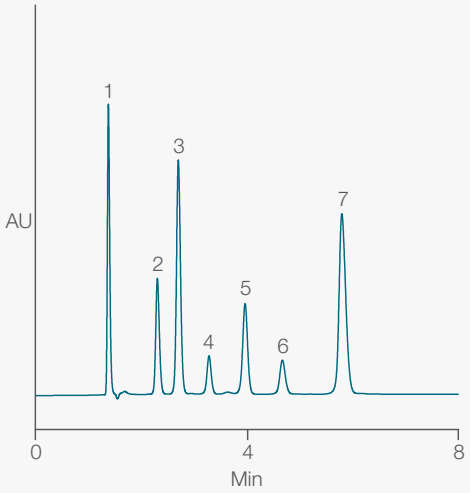
Acclaim Mixed-Mode WCX-1 column

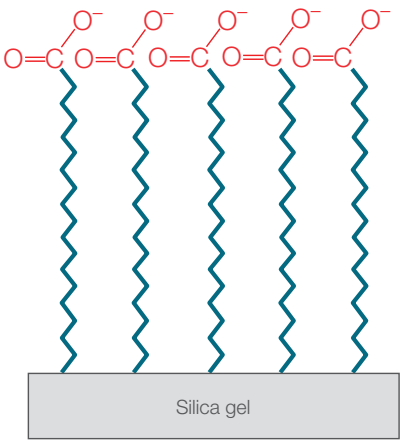
For separating basic molecules

Thermo Scientific™ Acclaim™ Mixed-Mode WCX-1 columns are a novel, high-efficiency, silica-based column specially designed for separating various basic analytes. This new packing material features a combination of both reversed-phase (RP) and weak cation-exchange (WCX) properties by incorporating a hydrophobic alkyl chain with carboxyl terminus (Figure 1). As a result, this new column provides total control of selectivity and great flexibility for method development, as well as a wide range of applications in pharmaceutical, chemical, food and beverages and other industries.

Simultaneous separation of acidic, neutral, and basic pharmaceuticals



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	40/60 v/v MeCN/NH_4OAc, pH 5.2 (20mM total)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 225 nm
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



Silica: High-purity, porous, spherical
Particle size: 5 µm
Surface area: 300 m^2/g
Pore size: 120 Å

Figure 1. Column chemistry of Acclaim Mixed-Mode WCX-1.

Features

- Adjustable selectivity
- Orthogonal selectivity
- Ideal for separating basic analytes: excellent peak shape, sufficient retention, high efficiency
- Multi-retention mechanisms: reversed-phase, cation-exchange, ion-exclusion, and hydrophilic interaction liquid chromatography (HILIC)
- Broad range of applications

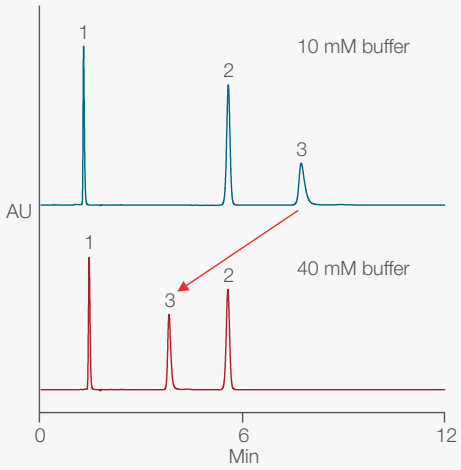
ThermoFisher SCIENTIFIC

Adjustable selectivity

Selectivity is the most important factor in determining the success of a separation. Although reversed-phase columns (i.e., C18) are most commonly used for small molecule separation, the selectivity is rather similar. Some polar-embedded columns provide somewhat different selectivity that is very often inadequate for many applications.

