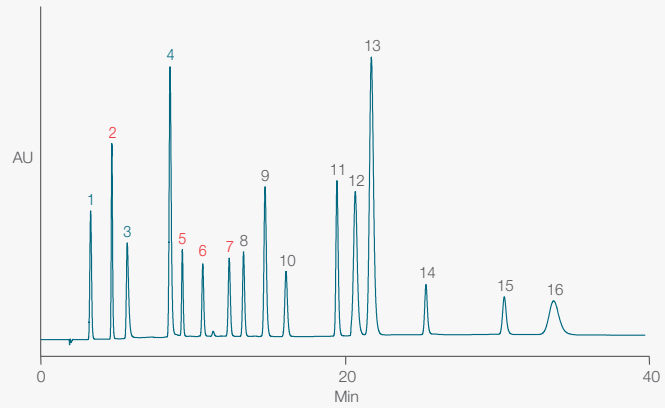
Acclaim Mixed-Mode WAX-1 column

Total control of selectivity

The Thermo Scientific™ Acclaim™ Mixed-Mode WAX-1 column is an important solution for a wide range of separation challenges, including samples in pharmaceutical, food and beverage, chemical, and other applications. The column packing features a new silica-based stationary phase that incorporates both hydrophobic and weak anion-exchange properties, as shown in Figure 1. Unlike traditional reversed-phase (RP) stationary phases, the new packing material features a hydrophobic alkyl chain with an ionizable terminus that gives the chromatographer total control of selectivity in the simultaneous separation of acids, bases, and neutral molecules.

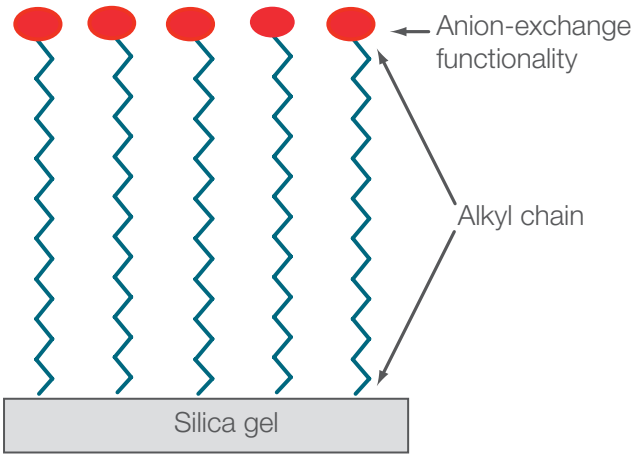
A simple solution for separating acids, bases, and neutral molecules



Blue - Basic, **Red - Neutral**, Grey - Acidic

Peaks

1. Caffeine
2. Acetaminophen
3. Dextromethorphan
4. Amitriptyline
5. Prednisone
6. Hydrocortisone
7. Dexamethasone
8. Acetyl salicylic acid
9. Sorbate, potassium
10. Benzoic acid
11. Salicylic acid
12. Ketoprofen
13. Naproxen, Na salt
14. Aconitic acid, *cis*-
15. Aconitic acid, *trans*-
16. Ibuprofen



Features

- Adjustable selectivity
- Selectivity complementary to RP columns
- High capacity and unique selectivity for anionic molecules
- Simultaneous separation of acidic, basic, and neutral molecules
- Multimode retention mechanisms: RP, anion-exchange, and hydrophilic interaction liquid chromatography (HILIC) modes

Figure 1. Surface chemistry of the Acclaim Mixed-Mode WAX-1 column.

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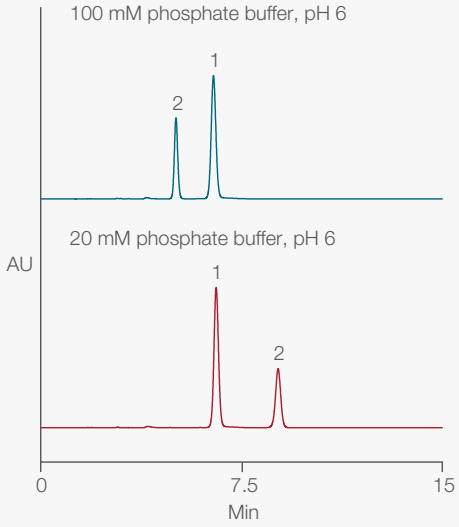
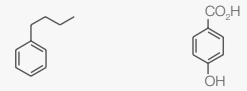
Adjustable selectivity

