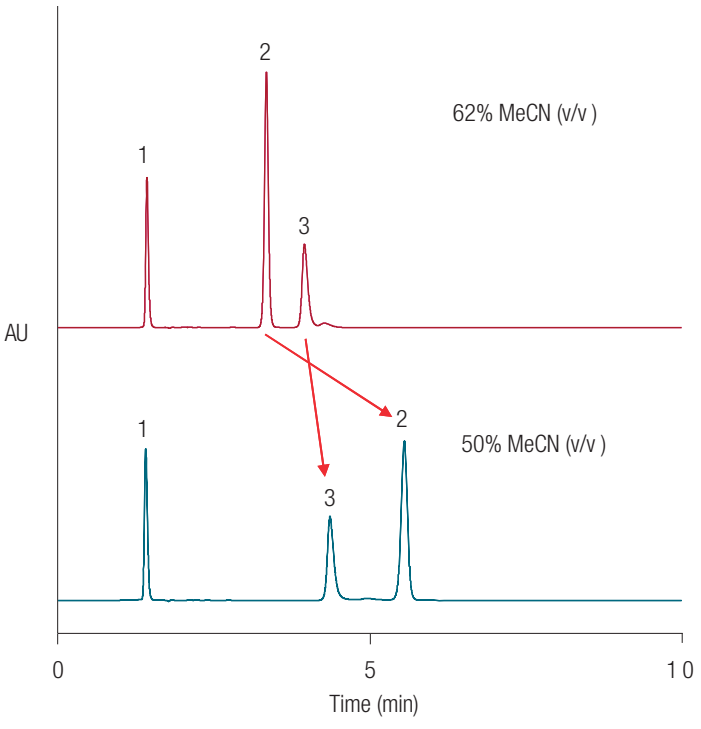

Figure 2. Adjustable selectivity - organic modifier effect

Method development (continued)

Mobile phase pH

Mobile phase pH affects the charge and hydrophobicity of the stationary phase. At a pH below the pKa of the stationary phase carboxylic group, the cation-exchange functionality is "OFF" so that hydrophobic interaction is the primary retention mechanism. At a pH above the pKa of the stationary phase carboxylic group, the cation-exchange functionality is "ON" so that both cation-exchange and hydrophobic interaction contribute to retention depending on the structures of analytes. Therefore, selectivity can be facilitated by modifying mobile phase pH.

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (215 nm)		
Mobile phase	50/50 v/v MeCN/10 mM sodium phosphate		
Peaks	1	Benzoic acid	200 µg/mL
	2	Naphthalene	50 µg/mL
	3	Benzyl amine	300 µg/mL

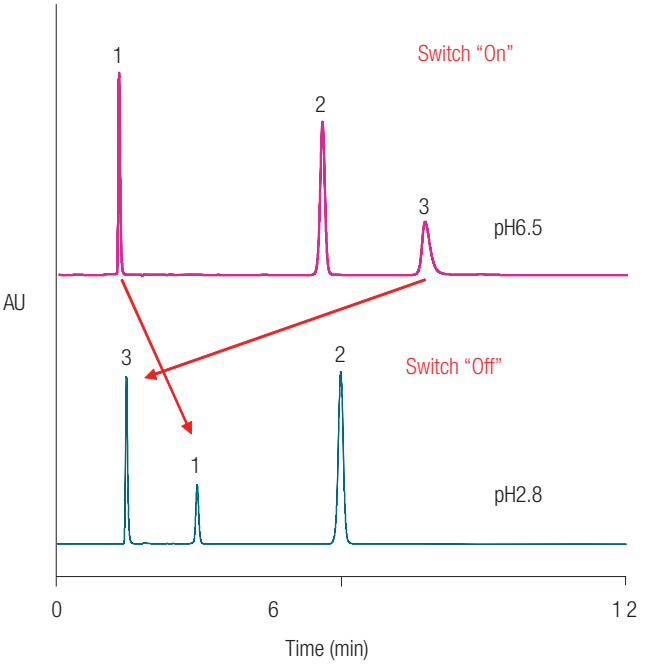

Figure 3. Adjustable selectivity - pH effect

Buffer types

The Acclaim Mixed-Mode WCX column should be used in buffered mobile phases. Within its operating pH range (pH 2.5 to 7.0), the column is compatible with a wide collection of typical HPLC mobile phases (e.g. phosphate buffers, acetate buffers, and etc.

Isocratic vs. gradient

For many applications that involve fewer than three molecules, it is usually easier to develop an isocratic method on the Acclaim Mixed-Mode WCX column than a RP column. For a more complicated separation, such as the one that concerns a mixture of molecules with different type and number of charge, as well as different hydrophobicity, a gradient method may be advantageous. In practical, ionic strength gradient, organic modifier gradient, or a combination of both has proven to be satisfactory with respect of reproducibility and simplicity.