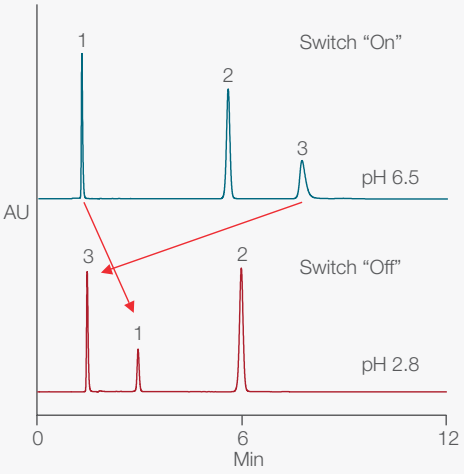
The Acclaim Mixed-Mode WCX-1 column combines both hydrophobic and cation-exchange characteristics, and thus facilitates adjustable selectivity through changes in mobile phase ionic strength, pH and the organic solvent content, individually or concurrently. With increasing ionic strength (cation concentration), the retention decreases for cationic analytes with virtually no change for neutral analytes (Figure 2).

Because of its weak cation-exchange nature, the capacity of the Acclaim Mixed-Mode WCX-1 column can be adjusted by modifying the mobile phase pH. As shown in Figure 3, at pH 6.5, the carboxyl group on the surface is negatively charged and both cation-exchange and hydrophobic interaction contribute to the retention. Benzoic acid elutes early due to electro-repulsion and benzyl amine elutes later because of the combination of electro-attraction and hydrophobic retention. At pH 2.8, on the other hand, the carboxyl group on the surface is protonated and exhibits no cation-exchange capacity, thus hydrophobic interaction is the major force for retention. As a result, protonated benzyl amine elutes first due to its low hydrophobicity, uncharged benzoic acid becomes more retained, and there is virtually no change for the neutral compound: naphthalene. Organic solvent changes hydrophobic retention and can also facilitate selectivity change on the Acclaim Mixed-Mode WCX-1 column (Figure 4).



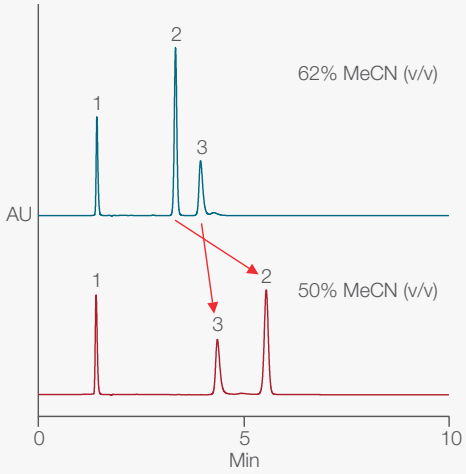
Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CH_3CN/sodium phosphate, pH 6.5
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm
Peaks	1. Benzoic acid 200 µg/mL 2. Naphthalene 50 µg/mL 3. Benzyl amine 300 µg/mL

Figure 2. Adjustable selectivity—ionic strength effect. Changing ionic strength affects cation retention without affecting neutral or anionic compounds.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CH_3CN/10 mM sodium phosphate
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm
Peaks	1. Benzoic acid 200 µg/mL 2. Naphthalene 50 µg/mL 3. Benzyl amine 300 µg/mL

Figure 3. Adjustable selectivity—pH effect. Changing the pH causes the capacity of weak cation-exchange functionality on the stationary phase, and can be switched "on" or "off."

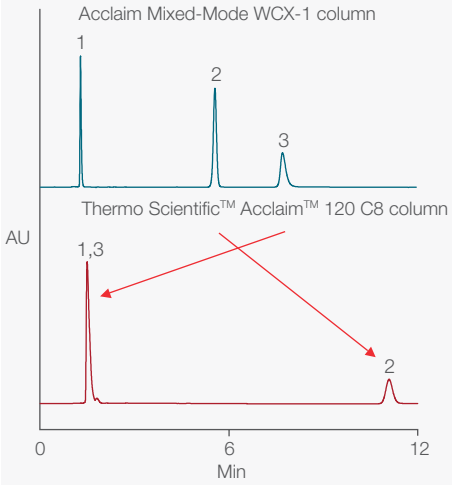


Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/30 mM sodium phosphate, pH 6.5
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm
Peaks	1. Benzoic acid 200 µg/mL 2. Naphthalene 50 µg/mL 3. Benzyl amine 300 µg/mL

Figure 4. Adjustable selectivity—organic solvent effect. Changing the mobile phase organic content affects selectivity.

