PRODUCT MANUAL

for

Acclaim®
Mixed-Mode WCX-1

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Product Manual

for

Acclaim® Mixed-Mode WCX-1 Columns

Acclaim Mixed-Mode WCX-1, 3µm, Analytical column, 3.0 x 50mm (P/N 071910)
Acclaim Mixed-Mode WCX-1, 3µm, Analytical column, 3.0 x 150mm (P/N 070092)
Acclaim Mixed-Mode WCX-1, 3µm, Analytical column, 2.1 x 150mm (P/N 070093)

Acclaim Mixed-Mode WCX-1, 5µm, Analytical column, 2.1 x 150mm (P/N 068371)
Acclaim Mixed-Mode WCX-1, 5µm, Analytical column, 4.6 x 150mm (P/N 068353)
Acclaim Mixed-Mode WCX-1, 5µm, Analytical column, 4.6 x 250mm (P/N 068352)

Acclaim Mixed-Mode WCX-1, 5µm, Guard column, 4.3 x 10mm (P/N 068354)
Acclaim Mixed-Mode WCX-1, 5µm, Guard column, 3.0 x 10mm (P/N 071911)
Acclaim Mixed-Mode WCX-1, 5µm, Guard column, 4.6 x 10mm (P/N 069705)

Document No. 065221
Revision 02
July 2009

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SECTION 1 – INTRODUCTION

The 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.

1.1. 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 (see figure 1). These two strong interactions of the phase with analytes allow for controlling retention of ionizable and neutral molecules independently. 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.

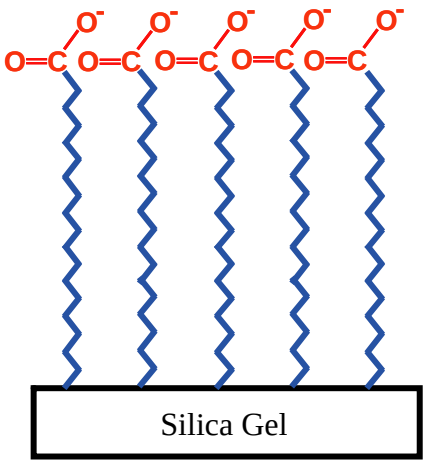


Figure 1
Acclaim Mixed-Mode WCX-1

1.2. Features

Adjustable selectivity (Figures 2-4)
Selectivity complementary to RP columns (Figure 6)
Orthogonal Selectivity (Figures 7-8)
Ideal selectivity for separating basic molecules (Figures 10-14)
Multimode separation mechanism: reversed-phase, cation-exchange, anion-exclusion, and HILIC (Figures 5, 16 and 17)
Simultaneous separation of acidic, neutral and basic compounds (Figure 9)

1.3. Specifications and Operating Conditions

Shipping solution: 50/50 v/v Acetonitrile/0.1M Ammonium acetate, pH5
Storage solution (recommended): 50/50 to 90/10 v/v Acetonitrile/ 10 to 100 mM Ammonium acetate, pH4 to 5

pH Range: 2.5 - 7.0
Recommended operating pH: 3 to 6.5
Temperature Range: < 50 ºC
Recommended operating temperature: < 40 ºC
Recommended operating pressure: < 3500 psi

	Recommended Maximum Pressure (psi)	**Typical Flow Rate (mL/min)**
3µm, 3.0 x 50mm	4500	0.2 – 1.2
3µm, 3.0 x 150mm	5800	0.2 – 1.2
3µm, 2.1 x 150mm	5800	0.1 – 0.60
5µm, 2.1 x 150mm	5800	0.1 – 0.60
5µm, 4.6 x 150mm	5800	0.5 – 3.0
5µm, 4.6 x 250mm	5800	0.5 – 3.0

1.4. Physical Characteristics

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

1.5. Acclaim Mixed-Mode WCX-1 Products

Part number **Description**

059526 Acclaim SST Guard Kit (Holder V-1 and coupler)
059456 Acclaim SST Guard Cartridge Holder V-1
059457 Guard to Analytical column Coupler (for V-1)
069580 Acclaim SST Guard Cartridge Holder V-2

Acclaim Mixed-Mode WCX-1				
	Particle Size	**Column Format**	**Part number**	**Required Holder**
Analytical Column	3µm	3.0 x 50mm	071910	-
		3.0 x 150mm	070092	-
		2.1 x 150mm	070093	-
	5µm	2.1 x 150mm	068371	-
		4.6 x 150mm	068353	-
		4.6 x 250mm	068352	-
Guard	5µm	4.3 x 10mm	068354	Holder V-1
		3.0 x 10mm	071911	Holder V-2
		4.6 x 10mm	069705	Holder V-2

SECTION 2 – INSTALLATION: STEP-BY-STEP USER GUIDE

Dionex 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 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 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.