HILIC mode

The Acclaim Mixed-Mode WCX column can operate in HILIC mode (Figure 4). In this mode, acetonitrile (not methanol) should be used in a range of 80 to 95% acetonitrile. The elution power can be modified by the employment of a polar solvent, such as an aqueous buffer. Using this column in HILIC mode provide increased retention for highly polar molecules. The higher the organic content in mobile phase, the higher the retention for a highly polar analyte.

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (270 nm)		
Mobile phase	MeCN/NH4OAc, pH5 (5 mM total) v/v 95/5 for HILIC mode operation v/v 50/50 for RP mode operation		
Peaks	1	Cytosine	100 ppm
	2	Naphthalene	100 ppm

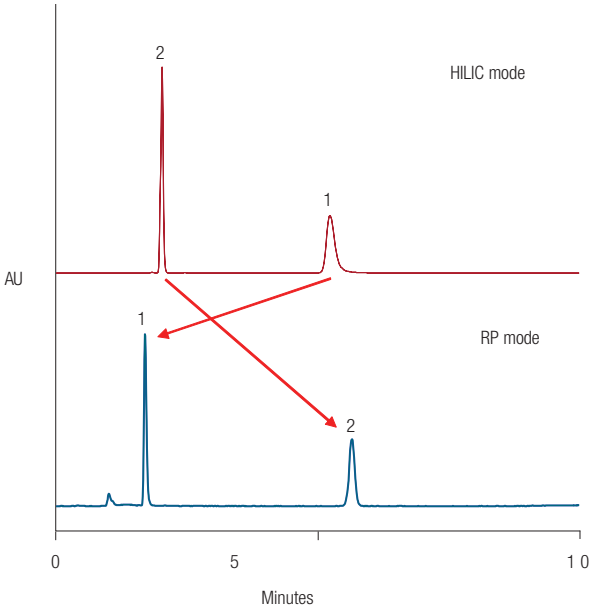

Figure 4. Adjustable selectivity - pH effect

Column care

Mobile phases

Mobile phases should be freshly prepared. All chemicals and solvents should be at the highest available quality. All mobile phases should be filtered before use. In-liner filters are recommended.

Guard cartridges

It is highly recommended that a guard cartridge 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 performance deterioration and short column lifetime.

Column storage

After use, flush with mobile phase without buffer and store in 100% acetonitrile.

Recommended operating pH range - pH 2.5 to 7.0

To obtain better column lifetime, it is highly recommended to use "silica friendlily" mobile phases. While the pH limit of the column is pH 2.5 to 7 the recommended operating pH range is between 3.0 - 6.5.

Recommended operating temperature limit (40 °C)

Although our experimental results indicated that the column could be used at 50 °C, the separation is usually optimized by modifying mobile phase ionic strength, pH, and/or organic modifier content. Elevated temperature is not recommended and should be avoided.

Flow rate and pressure limit

Usually, good column efficiency can be obtained at 1 mL/min at the recommended flow rate (see Table in Section 1.3) while a higher flow rate can be used for fast analysis provided that the pressure limit is not exceeded. It is important not to impose a sudden column pressure surge. Thus increase flow rate gradually from 0.2 mL/min up to the desired flow rate. The pressure limit for the column is 5800 psi.

Column washing procedure

When the column washing practice is needed, such as deteriorated column performance and/or excessively high backpressure, the following procedure can be used as a guideline:

1. Wash the column with 20 mM ammonium (or sodium) acetate buffer, pH 5/ acetonitrile v/v 50/50 for 3 column volumes at the recommended flow rate.
2. Wash the column with 100 mM ammonium (or sodium) acetate buffer, pH5/ acetonitrile v/v 50/50 for 20 column volumes at a flow rate between 0.2 to 1 mL/min (to remove strongly retained cationic species).
3. Wash the column with 0.1% oxalic acid in DI water for 20 column volumes at a flow rate between 0.2 to 1 mL/min (to remove metal contamination).
4. Wash the column with 20 mM ammonium (or sodium) acetate buffer, pH5/ acetonitrile v/v 50/50 for 3 column volumes at a flow rate between 0.2 to 1 mL/min.
5. Wash the column with 20 mM ammonium (or sodium) acetate buffer, pH5/ acetonitrile v/v 25/75 for 20 column volumes at a flow rate between 0.5 to 1 mL/min (to remove strongly retained hydrophobic compounds).
6. Equilibrate the column with the mobile phase.

Note

An ammonium acetate buffer can be used instead of a phosphate buffer if a LC-MS application is intended. If above treatment fails to improve the column performance, replace it with a new one.

Frequently asked questions (FAQs)

What is the Acclaim Mixed-Mode WCX-1 column?