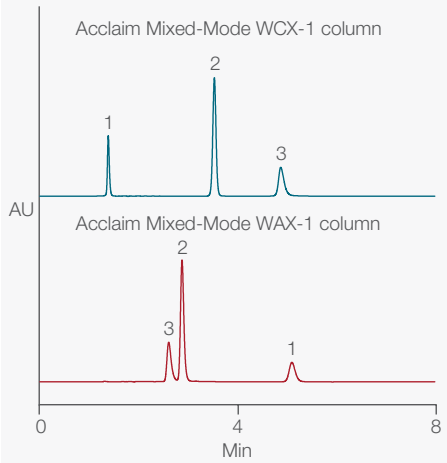
Orthogonal selectivity

Many applications are developed on reversed-phase C18 columns. For some applications, however, regulatory secondary analytical methods are required in addition to the primary method. Usually, secondary methods are developed on a column with selectivity orthogonal to the primary column. Because of its adjustable selectivity, the Acclaim Mixed-Mode WCX-1 column is an ideal column for this purpose. As shown in Figure 5, the Acclaim Mixed-Mode WCX-1 column exhibits different elution order compared to the C18 column under the same condition. In addition, this column provides the selectivity orthogonal to its weak anion-exchange (WAX) counterpart (Figures 6 and 7) because of their opposite retention mechanism. The Acclaim Mixed-Mode WCX-1 column not only provides selectivity orthogonal to other column types (RP and WAX), but can also achieve orthogonal selectivities on the same column through its adjustable selectivity. Therefore, along with RP columns, the Acclaim Mixed-Mode WCX-1 column is a useful tool for method development for a broad range of applications.



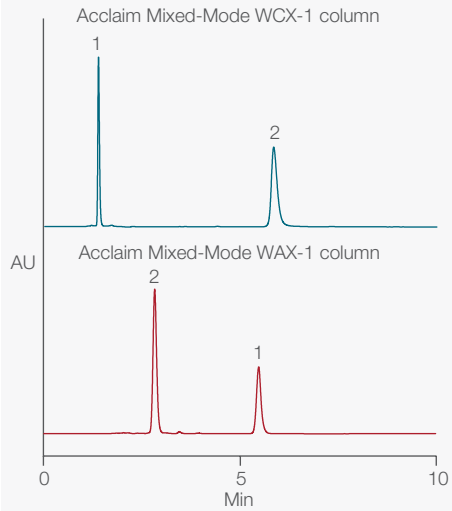
Column	5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CH_3CN/10 mM (total)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm
Peaks	1. Benzoic acid 200 µg/mL 2. Naphthalene 50 µg/mL 3. Benzyl amine 300 µg/mL

Figure 5. WCX versus RP—orthogonal selectivity.



Column	5 µm
Dimensions	4.6 × 150 mm
Mobile phase	60/40 v/v CH_3CN/20 mM sodium phosphate buffer, pH 6.5
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm
Peaks	1. Benzoic acid 200 µg/mL 2. Naphthalene 50 µg/mL 3. Benzyl amine 300 µg/mL

Figure 6. WCX versus WAX—orthogonal selectivity.