2.1. Step 1 - Visually inspect the column

Report any visual damage to Dionex Corporation.

2.2. 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 de-ionized 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. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

2.2.1. 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 de-ionized 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.***

2.2.2. 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.

2.2.3. 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.

2.2.3.1. Example A. Preparation of 50 mM, pH6 phosphate buffer
1. Weigh 13.6 g potassium monobasic phosphate.
2. Completely dissolve above salt in 2000 g of D.I. water.
3. Carefully adjust the solution to pH6 with HCl or NaOH.

* Sometimes, a small quantity of pyrophosphate (0.1 g/L) can be used to eliminate metal interference from mobile phase, instrument, and/or column hardware.

2.2.3.2. Example B. Preparation of 100 mM, pH5 ammonium acetate buffer
1. Weigh 50 g ammonium acetate buffer (2M, pH5.4) from Dionex.
2. Add 950 g of D.I. water to above solution.

2.3. 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 auto-sampler). The system should be thoroughly primed before use.

2.4. 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).

2.5. 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.

2.6. Step 6 - Real sample analysis

Once the satisfactory result is obtained, the column is ready for use (refer to Sections 3 and 4).

2.7. Quality Assurance Report Examples

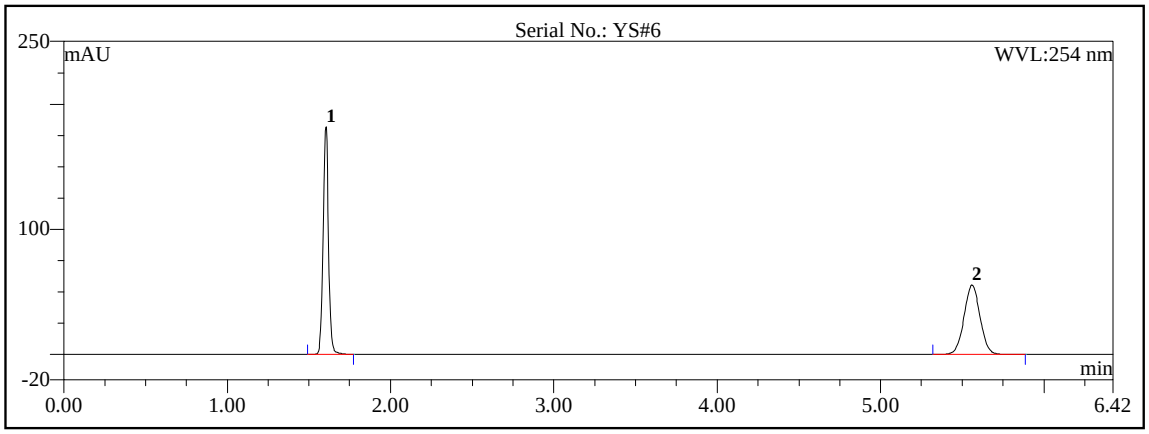
2.7.1. QAR Example 1 - 4.6 x 150 mm

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

Date: 12-Feb-08 15:31
Serial No. : YS#6
Lot No. : 112

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: 50:50 v/v Acetonitrile:0.10 M NH4OAc, pH 5.4



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (1) (EP)	Concentration (µg/mL)
1	Cytosine	1.6	0.99	12251	100
2	Naphthalene	5.6	1.03	15633	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	Passed

Production Reference:
Datasource: QAR
Directory: Acclaim\Mixed-Mode_WCX
Sequence: 068353_MMWCX_46X150MM
Sample No: 1