It is a new mixed-mode silica column that incorporates both hydrophobic and weak cation-exchange properties. Its surface chemistry features an alkyl long chain with a carboxylic terminus. This column has demonstrated great potentials for separating a wide range of cationic compounds containing mixtures, including pharmaceuticals, food & beverage, chemical, and more.

Why do I need the Acclaim Mixed-Mode WCX-1 column?

The mixed mode separation mechanism of the Acclaim Mixed-Mode WAX-1 column allows for controlling retention of ionizable and neutral molecules by changing the mobile phase ionic strength, pH, and organic composition, either independently or concurrently. As a result, many application challenges involving hydrophilic ionizable compounds that are difficult for C18 columns can be easily accomplished on this column.

When do I need the Acclaim Mixed-Mode WCX-1 column?

Whenever you encounter a separation involving basic analytes that is difficult and challenging on a regular C18 column, you can consider using the Acclaim Mixed-Mode WCX-1 column.

Here are some situations:

1. Separation of basic molecules, such as antidepressants, catecholamines, some inorganic cations (Li and Na), quaternary amines, etc..
2. Simultaneous separation of an acidic drug and the counterions.
3. You need selectivity orthogonal to a reversed-phase column.
4. Simultaneous separation of a mixture of basic, neutral and acidic molecules.

What factors should I consider for method development using this column?

There are three main factors that affect column selectivity: mobile phase ionic strength, mobile phase pH, and mobile phase organic composition. You can optimize your separation by changing one, two, or all three factors.

What mobile phases should I use with this column?

In principle, this column is compatible with most HPLC mobile phases. Our experimental data indicated that both phosphate buffers and ammonium acetate buffers worked satisfactorily. Depending on the application, the commonly used buffer concentrations range is 5 to 100 mM, and pH range should be in the range 2.5 to 7. When an organic modifier is used, make sure to keep it miscible with the buffer solution.

What should I do before starting using Acclaim Mixed-Mode WCX-1 column?

Read this product manual carefully, and contact Thermo Fisher Scientific Technical Support if you have any questions regarding the use of this column.

What types of basic compounds can be analyzed on this column?

You can use this column to separate a wide range of basic compounds that are difficult to separate on reversed-phase columns, such as antidepressant drugs, catecholamines, some inorganic cations (e.g. Na+ and Ca2+), some quaternary amines.

How to store the column?

Refer to "Column storage" for details.

Can I use this column to analyze acidic molecules?

Yes. Acidic molecules with medium to higher hydrophobicity can be retained and separated on this column at a pH between 2.5 to 4.5 depending on the nature of the analytes.

Frequently asked questions (FAQs) (continued)

Can I use this column to analyze neutral molecules?

Yes. This column provides intermediate hydrophobic retention so that neutral molecules with medium to high hydrophobic retention can be retained sufficiently. For highly hydrophilic/polar molecules, a HILIC mode separation should be considered.

Can I use this column to separate a mixture of basic, acidic, and neutral molecules?

Yes. As shown in Figure 9, the Acclaim Mixed-Mode WCX-1 separates a mixture of basic, neutral, and acidic molecules in a single, with excellent peak shape and resolution. It provides greater flexibility for application method development compared to both conventional reversed-phase and ion-exchange columns.

Do I need a guard cartridge with an Acclaim Mixed-Mode WCX-1 analytical column?

Yes. It is highly recommended to use guard cartridges with an Acclaim Mixed-Mode WCX-1 analytical column. The guard cartridge protects the more expensive analytical column by trapping highly retained components and particulates from the mobile phase or the sample.

What should I do if the column shows deteriorated performance?

Refer to "Column washing procedure" for details.

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

First, make sure that the mobile phase is freshly prepared and filtered before use and that the sample is free of particulates. Then, back flush the column for certain amount of time while monitoring the change in column pressure. If problem persists, purchase a new column.

Examples of orthogonal selectivity

WCX vs. RP – orthogonal selectivity

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (215 nm)		
Mobile phase	50/50 v/v MeCN/10 mM (total) sodium phosphate buffer, pH 6.5		
Peaks	1	Benzoic acid	200 µg/mL
	2	Naphthalene	50 µg/mL
	3	Benzyl amine	300 µg/mL

