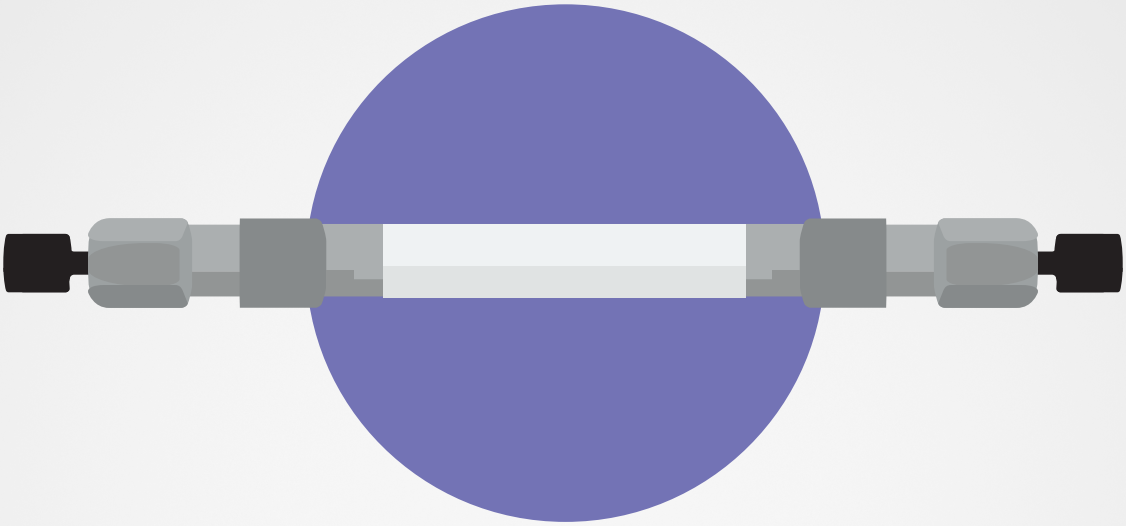
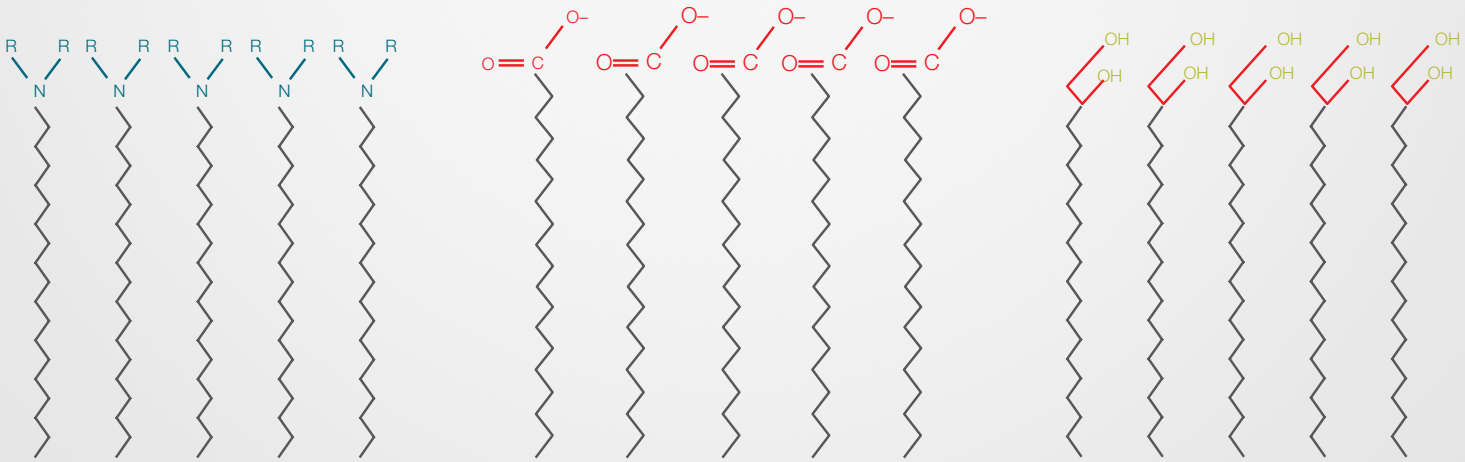
thermo scientific



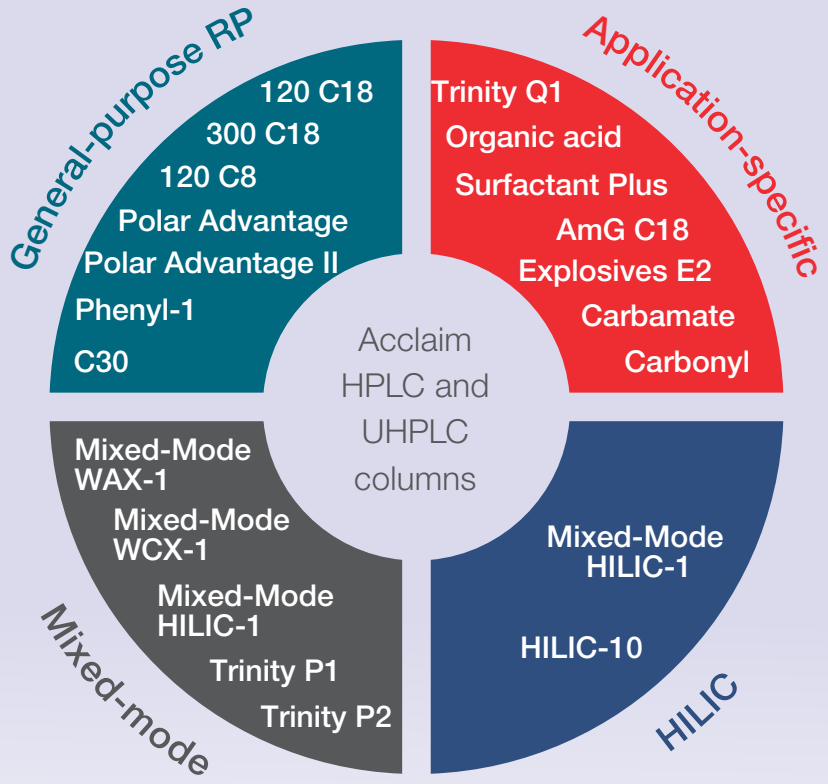


Acclaim Mixed-Mode HPLC columns

Columns for challenging separation needs

ThermoFisher SCIENTIFIC

Broad range of HPLC and UHPLC columns



Broad range of HPLC and UHPLC columns for challenging separation needs

- **General-purpose RP**
  Separate complex samples with high surface area columns (C18, etc)
- **Mixed-Mode**
  Retain multiple types of analytes on a single column
- **Application-specific**
  Unique columns for specific applications: surfactants, organic acids, pesticides, aminoglycosides, explosive residues
- **HILIC**
  Designed for the separation of polar compounds

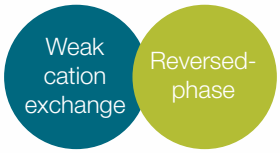
Contents

Gaining more resolution

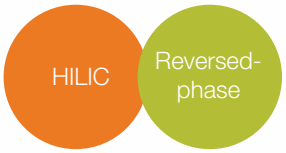
In liquid chromatography, good resolution of peaks is the goal in order to gain meaningful information. Mixed-Mode chromatography provides better control of resolution, as more parameters can be adjusted in order to optimize resolution. Thermo Scientific™ Acclaim™ Mixed-Mode columns provide unique surface chemistries which allows the chromatographer to adjust the elution of analytes easily and achieve maximum resolution, often providing resolution of compounds not possible by other conventional high performance liquid chromatography (HPLC) columns. The combination of ion-exchange with reversed-phase, or HILIC chemistries allows the chromatographer to exploit chemical properties of the analyte mixture to fully resolve the components of a complex sample or for sensitive detection of trace amounts of analytes.[1] Reversed-phase chromatography alone could not accomplish this because it would not retain the very polar analytes or charged analytes. Ion-exchange (IEX) chromatography alone would be insufficient for hydrophobic compounds. Having a column chemistry with a combination of functionalities gives you a whole new tool set to resolve and evaluate your samples.