Chromeleon® Dionex Corp. 1994-2008

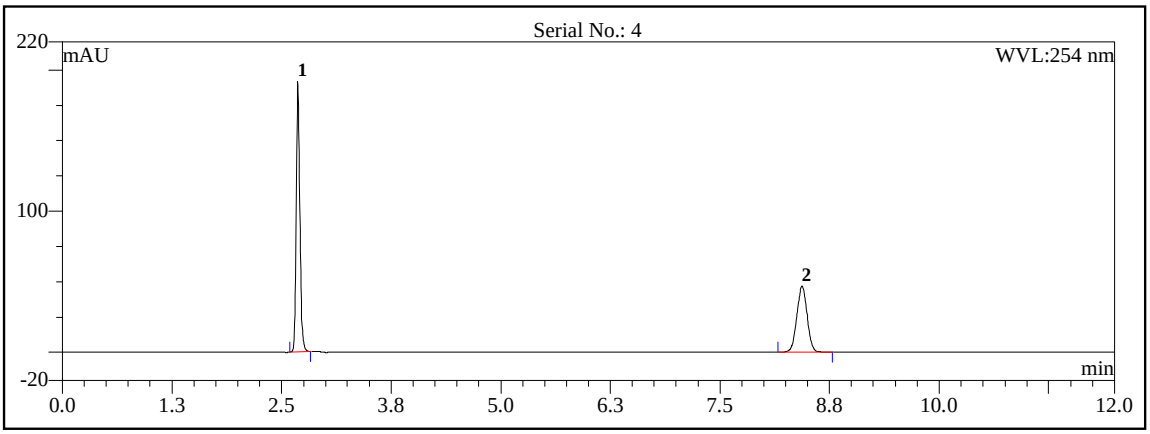
2.7.2. QAR Example 2 - 4.6 x 250 mm

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

Date: 18-Dec-07 13:00
Serial No. : 000004
Lot No. : 93

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: 50:50 v/v Acetonitrile:0.10 M NH4OAc, pH 5.4



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (1) (EP)	Concentration (µg/mL)
1	Cytosine	2.7	1.22	20607	100
2	Naphthalene	8.4	1.02	24885	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	1377

Production Reference:
Datasource: QAR
Directory: Acclaim\Mixed-Mode_WCX
Sequence: 068352_MMWcx_46X250mm
Sample No: 1

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SECTION 3 – 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.

3.1. 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.

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm	
Dimension:	4.6x150 mm	
Mobile Phase:	50/50 v/v MeCN/sodium phosphate, pH6.5	
Temperature:	30 °C	
Flow Rate:	1 mL/min	
Inj. Volume:	5 µL	
Detection:	UV (215 nm)	
Peaks:		µg/mL
	1. Benzoic acid	200
	2. Naphthalene	50
	3. Benzyl amine	300

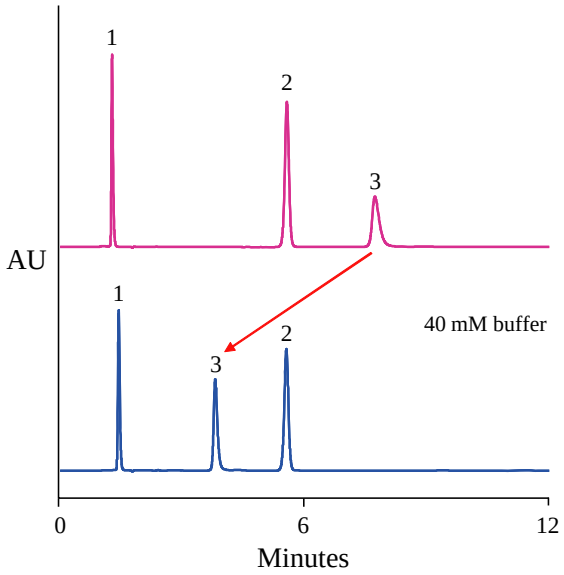


Figure 2
Adjustable Selectivity - Ionic Strength Effect

3.2. 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).

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	4.6x150 mm
Mobile Phase:	MeCN/30 mM sodium phosphate, pH6.5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV (215 nm)

Peaks:	µg/mL
1. Benzoic acid	200
2. Naphthalene	50
3. Benzyl amine	300

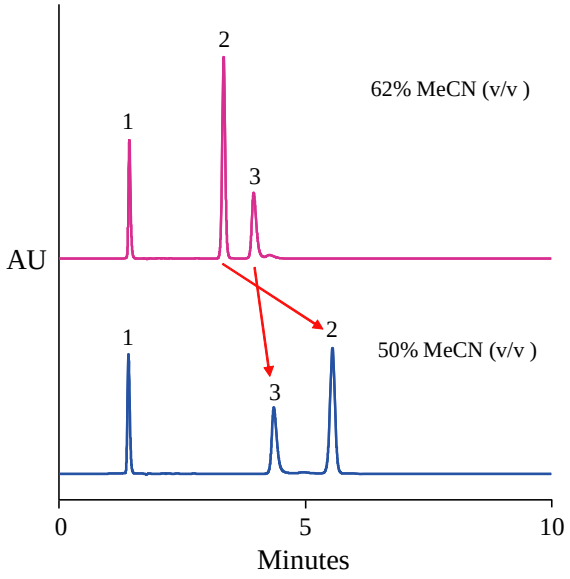


Figure 3
Adjustable Selectivity - Organic Modifier Effect

3.3. 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.