The Acclaim Mixed-Mode WAX-1 column combines both hydrophobic and anion-exchange characteristics, which facilitates selectivity adjustment by changing mobile phase ionic strength, pH, or organic content, either independently or concurrently (Figures 2–4). Ionic strength is crucial for adjusting selectivity. An increase in ionic strength results in retention decrease, increase, and no effect for acidic, basic, and neutral molecules, respectively. Hydrophobic retention is markedly affected by organic content of mobile phase. In general, all types of molecules (acids, bases, and neutrals) are less retained on this column with increased organic content in the mobile phase, when keeping other conditions constant (e.g., ionic strength, pH, temperature, etc.) Although pH has little effect on the retention of neutral molecules, it significantly affects anionic molecules. For example, lowering pH reduces the negative charge of carboxy-late-containing molecules, giving rise to decreased ion-exchange retention. As a result, many applications difficult with C18 columns are easily performed using the Acclaim Mixed-Mode WAX-1 column.

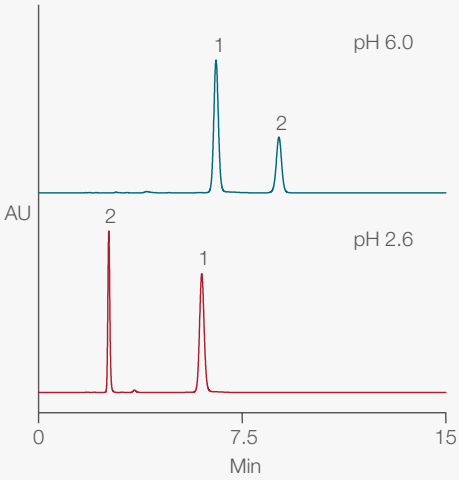


Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v acetonitrile/phosphate buffer
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene 0.1 mg/mL 2. 4-Hydroxybenzoic acid 0.5 mg/mL

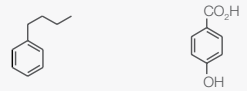


Butylbenzene 4-Hydroxybenzoic acid

Figure 2. Adjustable selectivity—changing ionic strength affects anion retention without affecting neutral compounds.

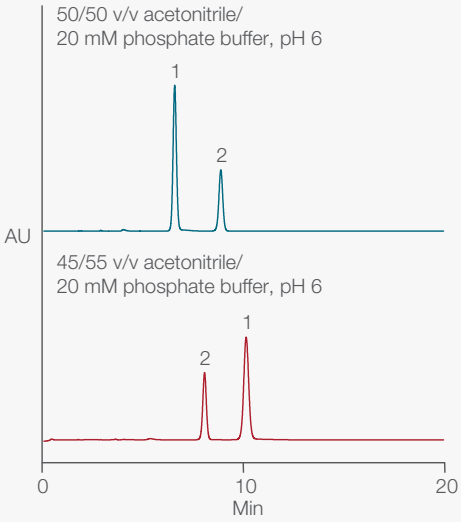


Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 v/v acetonitrile/ 20 mM phosphate buffer
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene 0.1 mg/mL 2. 4-Hydroxybenzoic acid 0.5 mg/mL

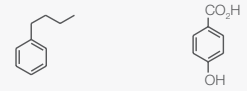


Butylbenzene 4-Hydroxybenzoic acid

Figure 3. Adjustable selectivity—changing pH affects anion retention without affecting neutral coumpounds.



Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	See chromatograms
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	UV, 210 nm
Peaks	1. Butylbenzene 0.1 mg/mL 2. 4-Hydroxybenzoic acid 0.5 mg/mL

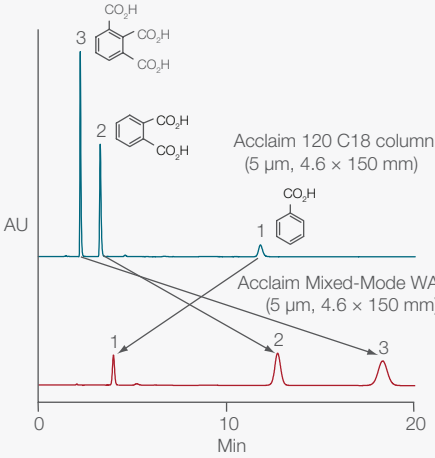


Butylbenzene 4-Hydroxybenzoic acid

Figure 4. Adjustable selectivity—changing the mobile phase organic content affects selectivity.