Acclaim Mixed-Mode columns are available in two functionalities (reversed-phase + ion-exchange) and three functionalities (reversed-phase or HILIC + ion-exchange). The greatest benefit of using Mixed-Mode columns is selectivity optimization, simply by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. With adjustable selectivity, it is also possible to separate analytes with dramatically different hydrophobicity and charge state, such as simultaneous separation of active pharmaceutical ingredients (API) and counterion, in a single analysis.

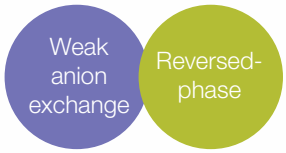
Acclaim Mixed-Mode
WCX-1 columns



Acclaim Mixed-Mode
HILIC-1 columns



Acclaim Mixed-Mode
WAX-1 columns



Acclaim Trinity
P1 columns

Acclaim Trinity
P2 columns

Mixed-Mode columns

Thermo Scientific™ Acclaim™ Mixed-Mode HPLC chromatography columns are packed with silica, which have been bonded with multiple functionalities on a single chromatographic support. For example, Thermo Scientific™ Acclaim™ Mixed-Mode WAX-1 columns combines both reversed-phase and anion-exchange chemistries on the silica particle. Other combinations include reversed-phase and Hydrophilic Interaction Liquid Chromatography (HILIC) and tri-modal phases which have three chemistries (Trinity) on the silica surface. These Mixed-Mode combinations allow the chromatographic conditions to be easily modified in order to optimize selectivity by adjusting either mobile phase ionic strength, pH and/or organic solvent concentration. The result is improved resolutions. Multiple complementary selectivities can be achieved on the same column under different, appropriate conditions. Mixed-Mode chromatography is well-suited to retaining ionic analytes, whether hydrophobic or hydrophilic and requires no ion-pairing agents in the method, significantly improving the MS compatibility.[2,3]

- Excellent performance: selectivity, resolution and retention
- Offers flexibility in method development
- Good when retention requirements are contradictory for a single-mode column
- Good for separations of active pharmaceutical ingredients (APIs), mixtures, formulations, ions etc.
- Compatible with MS (without ion-pairing reagents)

Mixed-Mode chromatography is a separation mode that utilizes RP, IEX, and HILIC interactions. Both bi-modal and tri-modal columns are available to meet the needs of a broad range of applications. In this table the various columns are described.

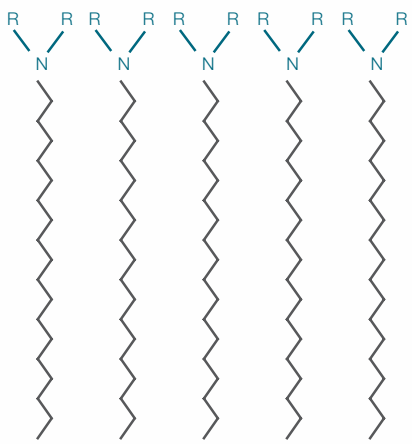
Multi-functional surface chemistries

Column		Mode of separation	Application
Mixed-Mode WAX-1	**Bi-modal**	• Reversed-phase • Weak anion-exchange	Neutral and anionic analytes, including • Acids/organic acids • Hydrophobic cations • Hydrophilic anions • Peptides • Fat-soluble vitamins • Pharmaceuticals
Mixed-Mode WCX-1		• Reversed-phase • Weak cation-exchange	Neutral, hydrophobic acids and cationic analytes, including • Hydrophilic cations • Hydrophobic anions • Bases /Basic pharmaceuticals • Aminophenols
Mixed-Mode HILIC-1		• Reversed-phase • HILIC	Highly polar molecules, including • Neutrals • Total content of ethoxylated nonionic surfactants • PEGS • Pharmaceutical • Consumer products • Chemical
Trinity P1	**Tri-modal**	• Reversed-phase • Strong cation-exchange • Weak anion-exchange	Higher hydrophobicity, including • API and counterion • Hydrophilic monovalent ions • Small pharmaceuticals
Trinity P2		• HILIC • Weak cation-exchange • Strong anion-exchange	Higher hydrophilicity, including • Multivalent ion and API • Hydrophilic ions • Zwitterions • Neutral sugars • Small pharmaceuticals

Bi-modal Mixed-Mode phases

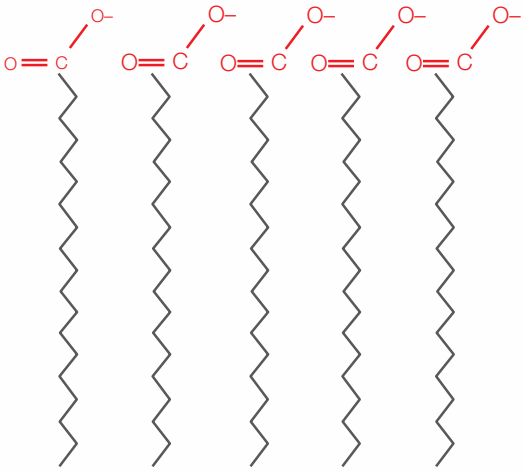
The Acclaim columns family contains three bi-modal Mixed-Mode columns and two tri-modal columns. As shown here, bi-modal columns have both a hydrophobic arm – providing reversed-phase retention, and an ion-exchange or diol group at the tip – providing ion-exchange or HILIC retention.

Acclaim Mixed-Mode
WAX-1 column



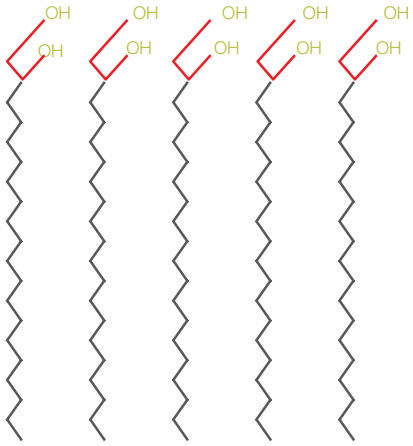
Silica gel

Acclaim Mixed-mMode
WCX-1 column



Silica gel

Acclaim Mixed-Mode
HILIC-1 column



Silica gel

Tri-modal Mixed-Mode phases

Nanopolymer Silica Hybrid technology: Tri-modal Mixed-Mode phases

Thermo Scientific™ Acclaim™ Trinity P1 and P2 columns are based on Thermo Scientific™ Nanopolymer Silica Hybrid (NSH™) technology. These are high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner pores are modified with a covalently bonded bi-modal functionality and the outer surface is modified with nanopolymers having a differing ion-exchange functionality. The spatial separation of the two ion-exchange regions allows both retention mechanisms to function simultaneously and be controlled independently.