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm	
Dimension:	4.6x150 mm	
Mobile Phase:	50/50 v/v MeCN/10 mM sodium phosphate	
Temperature:	30 °C	
Flow Rate:	1 mL/min	
Inj. Volume:	5 µL	
Detection:	UV (215 nm)	
Peaks:		µg/mL
	1. Benzoic acid	200
	2. Naphthalene	50
	3. Benzyl amine	300

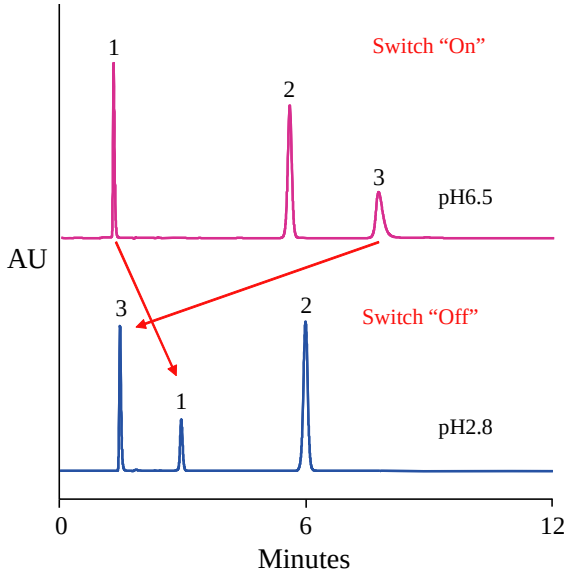


Figure 4
Adjustable Selectivity - pH Effect

3.4. 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.

3.5. HILIC Mode

The Acclaim Mixed-Mode WCX column can operate in HILIC mode (see figure 5). 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.

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	4.6x150 mm
Mobile Phase:	MeCN/NH_4OAc, pH5 (5 mM total)
	v/v 95/5 for HILIC mode operation
	v/v 50/50 for RP mode operation
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV (270 nm)
Peaks:	100 ppm each
	1. Cytosine
	2. Naphthalene

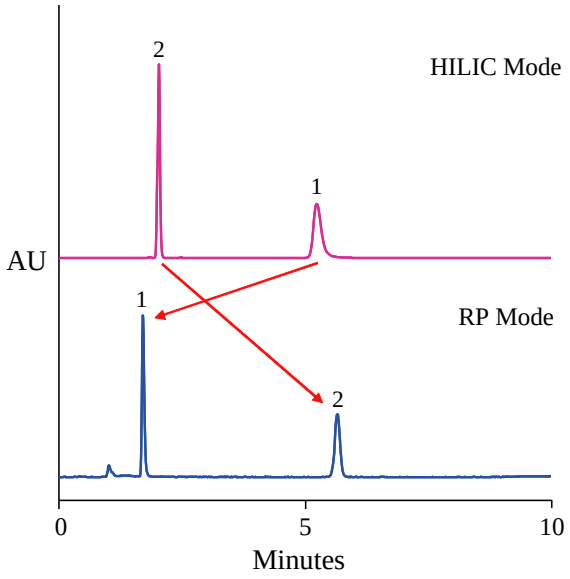


Figure 5
RP Mode vs. HILIC Mode

3.6. Buffer Types

The Acclaim Mixed-Mode WCX column should be used in buffered mobile phases. Within its operating pH range (pH2.5 to 7.0), the column is compatible with a wide collection of typical HPLC mobile phases (e.g. phosphate buffers, acetate buffers, and etc

SECTION 4 – COLUMN CARE

4.1. 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.

4.2. 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.

4.3. Column storage

The column can be stored in the mobile phase for a short period of time. For long term storage, use a buffer with higher organic content at a pH between 3 and 6. Recommended storage solution can be 50/50 to 90/10 v/v Acetonitrile (or methanol)/ 10 to 100 mM Ammonium acetate,
pH4 to 5

4.4. 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.

4.5. 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.

4.6. 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 4000 psi.

4.7. 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, pH5/ acetonitrile v/v 50/50 for 3 column volumes at the recommended flow rate (see Table in Section 1.3).
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 D.I. 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: before any injection is made, the column should be equilibrated with a mobile phase for at least 30 column volumes.

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.***

SECTION 5 – FREQUENTLY ASKED QUESTIONS

5.1. 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.

5.2. 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.

5.3. 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

5.4. 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.

5.5. 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.

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

Read this Product Manual carefully, and contact Dionex Technical Support if you have any questions regarding the use of this column.

5.7. 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 Ca^{2+}), some quaternary amines.

5.8. How to store the column?

Refer to "Section 4.3 Column storage" for details.

5.9. 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.

5.10. 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.

5.11. 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.

5.12. 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.

5.13. What should I do if the column shows deteriorated performance?

Refer to "Section 4.7 Column washing procedure" for details.

5.14. 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, try to replace the inlet bed support. If all above fail, purchase a new column.