Column	5 µm
Dimensions	4.6 × 150 mm
Mobile phase	60/40 v/v CH_3CN/sodium phosphate, pH 6.5 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

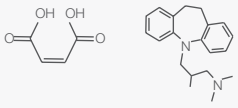
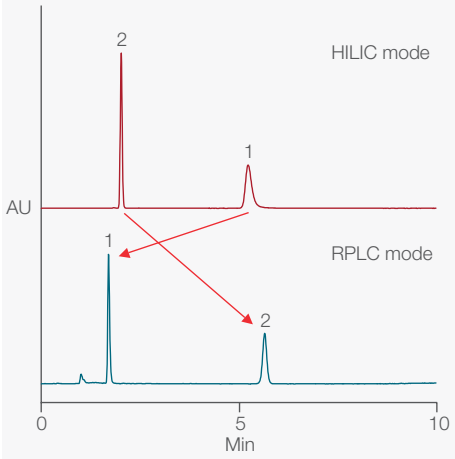


Figure 7. WCX versus WAX—orthogonal selectivity.

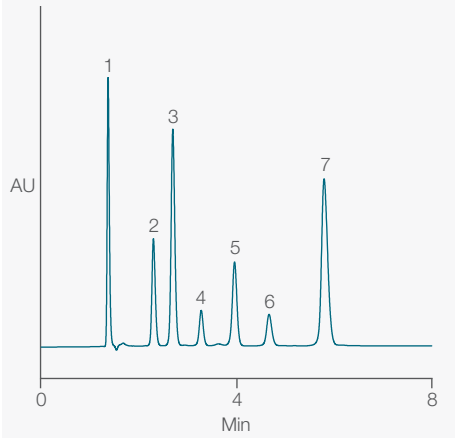
Ideal for basic analytes

Basic compounds are important in a variety of industrial applications, including pharmaceutical, chemical, consumer products, food, and beverages. However, analysis of these compounds is often challenging using reversed-phase silica columns. At neutral pH, these columns often exhibit tailing peaks for basic molecules because of the secondary interaction (ion-exchange) between unreacted silanol groups on the surface and the analyte. Polar-embedded columns were developed to minimize this difficulty and show optimal separation of a wide range of basic molecules. However, highly hydrophilic, basic molecules are not retained adequately without the use of ion-pairing agents to improve retention. As a consequence, the high performance liquid chromatography (HPLC) method requires a more complicated mobile phase that is incompatible with MS, and often a dedicated column. The Acclaim Mixed-Mode WCX-1 column circumvents these difficulties. Not only does it retain basic molecules (from highly hydrophilic such as sodium ion to highly hydrophobic such as amitriptyline), but it also elutes them with symmetrical peak shapes and excellent efficiency (see Figures 10–14).



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/NH_4OAc, pH 5 (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, 215 nm
Peaks	100 ppm each 1. Cytosine 2. Naphthalene

Figure 8. RPLC mode versus HILIC mode.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	40/60 v/v CN_3CN/NH_4OAc, pH 5.2 (20 mM total)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 225 nm
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 9. Simultaneous separation of acidic, neutral, and basic pharmaceuticals.

Multiple-retention mechanisms

Because of its novel column chemistry, the Acclaim Mixed-Mode WCX-1 column offers multiple retention mechanisms, including reversed-phase liquid chromatography (RPLC), cation-exchange, ion-exclusion and HILIC. Figure 8 demonstrates that in highly organic mobile phase, the Acclaim Mixed-Mode WCX-1 column can serve as a HILIC column. Hydrophilic analytes such as cytosine elute later than hydrophobic neutral analytes (naphthalene). The multiple retention mechanisms facilitate the column's effectiveness in many applications that require different selectivities.