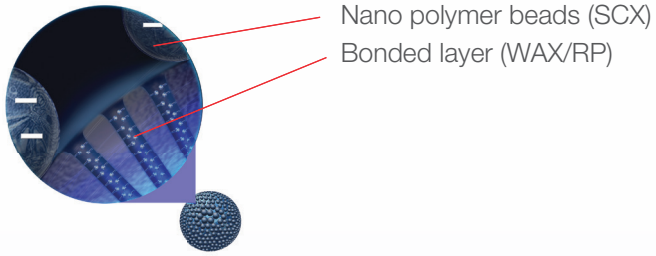
Acclaim Trinity P1 column

The inner-pore of the Acclaim Trinity P1 column is bonded with a bi-modal reversed-phase and anion-exchange functionality. The outer surface is modified with cation-exchange nano-polymer beads.

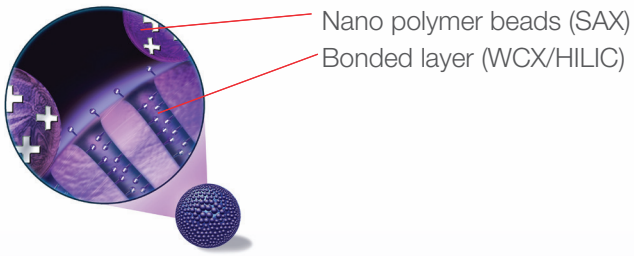
Acclaim Trinity P2 column

The inner-pore area of the Acclaim Trinity P2 consists of the bi-modal hydrophilic surface together with cation exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads.

Acclaim Trinity P1



Acclaim Trinity P2



Adjustable selectivity

Using Mixed-Mode columns, the selectivity can be adjusted by changing ionic strength, pH, and organic solvent content in the mobile phase.

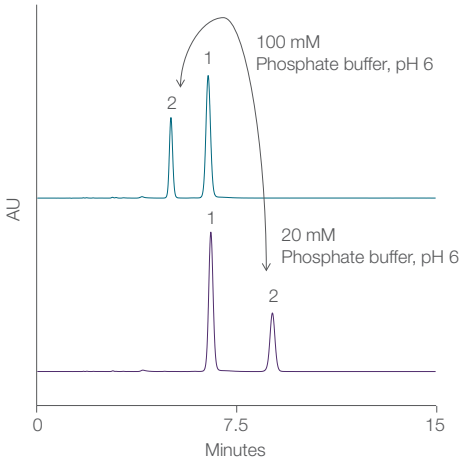
As an example, we will look at the elution of two probe molecules, one neutral and one acidic, separated on the Acclaim Mixed-Mode WAX-1 column. When is increased mobile phase ionic strength, from 20 to 100 mM, while keeping mobile phase pH and organic content constant, the negatively charged acid elutes earlier, but virtually has no effect on retention of the neutral molecule. As a result, the elution order is reversed.

Selectivity adjusted by ionic strength

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	50/50 v/v acetonitrile/phosphate buffer
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	2 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene (0.1 mg/mL) 2. 4-Hydrobenzoic acid (0.5 mg/mL)

Butylbenzene

4-Hydroxybenzoic acid



Selectivity can also be adjusted, or “tuned”, by mobile phase pH. At pH 6 the acid is negatively charged. The retention is mainly governed by both electro-static interaction and hydrophobic interaction. On the other hand, the neutral probe is retained solely by hydrophobic interaction. As a result, 4-hydroxybenzoic acid elutes after butylbenzene.

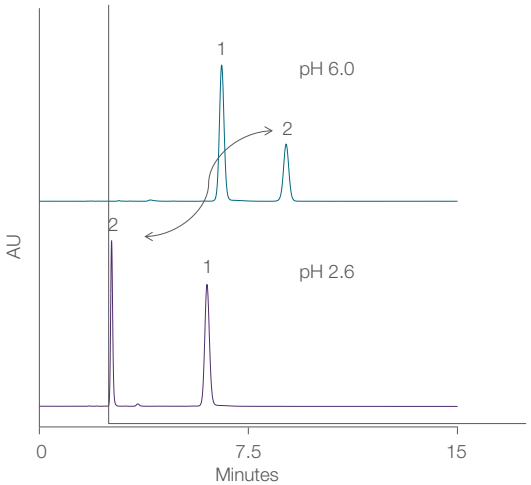
When lowering the pH to 2.6 the acid is protonated and less negatively charged, and the retention is mainly caused by hydrophobic interaction. In this case, the retention of both neutral and acidic molecules are mostly governed by hydrophobic interaction. Because 4-hydroxybenzoic acid is more hydrophilic than butylbenzene, it elutes first, causing selectivity change.

Selectivity adjusted by pH

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	50/50 v/v acetonitrile/20 mM phosphate buffer
Temperature	30°C
Flow rate	1 mL/min
Injection volume	2 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene (0.1 mg/mL) 2. 4-Hydrobenzoic acid (0.5 mg/mL)

Butylbenzene

4-Hydroxybenzoic acid



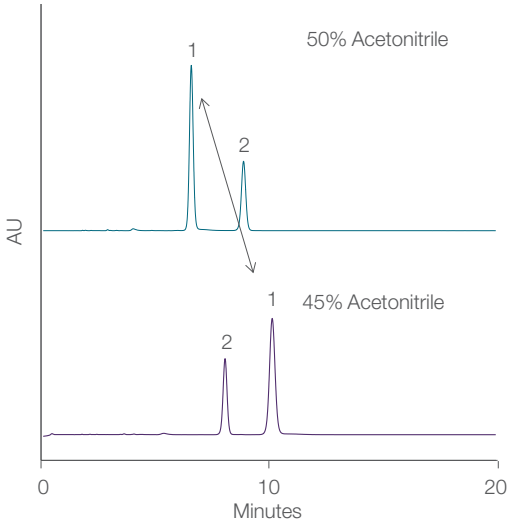
Mobile phase organic content mainly affects hydrophobic retention. As shown here, increasing acetonitrile content from 45 to 50%, the retention of the neutral molecule is affected more dramatically than the acidic molecule at pH 6, thus the elution order reversal is observed.