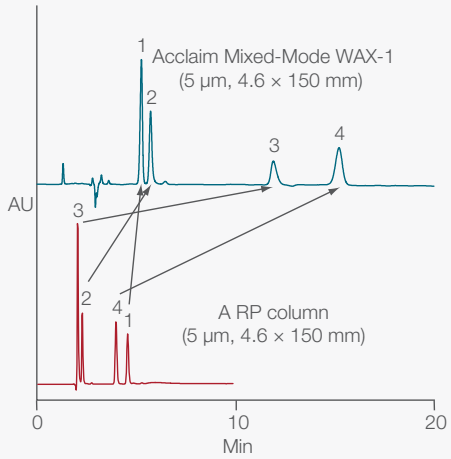
Selectivity complementary to RP columns

Because of its unique column chemistry, the Acclaim Mixed-Mode WAX-1 column provides “orthogonal” or complementary selectivity. As shown in Figures 5 and 6, different elution orders of organic acids can be obtained on this column as opposed to a conventional RP column. Although RP silica columns (e.g., C18) are the most widely used stationary phases for many HPLC separations, it is often desired and even required to have a secondary analytical method to complement a preexisting (primary) method for a given sample. The Acclaim Mixed-Mode WAX-1 column accommodates this need by providing complementary selectivity.



Mobile phase	50/50 v/v acetonitrile/ 50 mM phosphate buffer, pH 2.8 for Acclaim Mixed-Mode WAX-1, 20/80 v/v acetonitrile/ 50 mM phosphate buffer, pH 2.8 for Acclaim 120 C18 column
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	UV, 220 nm
Peaks	1. Benzoic acid 0.2 mg/mL
	2. Phthalic acid 0.2 mg/mL
	3. 1,2,3-Tricarboxylic benzene 0.2 mg/mL

Figure 5. Selectivity complementary to reversed-phase column (I).

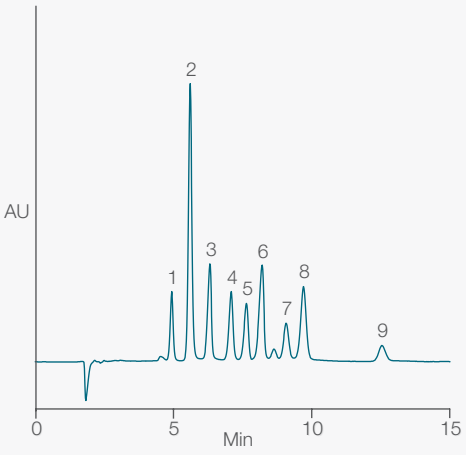


Mobile phase	50/50 v/v acetonitrile/100 mM phosphate buffer, pH 6.0 for Acclaim Mixed-Mode WAX-1 50 mM phosphate buffer, pH 2.6 for RP column
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	1. Succinic acid 0.5 mg/mL
	2. Tartaric acid 0.5 mg/mL
	3. Oxalic acid 0.1 mg/mL
	4. Citric acid 0.5 mg/mL

Figure 6. Selectivity orthogonal to reversed-phase column (II).

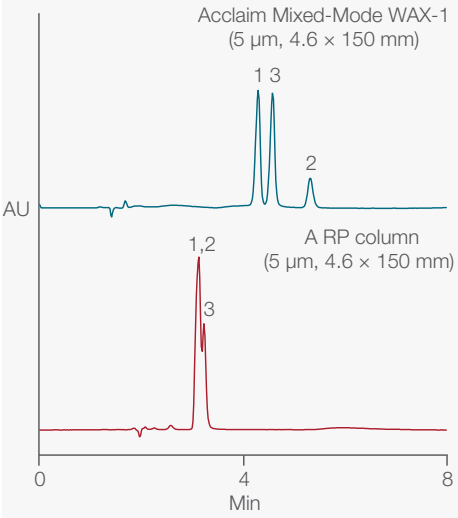
High capacity and unique selectivity for anionic molecules

Although small organic acids can sometimes be separated on an RP column in ion-suppression mode at low pH, de-wetting often causes sudden reversible retention loss on conventional RP column in highly aqueous conditions. Even with aqueous-compatible RP columns packed with polar-embedded or polar end-capped stationary phases, hydrophilic organic acids still cannot be separated, mainly because hydrophobic retention alone is inadequate to differentiate molecules with similar hydrophobicities. 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 any RP columns (Figures 7–9).