Simultaneous separation of basic, neutral, and acidic molecules

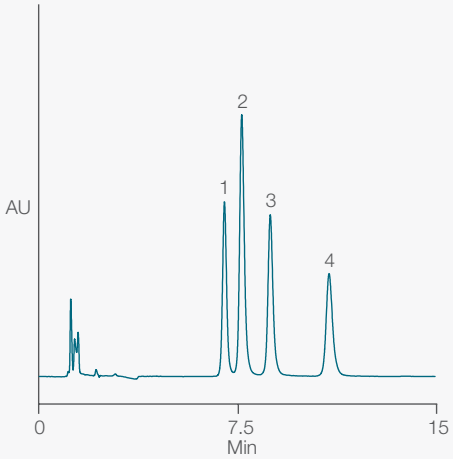
Separation of different types of molecules including bases, neutrals, and acids (BNAs) within a single chromatographic run on the same column is highly desirable but challenging. The unique column chemistry of the Acclaim Mixed-Mode WCX-1 provides multiple separation mechanisms. Consequently, retention of basic, neutral, and acidic molecules can be either independently or concurrently adjusted by changing mobile phase ionic strength, pH, or organic solvent content. While all types of molecules are retained by hydrophobic interaction, the cation-exchange functionality results in increased retention for cationic species through electrostatic attraction, decreased retention for anionic compounds through electrostatic repulsion, and virtually no effect on neutral molecules. Figure 9 demonstrates simultaneous separations of a mixture of basic, neutral, and acidic molecules using an isocratic method, with excellent peak shape and resolution.

Broad range of applications

The Acclaim Mixed-Mode WCX-1 column can serve as a primary column for a variety of basic analytes and as a secondary column that provides selectivity orthogonal to that of reversed-phase columns. Thus it is an invaluable tool for HPLC method development for a broad range of applications, such as pharmaceutical, food and beverage, and chemical analyses.

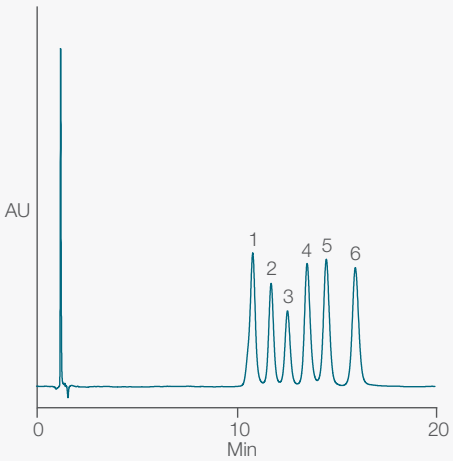
Figures 10 and 11 show that both hydrophilic basic pharmaceuticals (catecholamines) and hydrophobic basic drugs (antidepressants) are retained and separated on this column with excellent peak shape and efficiency without using ion-pairing agents.

Tris is known as trishydroxymethylaminomethane, with the formula $(HOCH_2)_3CNH_2$. In biochemistry, it is widely used as a component of buffer solutions, especially for solutions of nucleic acids. Due to its hydrophilic nature, it is difficult to determine Tris by RPLC. By comparison, it is an easy task when using the Acclaim Mixed-Mode WCX-1 column (Figure 12).



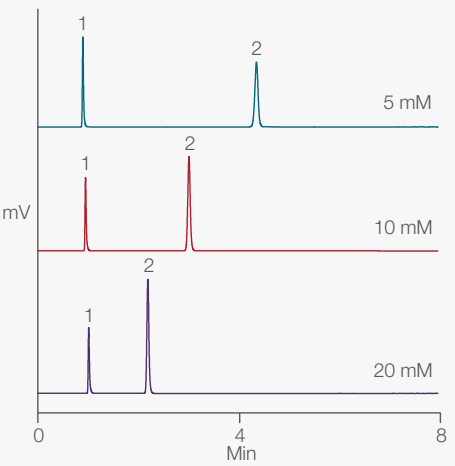
Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	2/98 v/v CN_3CN/sodium phosphate, pH 6.2 (10 mM total)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 215 nm (0.25 mM earch)
Peaks	1. NE 2. E 3. DHBA 4. DA

Figure 10. Separation of catecholamines.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CN_3CN/10 mM NH_4OAc, pH 5.3
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.