Selectivity adjusted by organic content

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	Acetonitrile/20 mM phosphate buffer, pH 6
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	210 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene (0.1 mg/mL) 2. 4-Hydrobenzoic acid (0.5 mg/mL)

Butylbenzene

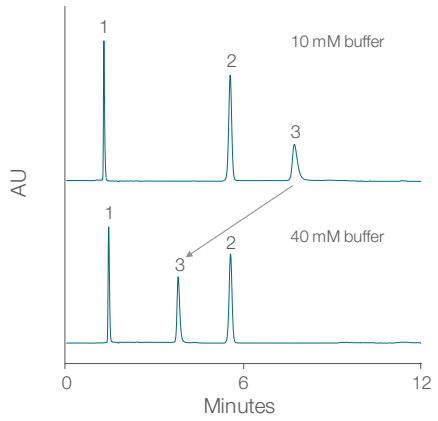
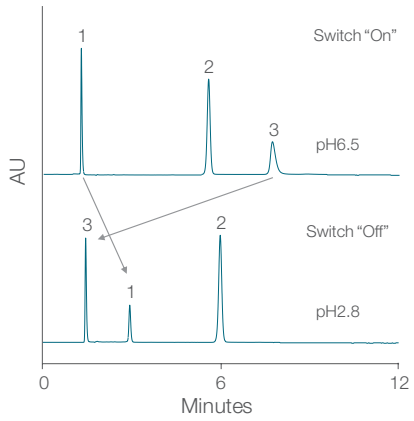
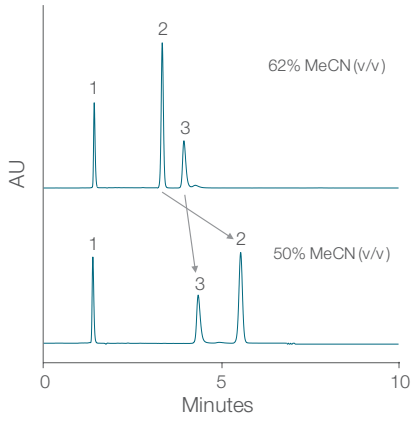
4-Hydroxybenzoic acid



Adjustable selectivity using Acclaim Mixed-Mode WCX-1 column

Selectivity is the most important factor in determining the success of a separation. The Thermo Scientific™ 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.

Mobile phase: 50/50 v/v CH3CN/sodium phosphate, pH 6.5 **Temperature:** 30 °C **Flow rate:** 1 mL/min **Inj. volume:** 5 µL **Detection:** UV (215 nm)	1. Benzoic acid, 200 µg/mL 2. Naphthalene, 50 µg/mL 3. Benzyl amine, 300 µg/mL

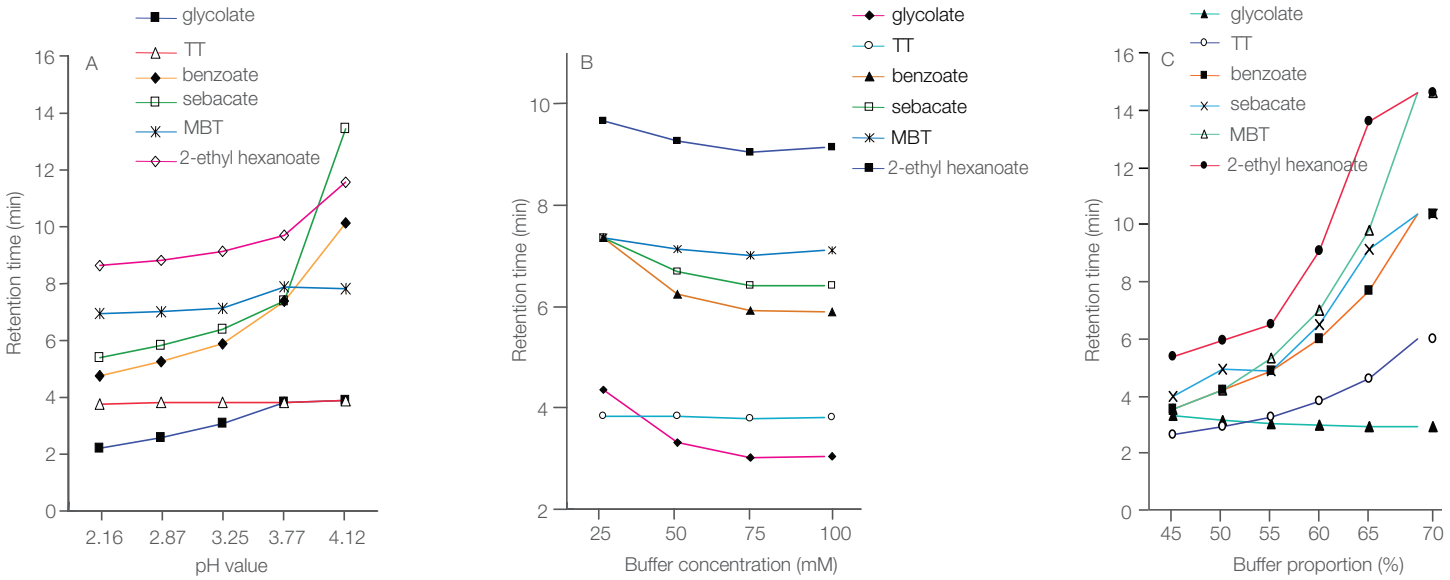
Adjust only ionic strength	Adjust only pH	Adjust only organic solvent
		
Starting conditions: At pH 6.5 the column surface is negatively charged and both cation-exchange and hydrophobic interaction are active. Benzoic acid elutes early due to electro-repulsion, Naphthalene is neutral and retained by hydrophobic interaction, and benzyl amine elutes later due to electro-attraction and hydrophobic retention.		
• Increasing ionic strength, is an increase of the cation concentration, therefore the benzyl amine (cationic analyte) elutes sooner • Neutral naphthalene molecule and benzoic acid not effected	• At pH 2.8 the column cation exchange surface is protonated and now cation-exchange activity is minimized and hydrophobic interaction becomes the primary mode: benzyl amine no longer retained by charge, all analytes retained by hydrophobic interaction (benzyl amine has low hydrophobicity and therefore retained weakly)	• Organic solvent changes hydrophobic retention and can facilitate selectivity changes.

Tracking selectivity changes

Selectivity changes can be tracked. For example, optimizing the chromatographic separation of engine coolant by varying the pH, ionic strength, and organic content of the mobile phase, to find the best resolution.

Ethylene glycol, with engine use, degrades to glycolic acid which can corrode the engine. For this reason, engine coolant often contains ethylene glycol as well as several corrosion inhibitors, as no single corrosion inhibitor is 100% effective. These inhibitors generally are from three compound classes; azoles, inorganic salts, and organic acids, and therefore represent both organic and anionic compounds. This mixture is very difficult to separate by reversed-phase mode alone but is possible using Acclaim Mixed-Mode WAX-1 column.

In the example below glycolate and the inhibitors are separated under varying pH, buffer concentrations and buffer proportions. Thus, determining the optimal chromatographic conditions for separation.



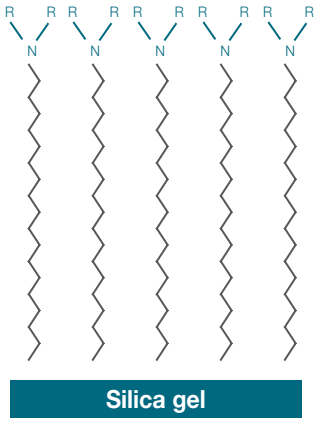
Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.5 mm	
Mobile phase	(A) 100 mM KH_2PO_4 buffer with different pH values (B) Acetonitrite, isocratic (A-60%, B-40%)
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	10 µL
Detection	UV, 214 nm
Peaks	1. Glycolate 2. Tolyl-triazole (TT) 3. Sebacate 4. 2-Mercaptobenzothiazole (MBT) 5. 2-Ethyl hexanoate

Bi-modal Mixed-Mode phases

Acclaim Mixed-Mode WAX-1 column

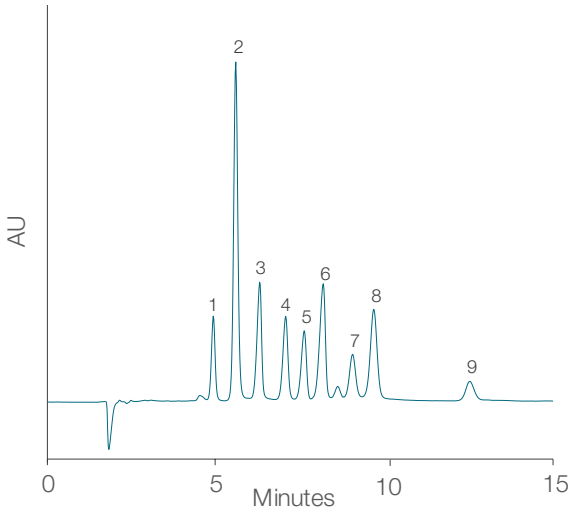
The column chemistry of the Acclaim Mixed-Mode WAX-1 column consists of an alkyl chain, an amide linker and a tertiary amine terminus. This combination offers multimode separation mechanisms including hydrophobic, anion-exchange, and cation-exclusion interactions.