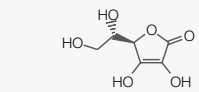


Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	25 mM phosphate buffer, pH 6
Temperature	30 °C
Flow rate	0.8 mL/min
Inj. volume	10 µL
Detection	UV, 210 nm
Peaks	1. Quinic acid 2. Shikimic acid 3. Glycolic acid 4. Lactic acid 5. Acetic acid 6. Formic acid 7. Ascorbic acid (Vitamin C) 8. *Iso*-ascorbic acid 9. Propionic acid

Figure 7. Separation of monocarboxylic acids.



Mobile phase	20 mM phosphate buffer, pH 6.0 for Acclaim Mixed-Mode WAX-1; 50 mM phosphate buffer, pH 2.6 for the RP column
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	1. Lactic acid 0.8 mg/mL 2. Ascorbic acid (Vitamin C) 0.25 mg/mL 3. Acetic acid 0.8 mg/mL

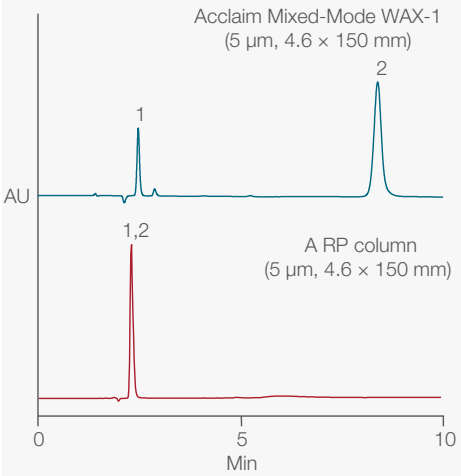


Ascorbic acid (Vitamin C)

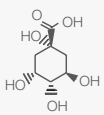
Lactic acid

Acetic acid

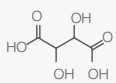
Figure 8. Separation of lactate, acetate, and ascorbate.



Mobile phase	40/60 v/v acetonitrile/ 50 mM phosphate buffer, pH 6.0 for Acclaim Mixed-Mode WAX-1 50 mM phosphate buffer, pH 2.6 for a RP column
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	1. Quinic acid 1 mg/mL 2. Tartaric acid 1 mg/mL



Quinic acid



Tartaric acid

Figure 9. Separation of quinic acid and tartaric acid.

Simultaneous separation of acidic, basic, and neutral molecules

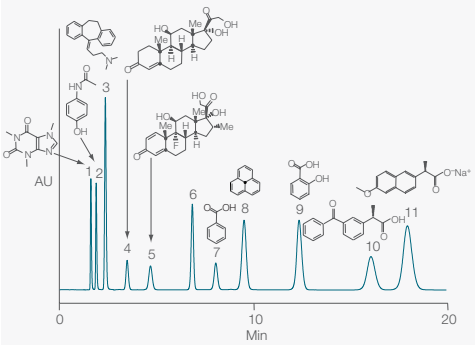
Separation of different types of molecules including bases, neutrals, and acids, in complicated mixtures within a single chromatographic run on the same column is challenging. The novel column chemistry of the Acclaim Mixed-Mode WAX-1 column results in a multimode separation mechanism including hydrophobic, anion-exchange, and ion-exclusion interactions. Consequently, retention of basic, neutral, and acidic molecules can be either independently or concurrently adjusted by changing ionic strength, pH, and organic solvent content in the mobile phase. While all types of molecules are retained by hydrophobic retention, the presence of anion-exchange functionality results in increased retention for anionic species through electrostatic attraction, decreased retention for cationic compounds through electrostatic repulsion, and virtually no effect on neutral molecules.

Figures 10 and 11 demonstrate simultaneous separations of a mixture of basic, neutral, and acidic molecules by both isocratic and gradient methods, with excellent peak shape and resolution. Conventional C18 columns fail to provide separation with comparable resolution because hydrophobic retention alone often provides inadequate leverage for selectivity manipulation in method development.