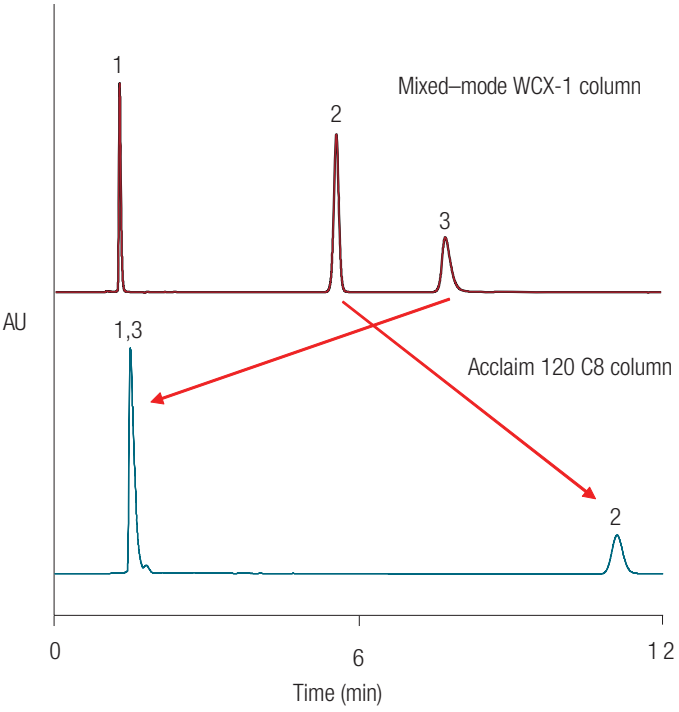

Figure 5. WCX vs. RP – orthogonal selectivity

WCX vs. WAX – orthogonal selectivity

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (215 nm)		
Mobile phase	60/40 v/v MeCN/20 mM sodium phosphate buffer, pH6.5		
Peaks	1	Benzoic acid	200 µg/mL
	2	Naphthalene	50 µg/mL
	3	Benzyl amine	300 µg/mL

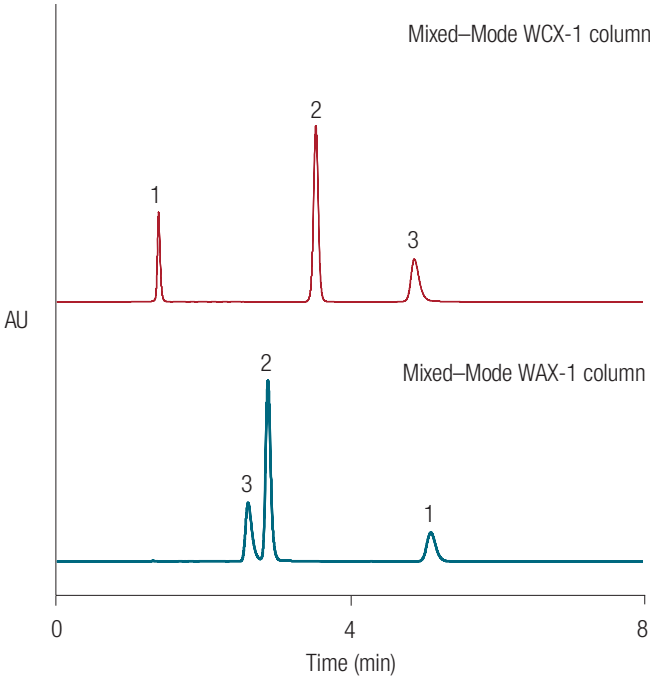

Figure 6. WCX vs. WAX – orthogonal selectivity

Examples of orthogonal selectivity (continued)

WCX vs. WAX – orthogonal selectivity

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)	
Flow rate	1 mL/min
Injection volume	2 µL
Temperature	30 °C
Detection	UV (215 nm)
Mobile phase	60/40 v/v MeCN/sodium phosphate, pH6.5 40 mM for Mixed-Mode WCX-1 10 mM for Mixed-Mode WAX-1
Sample	Trimipramine Maleate (250 ppm
Peaks	1 Maleate
	2 Trimipramine