Hydrophilic organic acids, shown below, are difficult to separate on a reversed-phase column, but the combined functionality of the anion-exchange and reversed-phase on the Acclaim Mixed-Mode WAX-1 column provides very nice resolution. Even weakly charged hydrophilic monocarboxylic organic acids are nicely separated in the example below.



Separation of mono-carboxylic acids

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	25 mM phosphate buffer, pH 6
Temperature	30 °C
Flow rate	0.8 mL/min
Injection volume	210 µL
Detection	UV, 210 nm
Peaks	1. Quinic acid 2. Shikimic acid 3. Glykolic acid 4. Lactic acid 5. Acetic acid 6. Formic 7. Ascorbic acid (vitamin C) 8. Iso-ascorbic acid 9. Propionic acid

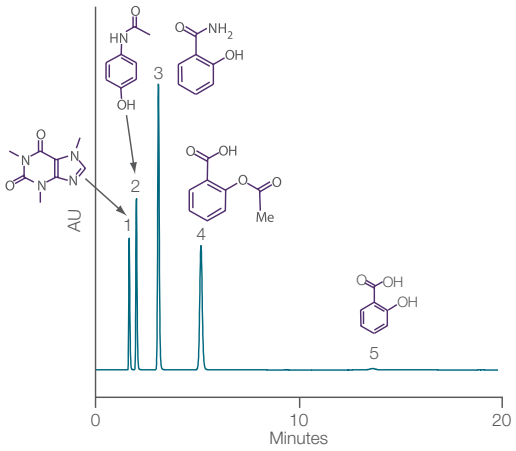


Combining RP and anion-exchange characteristics, the Acclaim Mixed-Mode WAX-1 column provides not only sufficient retentions, but also ideal selectivity for a variety of anionic molecules, even for weakly charged ones like hydrophilic mono-carboxylic organic, which would be difficult if not impossible to separate with RP columns.

Pain relief medicine

The Acclaim Mixed-Mode WAX-1 column is used below for the separation of pain relief pharmaceutical compounds. This example shows the simultaneous separation of the basic, acidic and neutral components with excellent resolution and symmetrical peak shape.

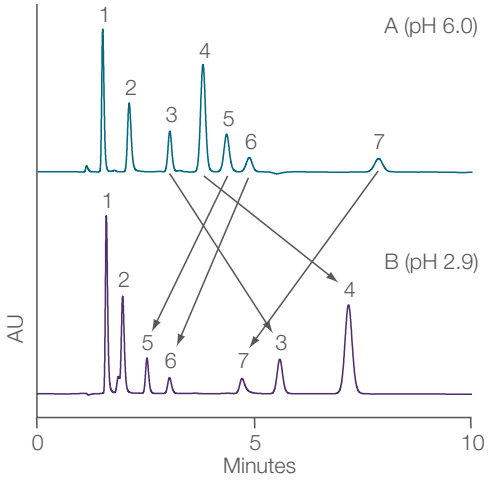
Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	40/60 v/v Acetonitrile/buffer (6.8 g potassium monophosphate and 0.5 g pyrophosphate in 1000 g D.I. H_2O, pH is adjusted to 6.0 with NaOH)
Flow rate	1 mL/min
Injection volume	1 µL
Detection	UV, 220 nm
Temperature	30 °C
Analytes	1. Caffeine 2. Acetaminophen 3. Salicylamide 4. Acetyl salicylic acid (Aspirin) 5. Salicylic acid

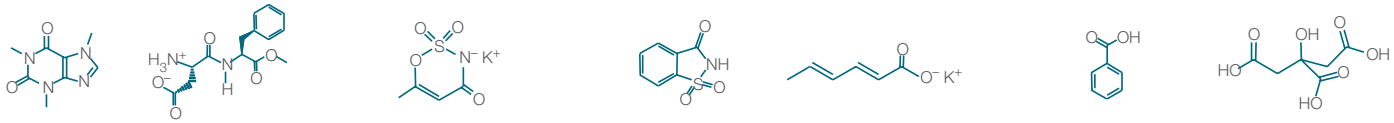


Analysis of soft drinks

The adjustable selectivity of Mixed-Mode separation is a powerful tool in optimizing separations as is shown here in the separation of these sweeteners and soft drink additives. The chromatographic separation can be run a pH 6.0 and 2.9 to achieve complimentary results.

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	(A) 55/45 v/v Acetonitrile/ 0.2 M phosphate buffer, pH 6.0 (B) 57/43 v/v Acetonitrile/ 0.12 M phosphate buffer, pH 2.9
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	2.5 µL
Detection	UV, 210 nm
Peaks	1. Caffeine 100 µg/mL 2. Aspartame 100 3. Acesulfame, potassium 100 4. Saccharin 100 5. Sorbate, poassium 100 6. Benzoic acid 100 7. Citrin acid 300

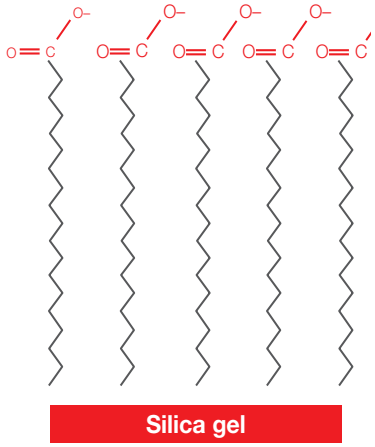




1. Caffeine 2. Aspartame 3. Acesulfame, potassium 4. Saccharin 5. Sorbate, potassium 6. Benzoic acid 7. Citric acid

Acclaim Mixed-Mode WCX-1 column

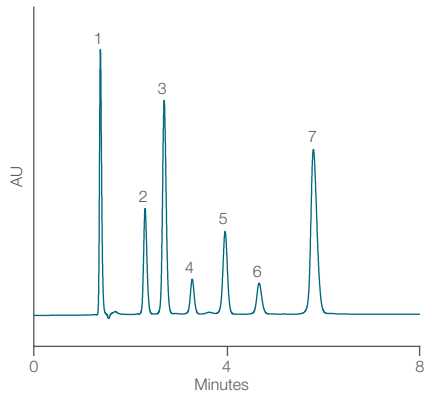
The combination of both reversed-phase and weak cation exchange properties of the Acclaim Mixed-Mode WCX-1 column is achieved by incorporating a hydrophobic alkyl chain with carboxyl terminus. The multiple retention mechanisms of the Acclaim Mixed-Mode WCX-1 column, including reversed-phase, cation exchange, and ion-exclusion results in the ability to separate highly hydrophilic, basic molecules. For example, below is the simultaneous separation of acidic, neutral and basic pharmaceuticals.