SECTION 6 – EXAMPLES OF ORTHOGONAL SELECTIVITY

6.1. WCX vs. RP – Orthogonal Selectivity

Column:	5 µm	
Dimension:	150x4.6 mm	
Mobile Phase:	50/50 v/v MeCN/10 mM (total) sodium phosphate buffer, pH6.5	
Temperature:	30 °C	
Flow Rate:	1 mL/min	
Inj. Volume:	5 µL	
Detection:	UV (215 nm)	
Peaks:		µg/mL
	1. Benzoic acid	200
	2. Naphthalene	50
	3. Benzyl amine	300

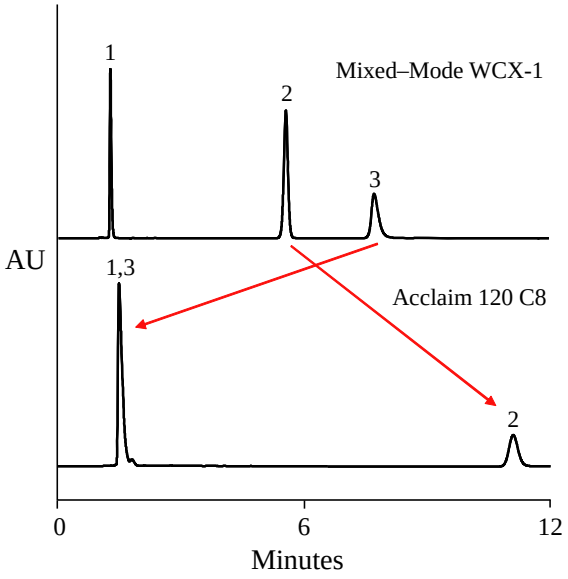


Figure 6
WCX vs. RP – Orthogonal Selectivity

6.2. WCX vs. WAX – Orthogonal Selectivity

Column:	5 µm	
Dimension:	150x4.6 mm	
Mobile Phase:	60/40 v/v MeCN/20 mM sodium phosphate buffer, pH6.5	
Temperature:	30 °C	
Flow Rate:	1 mL/min	
Inj. Volume:	5 µL	
Detection:	UV (215 nm)	
Peaks:		µg/mL
	1. Benzoic acid	200
	2. Naphthalene	50
	3. Benzyl amine	300

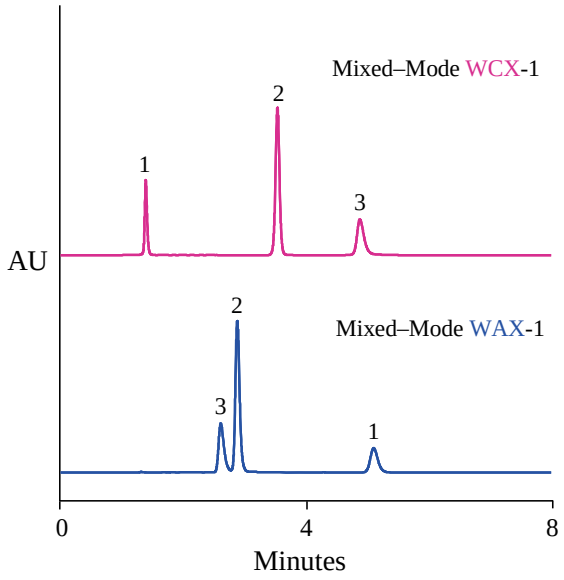
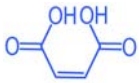


Figure 7
WCX vs. WAX – Orthogonal Selectivity

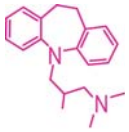
6.3. WCX vs. WAX – Orthogonal Selectivity

Column:	5 µm
Dimension:	4.6x150 mm
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
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	UV (215 nm)
Sample:	Trimipramine Maleate (250 ppm)
Peaks:	

1. Maleate



2. Trimipramine



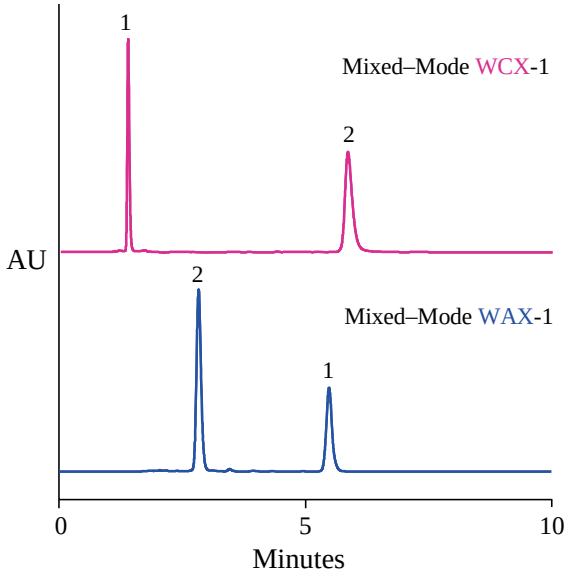


Figure 8
WCX vs. WAX – Orthogonal Selectivity

SECTION 7 – APPLICATIONS

7.1. Simultaneous Separation of Acidic, Neutral and Basic Pharmaceuticals

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	150x4.6 mm
Mobile Phase:	40/60 v/v MeCN/NH_4OAc, pH5.2 (20 mM total)
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV (225 nm)