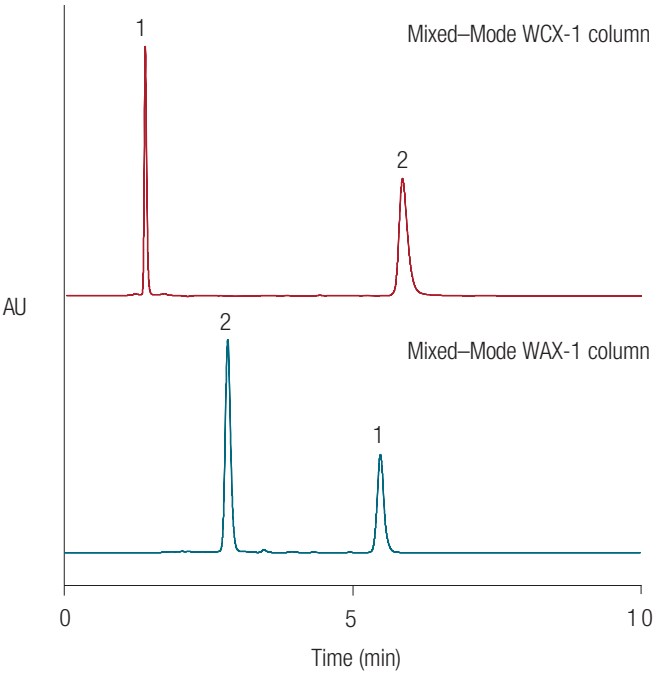

Figure 7. WCX vs. WAX – orthogonal selectivity

Applications

Simultaneous separation of acidic, neutral and basic pharmaceuticals

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (225 nm)		
Mobile phase	40/60 v/v MeCN/NH_4OAc, pH 5.2 (20 mM total)		
Peaks	1	Maleate	50 µg/mL
	2	Ketoprofen	30 µg/mL
	3	Naproxen	30 µg/mL
	4	Hydrocortisone	60 µg/mL
	5	Dexamethasone	60 µg/mL
	6	Oxprenolol	300 µg/mL
	7	Timolol	250 µg/mL

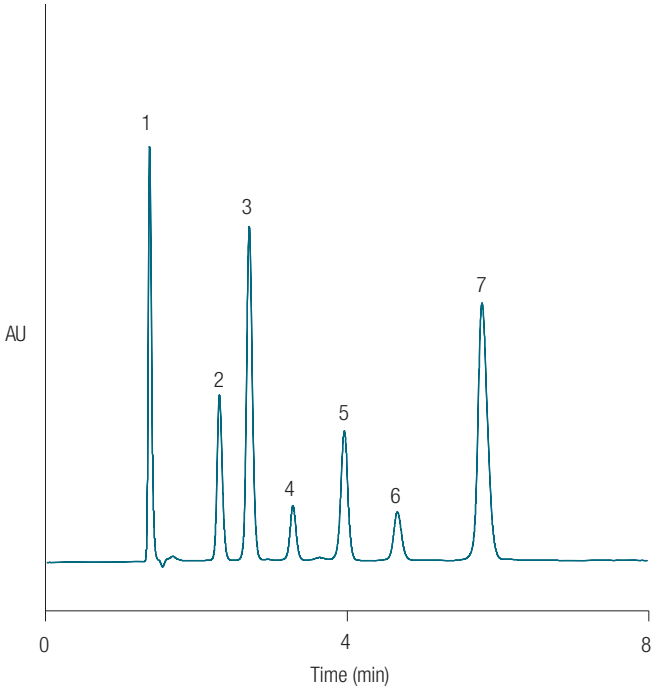

Figure 8. Simultaneous separation of acidic, neutral and basic pharmaceuticals

Separation of catecholamines

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)		
Flow rate	1 mL/min	
Injection volume	5 µL	
Temperature	30 °C	
Detection	UV (215 nm)	
Mobile phase	2/98 v/v MeCN/sodium phosphate, pH6.2 (10 mM total concentration)	
Peaks	1	NE
	2	E
	3	DHBA
	4	DA

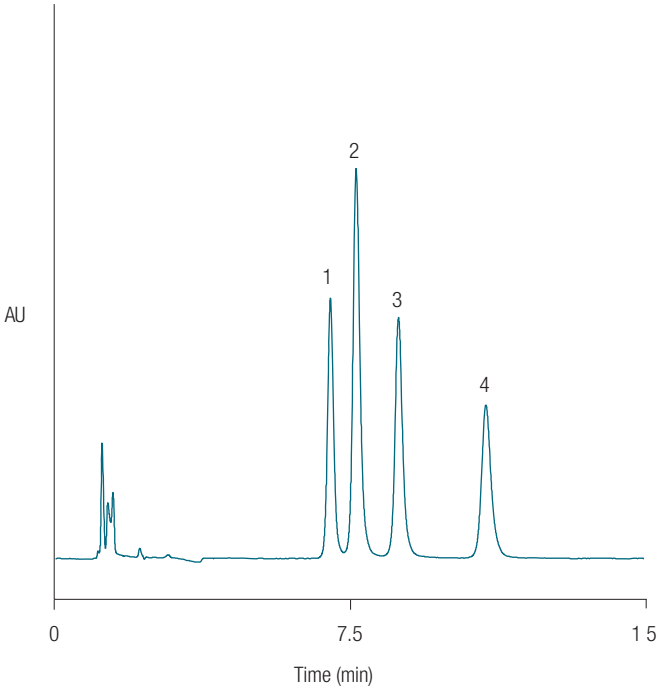

Figure 9. Separation of catecholamines

Applications (continued)

Separation of antidepressants

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	UV (225 nm)		
Mobile phase	50/50 v/v MeCN/10 mM NH_4OAc, pH 5.2		
Peaks	1	Doxepin (mixture of isomers)	100 ppm
	2	Imipramine	100 ppm
	3	Trimipramine	100 ppm
	4	Amitriptyline (As.=1.08, 11623 plates/column)	100 ppm
	5	Protriptyline	100 ppm
	6	Nortriptyline	100 ppm