Separation of basic, neutral, and acidic pharmaceuticals

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 column 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. This figure demonstrates simultaneous separations of a mixture of basic, neutral, and acidic molecules using an isocratic method, with excellent peak shape and resolution.

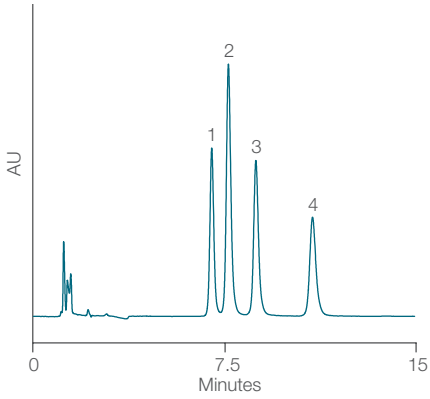
Acclaim Mixed-Mode WCX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	40/60 v/v CH_3CN/NH_4OAc, pH 5.2 (20 mM total)
Temperature	30°C
Flow rate	1 mL/min
Injection volume	5 µL
Detection	UV, 225 nm
Peaks	1. Maleate 50 µg/mL 2. Ketoprofen 30 µg/mL 3. Naproxen 30 µg/mL 4. Hydrocortisone 60 µg/mL 5. Dexamethasone 60 µg/mL 6. Oxprenolol 300 µg/mL 7. Timolol 250 µg/mL



Separation of catecholamines

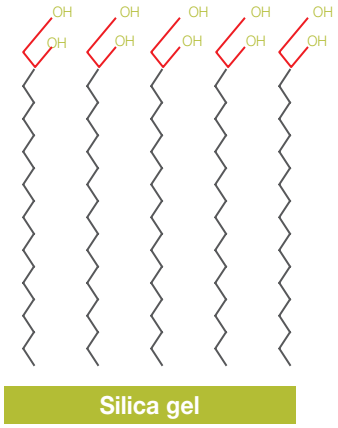
Hydrophilic basic pharmaceuticals, such as catecholamines can be nicely separated with this column.

Acclaim Mixed-Mode WCX-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	2/98 v/v CH_2CN/sodium phosphate, pH 6.2 (10 mM total concentration)
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	5 µL
Detection	UV, 215 nm
Peaks (0.25 nM each)	1. Norepinephrine 2. Epinephrine 3. 4-Dihydroxybenzylamine (DHBA) 4. Dopamine



Acclaim Mixed-Mode HILIC-1 column

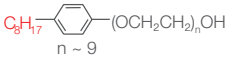
The bi-modal Thermo Scientific™ Acclaim™ Mixed-Mode HILIC-1 column combines both reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) properties. The surface chemistry features a long-chain hydrophobic alkyl group with a hydrophilic diol polar terminus, which provides great potential for separating a wide range of polar and non-polar molecules.



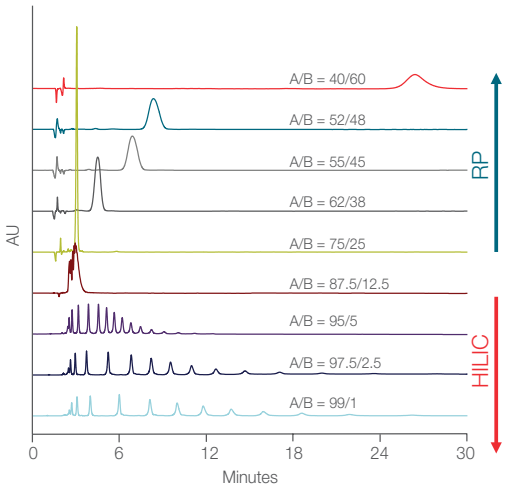
Separation dependency of organic solvent

This surfactant alkylphenol ethoxylate (IGEPAL CA-630) can be measured in two modes of separation. On top, in the reversed-phase mode when the mobile phase contains less than 75% acetonitrile, the ethoxylated surfactant elutes as one peak, for easy total content measurements. The same column, when eluted with greater organic solvent concentration in the mobile phase (the HILIC mode), separates all the ethoxylated components individually which can be used to determine the degree of ethoxylation of the surfactant.

Acclaim Mixed-Mode HILIC-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	(A) CH_3CN (B) 0.1 M NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	10 µL
Detection	UV, 225 nm
Sample	IGEPAL CA-630 (0.1%)



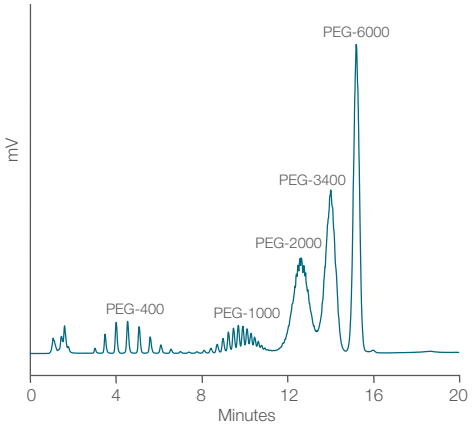
IGEPAL CA-630



Analysis of polyethylene glycols (PEGs)

Polyethylene glycols (PEGs) have a wide variety of uses, including medical formulations, personal care products, and industrial applications. Separation of PEGs with different molecular weights are shown below.

Acclaim Mixed-Mode HILIC-1 column, 5 µm, 150 x 4.6 mm	
Mobile phase	(A) MeOH (B) D.I. H_2O 20% to 95% A in 20 min
Temperature	30 °C
Flow rate	1 mL/min
Injection volume	25 µL
Detection	ELS detector
Sample	Various PEGs (0.04% each)



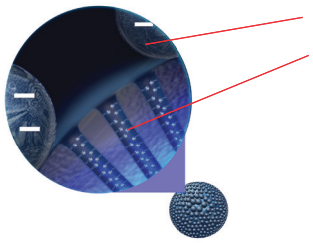
Tri-modal Mixed-Mode phases

Acclaim Trinity P1 column applications

The Thermo Scientific™ Acclaim™ Trinity P1 column is a tri-modal surface functionality; the column inner-pore is bonded with a bi-modal reversed-phase and anion-exchange functionality and the outer surface is modified with cation-exchange nano-polymer beads.

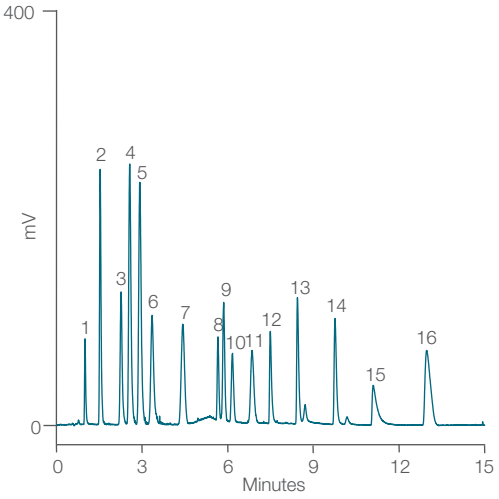
The Acclaim Trinity P1 column can separate both pharmaceutically related cations and anions on one column. The selectivity is good and peaks are symmetrical. The column is designed such that cations elute before anions.