Peaks:	µg/mL
1. Maleate	50
2. Ketoprofen	30
3. Naproxen	30
4. Hydrocortisone	60
5. Dexamethasone	60
6. Oxprenolol	300
7. Timolol	250

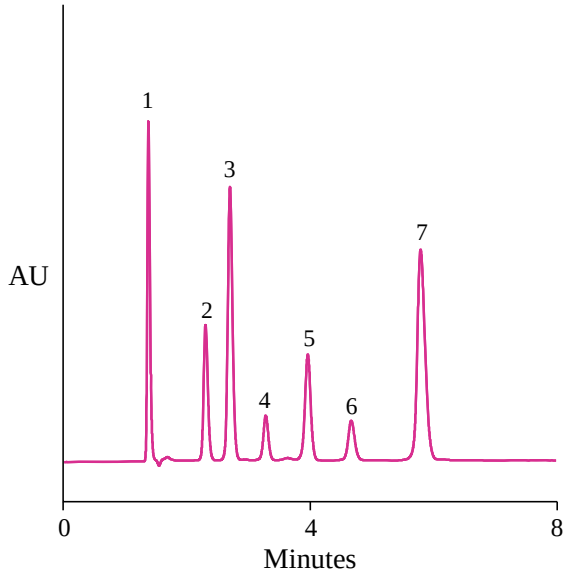


Figure 9
Simultaneous Separation of Acidic, Neutral and Basic Pharmaceuticals

7.2. Separation of Catecholamines

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	4.6x150 mm
Mobile Phase:	2/98 v/v MeCN/sodium phosphate, pH6.2 (10 mM total concentration)
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	UV (215 nm)
Peaks:	(0.25 mM each)
	1. NE
	2. E
	3. DHBA
	4. DA

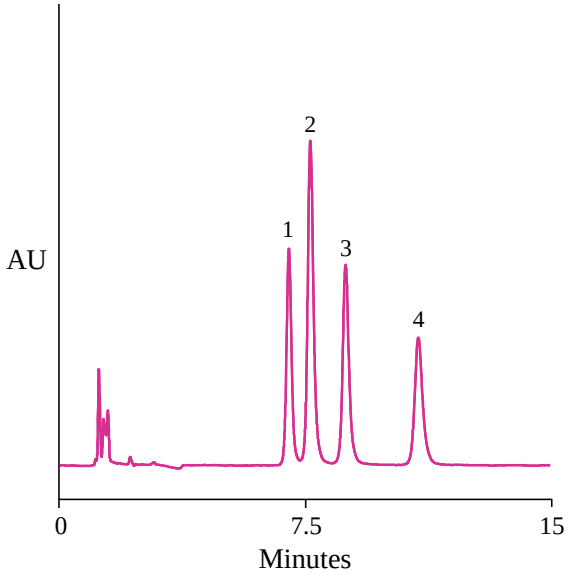


Figure 10
Separation of Catecholamines

7.3. Separation of Antidepressants

Column: Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 50/50 v/v MeCN/10 mM NH_4OAc, pH5.2
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV (215 nm)
Peaks: 100 ppm each

1. Doxepin (mixture of isomers)
2. Imipramine
3. Trimipramine
4. Amitriptyline (As.=1.08, 11623 plates/column)
5. Protriptyline
6. Nortriptyline

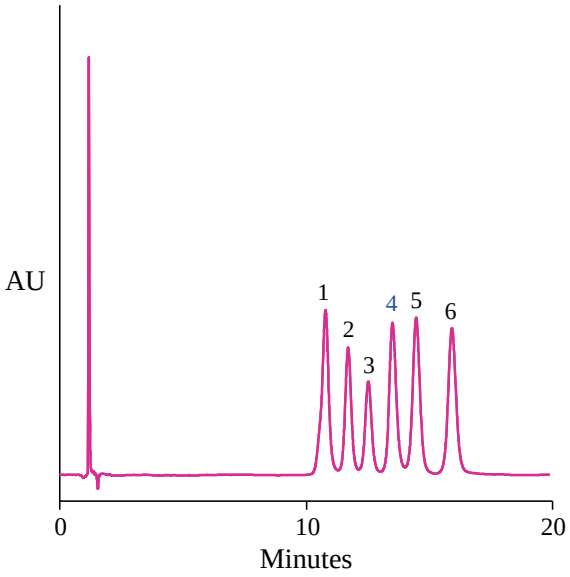


Figure 11
Separation of Antidepressants

7.4. Analysis of Quaternary Amines

Column: Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 50/50 v/v MeCN/NH_4OAc, pH5.2
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: ELS detector
Peaks: (300 ppm each)
1. $(CH_3CH_2CH_2)_4N^+$
2. $(CH_3CH_2CH_2CH_2)_4N^+$
3. $(CH_3CH_2CH_2CH_2CH_2)_4N^+$