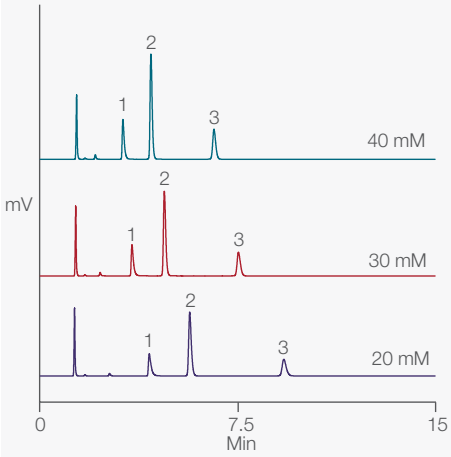


Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CN_3CN/NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	ELS detector
Sample	Tris HCl (1 mg/mL)
Peaks	1. Cl^- 2. $TrisH^+$

Figure 12. Analysis of tris HCl salt.

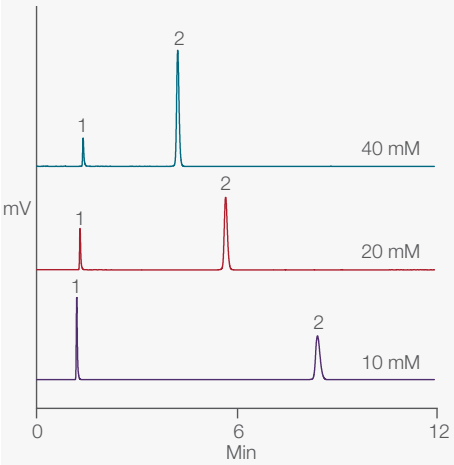
Quaternary amines are used as oil-wetting agents, corrosion and shale inhibitors and bactericides. Figure 13 demonstrates the capability of the new column for separating a series of alkyl quaternary amines.

Phosphonium salts are used as chemical reagents for witting reaction, corrosion inhibitors, and flame-retardants. As shown Figure 14, the phosphonium cation elutes on the Acclaim Mixed-Mode WCX-1 column with excellent peak shape and adequate retention that can be tuned by changing mobile phase ionic strength.



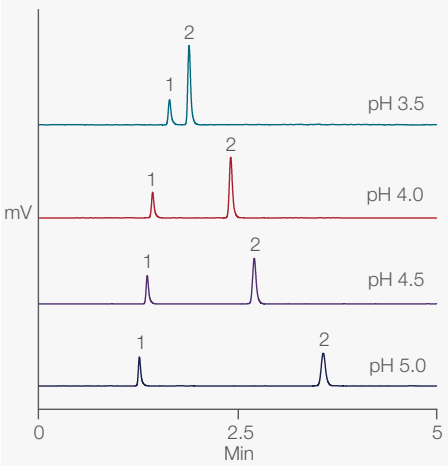
Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v CH_3CN/NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	ELS detector
Peaks	(300 ppm each) 1. $(CH_3CH_2CH_2)4N^+$ 2. $(CH_3CH_2CH_2CH_2)4N^+$ 3. $(CH_3CH_2CH_2CH_2CH_2)4N^+$

Figure 13. Analysis of quaternary amines.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	60/40 v/v CH_3CN/NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	ELS detector
Sample	Tetrabutylphosphonium bromide (0.1%)
Peaks	1. Br^- 2. $(CH_3CH_2CH_2CH_2)4P^+$

Figure 14. Analysis of phosphonium salt.



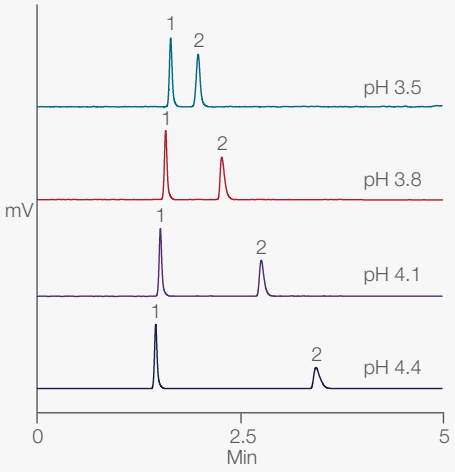
Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	5 mM NH_4OAc buffer
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	ELS detector
Sample	NaCl (2 mM)
Peaks	1. Naproxen 2. Na^+

Figure 15. Analysis of NaCl.