Acclaim Trinity P1



Simultaneous separation of pharmaceutical counterions (gradient method)

Acclaim Trinity P1 column, 3 µm, 50 x 3.0 mm	
Mobile phase	(A) CH_3CN (B) DI H_2O (C) 0.2 M NH_4OAc, pH 4
Temperature	30 °C
Flow rate	0.5 mL/min
Injection volume	2 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30°C)
Peaks	1. Procaine 2. Choline 3. Tromethamine 4. Sodium 5. Potassium 6. Meglumine 7. Mesylate 8. Maleate 9. Chloride 10. Bromide 11. Iodide 12. Phosphate 13. Malate 14. Tartrate 15. Citrate 16. Sulfate



Gradient	-10	0	2	7	15
A%	60	60	60	10	10
B%	35	35	35	0	0
C%	5	5	5	90	90

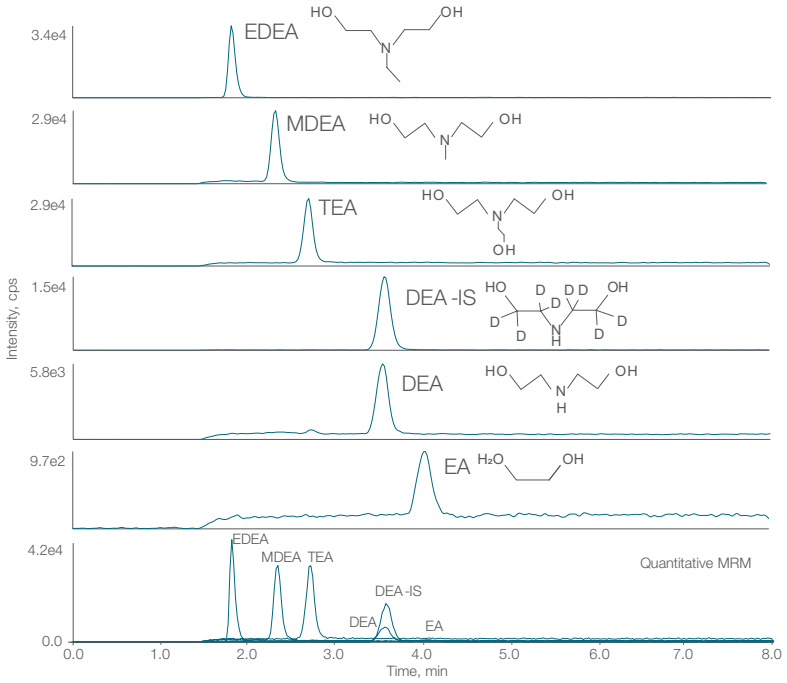
LC-MS-MS analysis of ethanol amines (SCX mode)

Below is the separation of five common ethanolamines with excellent retention and resolution using liquid chromatography with tandem mass spektrometry (LC-MS-MS).

Ethanolaines are widely used as emulsifying agents, detergents, ingredients in bactericides and cosmetics, and also in the pesticide manufacturing process. Inefficient removal and/or inappropriate disposal of ethanolamimes may cause adverse effects to the environment. In addition, ethanolamines have been used as bio- and environmental markers for nitrogen mustards (HN1, HN2, and HN3), thus they are important for monitoring human and environmental exposure to nitrogen mustards.

As shown here, five common ethanolamines can be separated on the Acclaim Trinity P1 column with excellent retention and resolution.

Acclaim Trinity P1 column, 3 µm, 100 x 2.1 mm	
Chromatography conditions	
System	Dionex RSLC LC LCi system
Temperature	20 °C
Mobile phase	90% CH_3CN, 10% NH_4OOCH buffer
Flow rate	600 µL/min
Injection volume	20 µL
Mass spectrometric conditions	
System	LC-MS-MS QTRAP
Interface	TurboSpray with Electrospray ionization
Curtain gas	15
Collision gas	Medium
IonSpray voltage	4500 V
Temperature	700 °C
Ion source gas 1	50
Ion source gas 2	20
Detection mode	Multiple reaction monitoring (MRM)

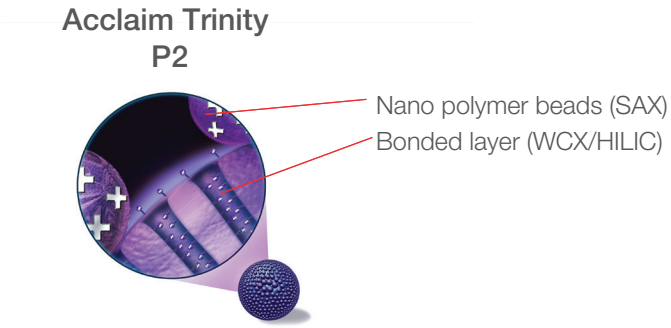


Analyte	Q1MS	Q3MS	DP	CE	CXP
EA	62	44	46	15	6
DEA	106	88	66	19	6
DEA-IS	114	78	53	24	6
MDEA	120	102	46	19	8
EDEA	134	116	51	21	8
TEA	150	132	61	19	0

Acclaim Trinity P2 column applications

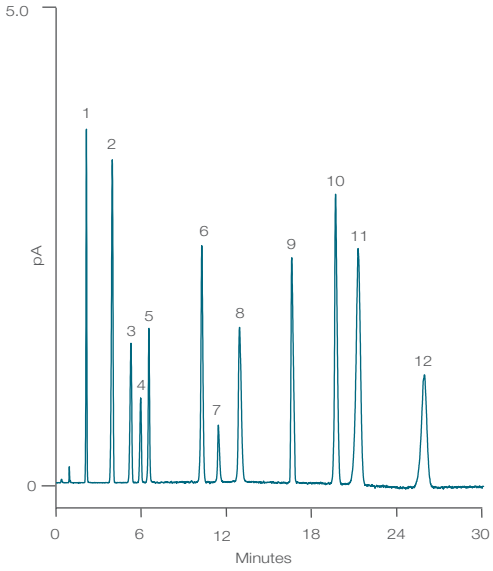
The Thermo Scientific™ Acclaim™ Trinity P2 column is based on HILIC/strong anion exchange/weak cation exchange trimodal phase.

Acclaim Trinity P2 column can separate both pharmaceutical API and related mono- and multi-valent counterions on a single column. The selectivity is good and peaks are symmetrical.