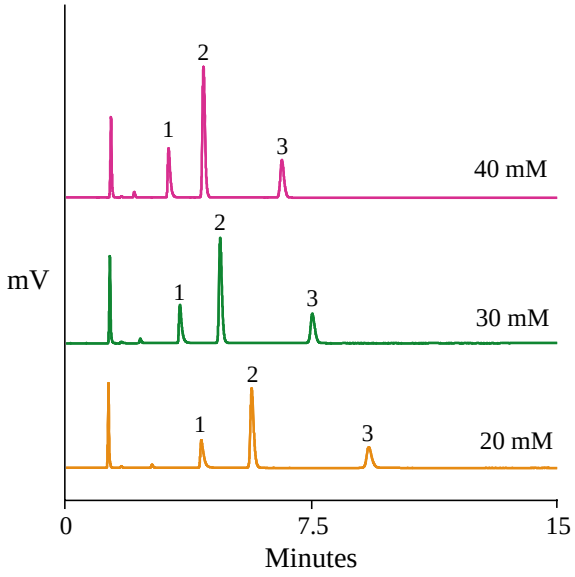


Figure 12
Analysis of Quaternary Amines

7.5. Analysis Tris HCl Salt

Column: Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 50/50 v/v MeCN/NH_4OAc, pH5.2
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: ELS detector
Sample: Tris HCl (1 mg/mL)
Peaks:

1. Cl^-
2. $TrisH^+$

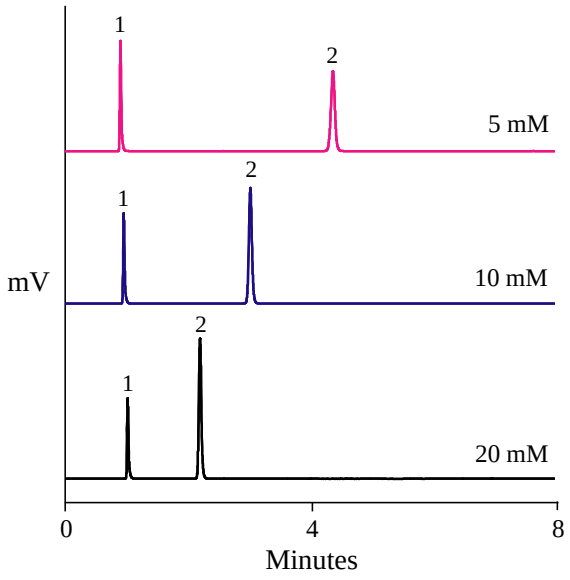


Figure 13
Analysis Tris HCl Salt

7.6. Analysis of Alkyl Phosphonium Salt

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	150x4.6 mm
Mobile Phase:	60/40 v/v MeCN/NH_4OAc, pH5.2
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELS detector
Sample:	Tetrabutylphosphonium bromide (0.1%)
	1. Br-
	2. $(CH_3CH_2CH_2CH_2)_4P^+$

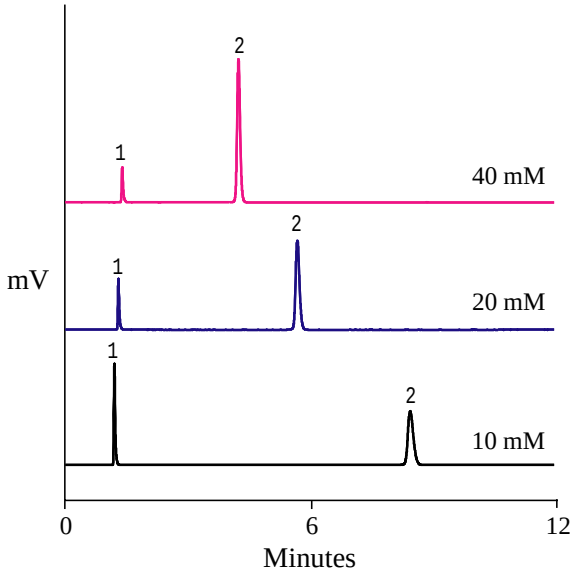


Figure 14
Analysis of Alkyl Phosphonium Salt

7.7. Analysis of Glucosamine Tablet

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	150x4.6 mm
Mobile Phase:	50/50 v/v MeCN/NH_4OAc, pH5.2, 5 mM (total)
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELS detector
Sample:	Glucosamine tablet