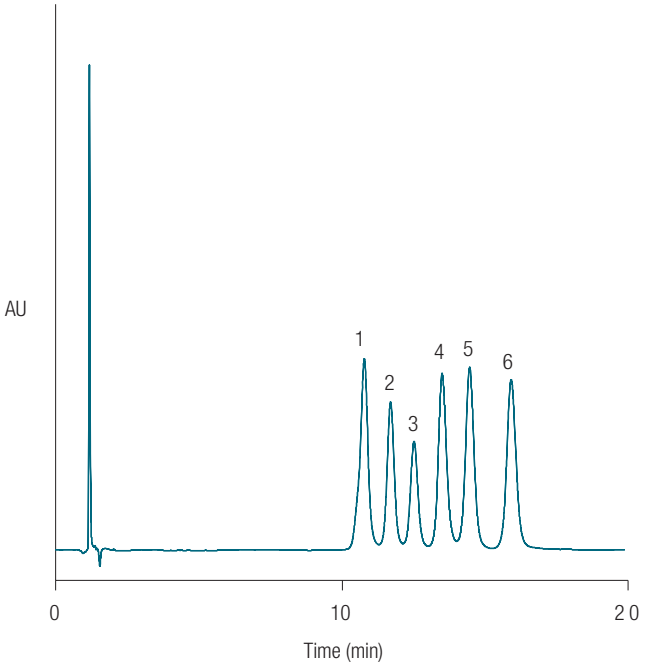

Figure 10. Separation of antidepressants

Analysis of quaternary amines

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)			
Flow rate	1 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	ELS detector		
Mobile phase	50/50 v/v MeCN/NH_4OAc, pH 5.2		
Peaks	1	$(CH_3CH_2CH_2)4N^+$	300 ppm
	2	$(CH_3CH_2CH_2CH_2)_4N^+$	100 ppm
	3	$(CH_3CH_2CH_2CH_2CH_2)_4N_+$	100 ppm

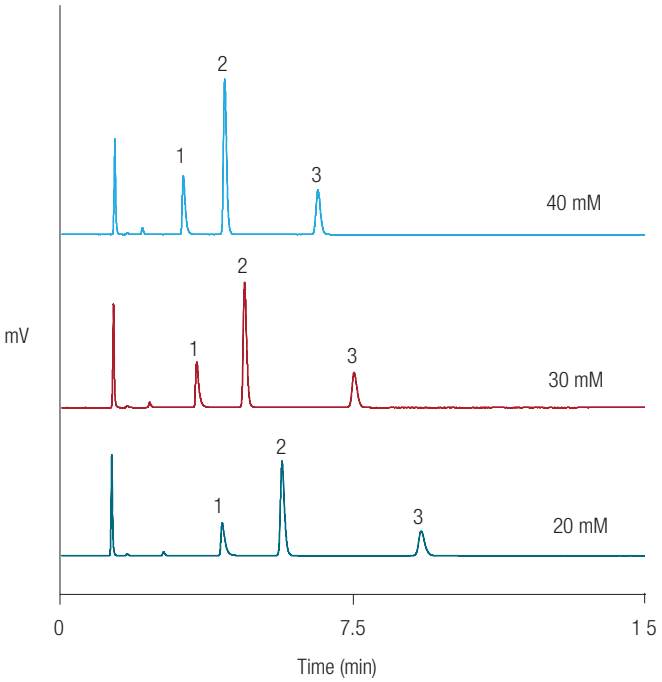

Figure 11. Analysis of quaternary amines

Applications (continued)

Analysis Tris HCl salt

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)		
Flow rate	1 mL/min	
Injection volume	5 µL	
Temperature	30 °C	
Detection	ELS detector	
Mobile phase	50/50 v/v MeCN/NH_4OAc, pH 5.2	
Sample	Tris HCl (1 mg/mL)	
Peaks	1	Cl^-
	2	$TrisH^+$