Pharmaceutical-related anions and cations

Acclaim Trinity P2 column, 3 µm, 100 x 3.0 mm	
Mobile phase	D.I. water and 100 mM NH_4OFm, pH 3.65 gradient
Temperature	30 °C
Flow rate	0.60 mL/min
Injection volume	2 µL
Detection	Corona Veo Charged Aerosol
Sample	Water: 0.02 – 0.10 mg/mL each in D.I.
Analytes	1. Phosphate 2. Sodium 3. Potassium 4. Chloride 5. Malate 6. Bromide 7. Nitrate 8. Citrate 9. Fumarate 10. Sulfate 11. Magnesium 12. Calcium



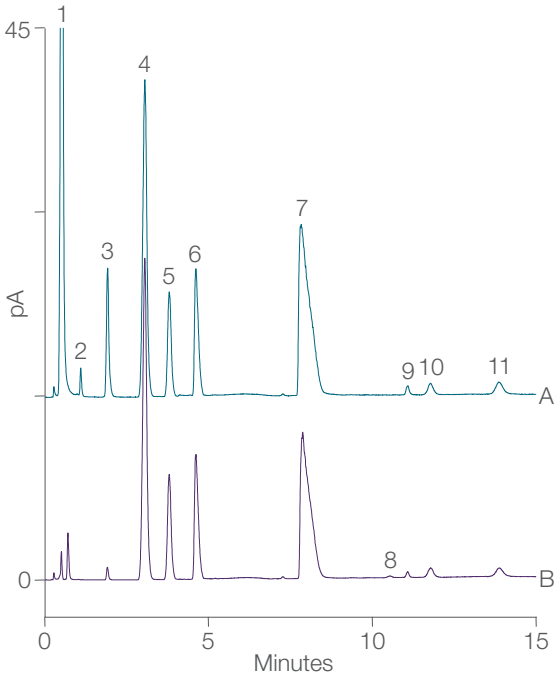
Time (min)	H_2O	0.1 M Ammonium formate, pH 3.65
-10	0.760	1.474
0	80	20
2	80	20
22	0	100
30	0	100

Electrolytes in sports beverages

These sports beverages are commonly advertised to replenish electrolytes after vigorous exercise. The product labels indicate they contain sodium, potassium, magnesium and calcium. The Acclaim Trinity P2 column is designed to resolve a broad range of anions and cations, mono- or multi-valent, in a single analysis using a simple gradient method.

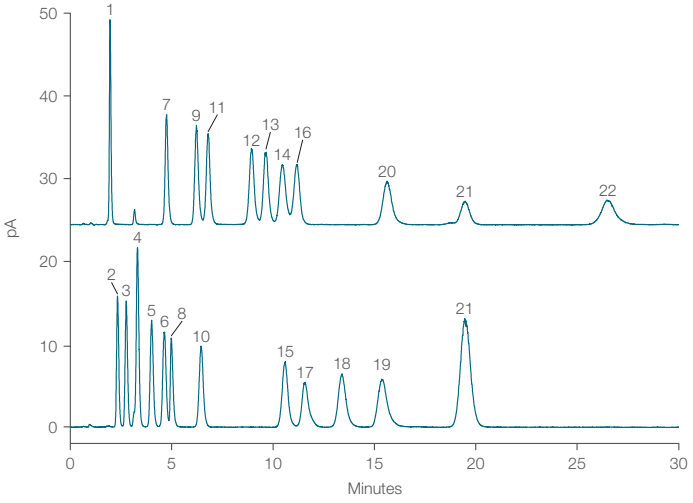
Acclaim Trinity P2 column, 3 µm, 50 x 3.0 mm	
Mobile phase	(A) Water (B) 100 mM Ammonium formate, pH 3.65
System	UltiMate 3000 RS
Temperature	30 °C
Flow rate	0.60 mL/min
Injection volume	2 µL
Detection	Corona Veo Charged Aerosol (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.5)
Sample	(A) Sports drink (Orange flavor) (B) Sports drink, zero-calorie (Fruit punch flavor)
Sample prep	Decolorized with Dionex OnGuard-II P cartridge
Peaks	1. Sugars 2. Ascorbic acid 3. Phosphate 4. Sodium 5. Potassium 6. Chloride 7. Citrate 8. Acesulfame 9. Unknown 10. Magnesium 11. Calcium

Gradient	-8.0	0.0	1.0	11.0	20.0
A%	90	90	90	0	0
B%	10	10	10	100	100



Separation of buffering agents

Acclaim Trinity P2 column, 3 µm, 100 x 3.0 mm		
Mobile phase	(A) Acetonitrile (B) 100 mM ammonium formate, pH 3.65	
Isocratic elution	79% A, 21% B (v/v)	
Temperature	320 °C	
Flow rate	0.60 mL/min	
Injection volume	5 µL	
Detection	Corona Veo charged aerosol detector, evaporator temperature 55 °C, gas pressure 60 psi, data rate 5 Hz, filter 2 s, power function 1.0	
Peaks	1. CHES 2. CAPS 3. CAPSO 4. MES 5. MOPS 6. MOPSO 7. AMPSO 8. Chloride 9. Bicine 10. TES, 11. ACES	12. TAPS 13. Tricine 14. TAPSO 15. HEPES 16. Glycine 17. PIPES 18. HEPPS 19. Phosphate, 20. POPSO 21. Sodium 22. TRIS



Taken from Application Note 20977.

Acclaim Mixed-Mode HPLC column specifications

Acclaim Mixed-Mode HPLC column specifications

	Acclaim Trinity P1	Acclaim Trinity P2	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode HILIC-1
Functionality	• Reversed-phase • Weak anion exchange • Strong cation exchange	• HILIC • Strong anion exchange • Weak cation exchange	• Reversed-phase • Weak cation exchange	• Reversed-phase • Weak anion exchange	• Reversed-phase • HILIC
USP type	–	–	L85	L78	–
Starting material	Ultrapure silica				
Particle shape	Spherical				
Particle sizes	3 µm	3 µm	3 µm 5 µm	3 µm 5 µm	3 µm 5 µm
Average pore diameter	300 Å	300 Å	120 Å	120 Å	120 Å
Surface area	100 m^2/g	100 m^2/g	300 m^2/g	300 m^2/g	300 m^2/g
pH range	2.5–7.5	2.5–7.5	2.5–7.5	2.5–7.5	2.5–7.5

References

1. Kelly Zhang, Xiaodong Liu, J. of Pharmaceutical and Biomedical Analysis, Volume 128, 5 September 2016, Pager 73-88
2. Exploring Mixed-Mode Chromatography: Column Chemistry, Properties, and Applications, Xiaodong Liu, and Christopher Pohl; Thermo Fisher Scientific, Sunnyvale, CA, USA, Thermo Publication PN21137
3. http://files.alfresco.mjh.group/alfresco_images/pharma//2015/03/24/17516363-2762-4507-b9a8-6487ae731afc/PN-PITTCON-MixedModeChrom.pdf
4. Mixed-Mode chromatography in pharmaceutical and biopharmaceutical applications, Journal of Pharmaceutical and Biomedical Analysis, K. Zhang, X. Liu, 128 (2016) 73-88

Ordering information

Particle size (µm)	Format	Length (mm)	ID (mm)	Mixed-Mode HILIC-1	Mixed-Mode WAX-1	Mixed-Mode WCX-1	Trinity P1	Trinity P2
Acclaim analytical columns								
3.0	HPLC column	50	2.1				075565	085431
			3.0	071912	071908	071910	071388	085433
		100	2.1				071389	085432
			3.0				071387	085434
		150	2.1	070091	070089	070093	075564	
			3.0	070090	070088	070092	075563	
5.0	HPLC column	150	2.1	066847	067084	068371		
			4.6	066843	064984	068353		
		250	4.6	066844	064985	068352		
Acclaim guards								
3.0	Guard cartridge	10	2.1				071391	085435
			3.0				071390	085436
5.0	Guard cartridge	10	2.1	069694	069686	-		
			3.0	071913	071909	071911		
			4.6	069706	069704	069705		
Acclaim guard cartridge holder				069580	069580	069580	069580	069580
Acclaim guard cartridge/column coupler				074188	074188	074188	074188	074188
Acclaim guard kit (holder and coupler)				069707	069707	069707	069707	069707

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