Inorganic cations (i.e. Na^+ and Ca^{2+}) are very important analytes in many samples: pharmaceutical, food, biosciences, chemical, and environmental. While reversed-phase columns fail to retain Na^+ and Ca^{2+} ions, the Acclaim Mixed-Mode WCX-1 column provides adequate retention for both using MS friendly mobile phases (Figures 15 and 16). In pharmaceutical formulation, Na^+ is often used as the counter ion for acidic active pharmaceutical ingredients (APIs). Figure 17A demonstrates that not only can the Acclaim Mixed-Mode WCX-1 column retain and measure Na^+ ion, but it can also measure the acidic API in the same analysis. The elution order can be changed by adjusting mobile phase ionic strength (Figure 17B).

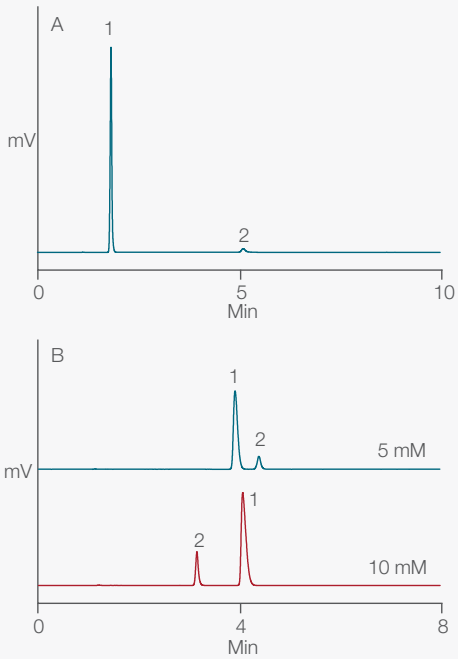
Reproducible manufacturing

Each Acclaim Mixed-Mode WCX-1 column is manufactured to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a qualification assurance report.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 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 16. Analysis of $CaCl_2$.



Column	Acclaim Mixed-Mode WCX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	A: 50/50 v/v CH_3CN/NH_4OAc, pH 5.2, 5 mM (total) B: 30/70 v/v CH_3CN/NH_4OAc, pH 5.2, 5mM (total)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	ELS detector
Sample	Naproxen sodium salt (1 mg/mL)
Peaks	1. Naproxen 2. Na^+

Figure 17. Analysis of Naproxen sodium salt. A) Analysis of counterion only. B) Simultaneous separations of both API and its counterion (with adjustable selectivity).

thermo scientific

Acclaim Mixed-Mode WCX-1 column specifications

Specifications	
Column chemistry	Proprietary carboxylic group
Base silica	High purity, spherical, porous
Particle sizes	3 µm and 5 µm
Pore size	120 Å
Surface area	300 m^2/g

Ordering information

Column	Particle size (µm)	Format	Length (mm)	ID (mm)	Part number
Mixed-Mode WCX-1	3.0	HPLC column	50	3.0	071910
			150	2.1	070093
				3.0	070092
	5.0		150	2.1	068371
				4.6	068353
			250	4.6	068352
	5.0	Guard cartridge (2/pk)	10	3.0	071911
				4.6	069705

Acclaim Guard Holder ordering information

Guard holder	Part number
Thermo Scientific™ Acclaim™ Guard Cartridge Holder V-2	069580
Thermo Scientific™ Acclaim™ Guard Kit (Holder and coupler) V-2	069707
Guard to Analytical Column Coupler V-2	074188

Expect reproducible results with sample prep, columns and vials

Don't see what you need? We would be happy to discuss your specific requirements. Please contact your local sales representative for custom orders.

Find out more at **thermofisher.com/acclaim**

ThermoFisher SCIENTIFIC