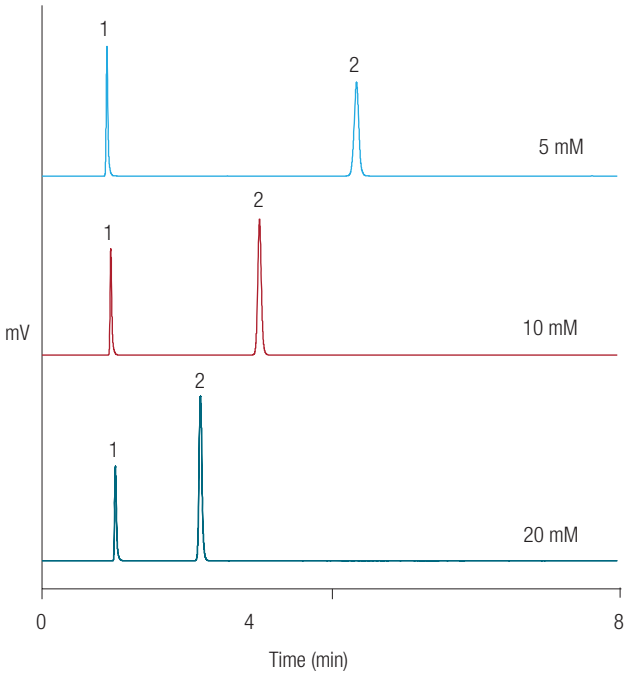

Figure 12. Analysis Tris HCl salt

Analysis of alkyl phosphonium salt

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)		
Flow rate	1 mL/min	
Injection volume	2 µL	
Temperature	30 °C	
Detection	ELS detector	
Mobile phase	60/40 v/v MeCN/NH_4OAc, pH 5.2	
Sample	Tetrabutylphosphonium bromide (0.1%)	
Peaks	1	Br^-
	2	$(CH_3CH_2CH_2CH_2)_4P^+$

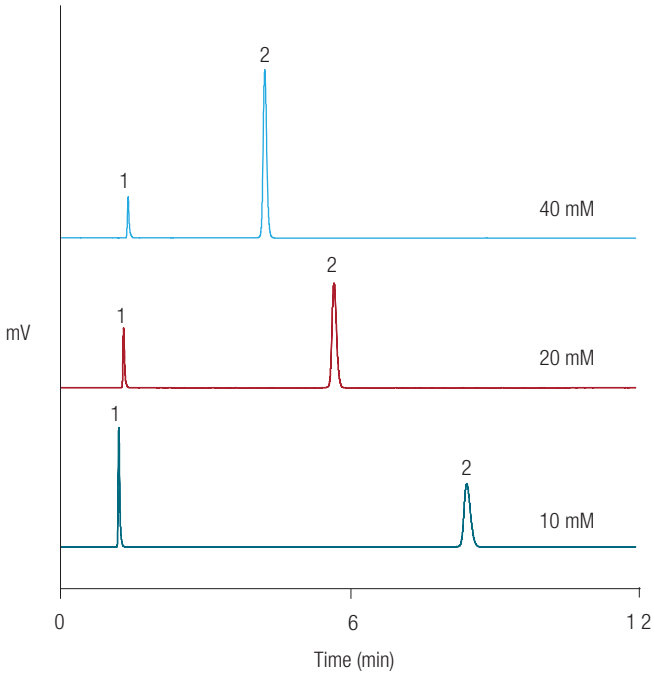

Figure 13. Analysis of alkyl phosphonium salt

Applications (continued)

Analysis of glucosamine tablet

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)		
Flow rate	1 mL/min	
Injection volume	2 µL	
Temperature	30 °C	
Detection	ELS detector	
Mobile phase	50/50 v/v MeCN/NH_4OAc, pH 5.2, 5 mM (total)	
Sample	Glucosamine tablet	
Sample preparation	1	Finely grind a 1500 mg tablet
	2	Mix 0.2 g material in 7.5 mL DI water and 2.5 mL MeOH
	3	Sonicate for 20 min
	4	Dilute 20 times
	5	Filter through 0.1 µm membrane filter

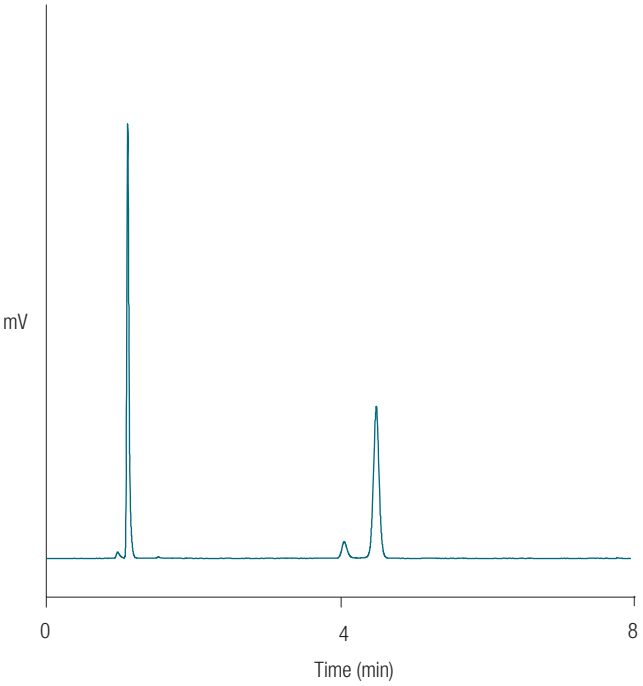

Figure 14. Analysis of glucosamine tablet

Analysis of NaCl – ionic strength effect

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)		
Flow rate	1 mL/min	
Injection volume	5 µL	
Temperature	30 °C	
Detection	ELS detector	
Mobile phase	50/50 v/v MeCN/NH_4OAc, pH 5	
Sample	NaCl (20 mM)	
Peaks	1	Cl^-
	2	Na^+