Sample preparation:
1. Finely grind a 1500 mg tablet
2. Mix 0.2 g material in 7.5 mL D.I. water and 2.5 mL MeOH
3. Sonicate for 20 min
4. Dilute 20 times
5. Filter through 0.1 µm membrane filter

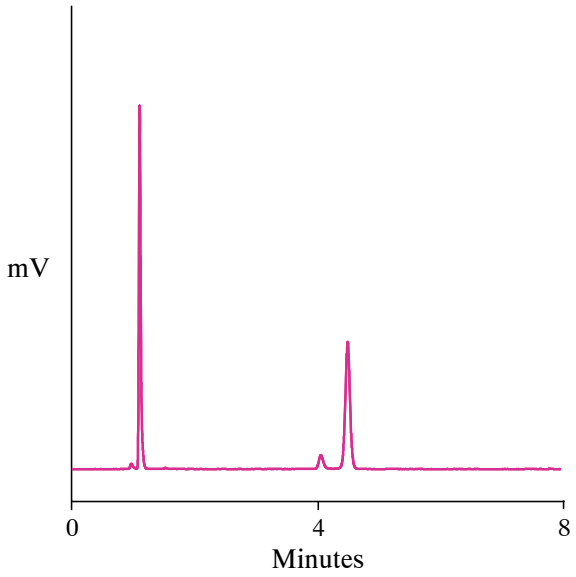


Figure 15
Analysis of Glucosamine Tablet

7.8. Analysis of NaCl – Ionic Strength Effect

Column:	Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension:	4.6x150 mm
Mobile Phase:	50/50 v/v MeCN/NH_4OAc, pH5
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	2 µL
Detection:	ELS detector
Sample:	NaCl (20 mM)
Peaks:	
	1. Cl^-
	2. Na^+

* Total concentration

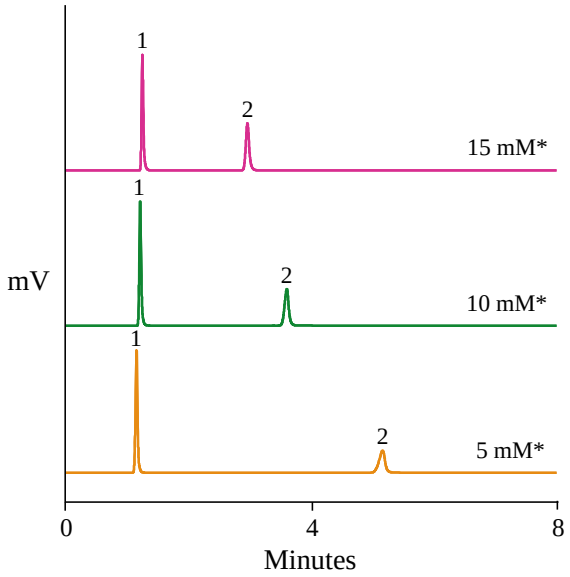


Figure 16
Analysis of NaCl – Ionic Strength Effect

7.9. Analysis of CaCl2 – pH Effect

Column: Acclaim® Mixed-Mode WCX-1, 5 µm
Dimension: 4.6x150 mm
Mobile Phase: 10 mM NH_4OAc buffer
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 2 µL
Detection: ELS detector
Sample: $CaCl_2$ (1 mg/mL)
Peaks:

1. Cl^-
2. Ca^+

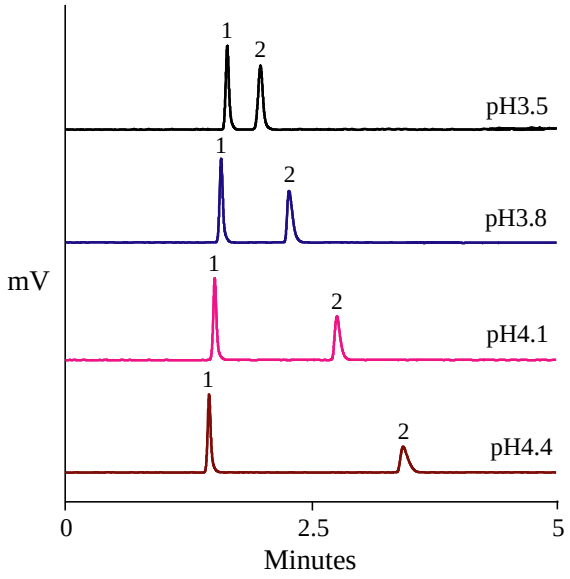


Figure 17
Analysis of CaCl2 – pH Effect