* Total concentration

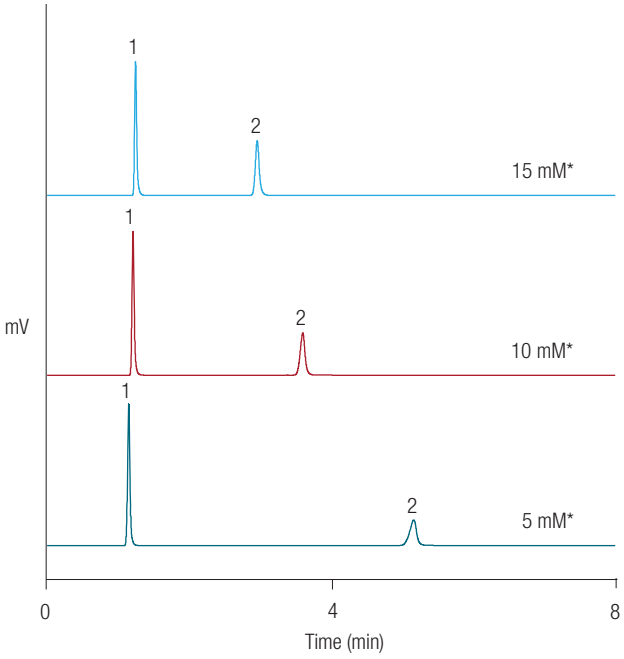

Figure 15. Analysis of NaCl – ionic strength effect

Applications (continued)

Analysis of $CaCl_2$ – pH effect

Acclaim Mixed-Mode WCX-1 column, 5 µm (4.6 x 150 mm)	
Flow rate	1 mL/min
Injection volume	2 µL
Temperature	30 °C
Detection	ELS detector
Mobile phase	10 mM NH_4OAc buffer
Sample	Glucosamine tablet
Sample preparation	1 Cl^-
	2 Ca^+

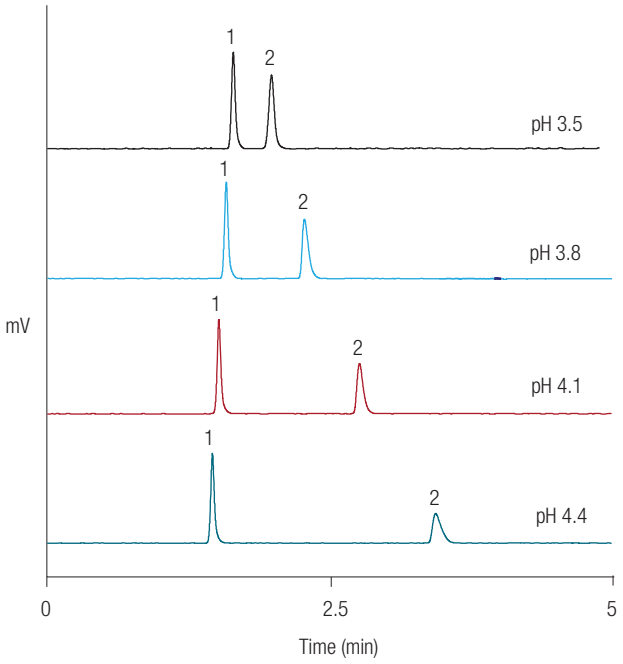

Figure 16. Analysis of $CaCl_2$ – pH effect

Ordering information

Acclaim Mixed-Mode WCX-1 columns ordering information

	Particle size	Column format	Holder required	Quantity	Cat. no
Analytical column	3 µm	3.0 x 50 mm	—	Each	071910
		3.0 x 150mm	—	Each	070092
		2.1 x 150 mm	—	Each	070093
	5 µm	2.1 x 150 mm	—	Each	068371
		4.6 x 150 mm	—	Each	068353
		4.6 x 250 mm	—	Each	068352
Guard column	5 µm	3.0 x 10 mm	Yes	Each	071911
		4.6 x 10 mm	Yes	Each	069705

Thermo Scientific™ Acclaim™ Guard Holder and Coupler ordering information

Description	Quantity	Cat. no
Acclaim Guard Cartridge Holder V-2	Each	069580
Acclaim Guard Cartridge Coupler V-2	Each	074188
Acclaim Guard Cartridge Holder-Coupler Kit V-2 Kit consists of (P/N 069580 + P/N 074188)	Each	069707

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