Multimode retention mechanisms: RP, anion-exchange, and HILIC

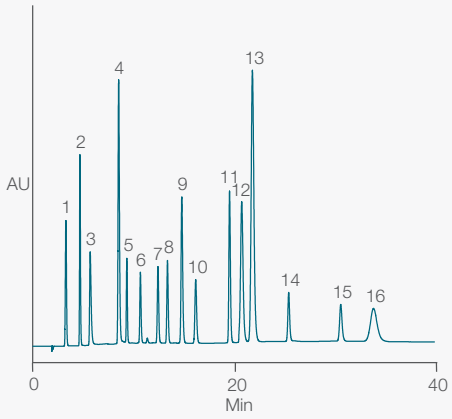
The Acclaim Mixed-Mode WAX-1 column offers multiple retention modes: RP, anion-exchange, cation-exclusion, and HILIC.

In HILIC mode, the column separates highly polar compounds such as amino acids (Figure 12), water-soluble vitamins, and mono- and disaccharides.



Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	50/50 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)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 220 nm
Peaks	1. Caffeine 2. Acetaminophen 3. Amitriptyline 4. Hydrocortisone 5. Dexamethasone 6. Iodide 7. Benzoic acid 8. Phenanthrene 9. Salicylic acid 10. Ketoprofen 11. Naproxen, Na salt

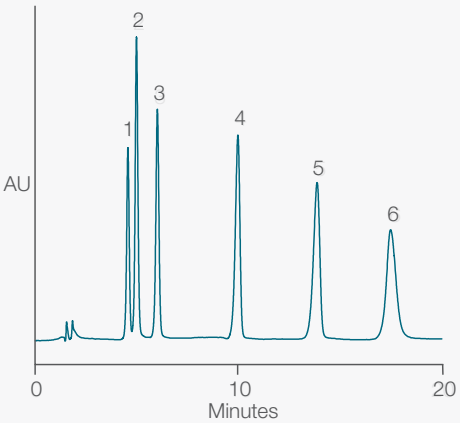
Figure 10. Isocratic separation of basic, neutral, and acidic molecules.



Time (min)	%A	%B	%C
-15	10	80	10
0	10	80	10
10	50	40	10
12	50	40	10
25	50	0	50
40	50	0	50

Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	A: Acetonitrile; B: D.I. water; C: 150 mM phosphate buffer, pH 6.0
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	15 µL
Detection	UV, 220 nm
Peaks	1. Caffeine 50 µg/mL 2. Acetaminophen 100 µg/mL 3. Dextromethorphan 100 µg/mL 4. Amitriptyline 100 µg/mL 5. Prednisone100 µg/mL 6. Hydrocortisone100 µg/mL 7. Dexamethasone 100 µg/mL 8. Acetyl salicylic acid 50 µg/mL 9. Sorbate, potassium 100 µg/mL 10. Benzoic acid 100 µg/mL 11. Salicylic acid 50 µg/mL 12. Ketoprofen 100 µg/mL 13. Naproxen, Na salt 100 µg/mL 14. Aconitic acid, *cis*- 50 µg/mL 15. Aconitic acid, *trans*- 50 µg/mL 16. Ibuprofen 100 µg/mL

Figure 11. Gradient separation of basic, neutral, and acidic pharmaceuticals.

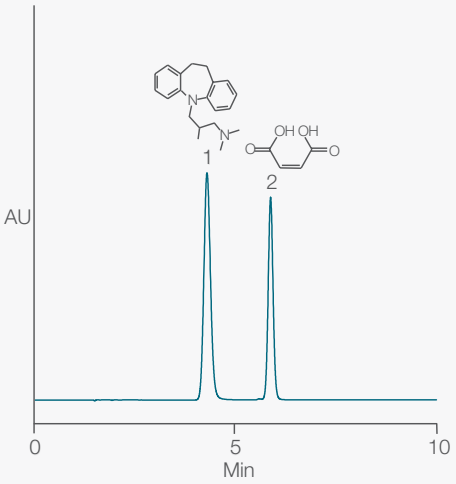


Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	25/75 v/v 25 mM phosphate buffer, pH 6.0/acetonitrile
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 210 nm
Sample	0.5 mg/mL of trimipramine maleate
Peaks	1. Leucine 2. Isoleucine 3. Valine 4. Alanine 5. Serine 6. Glycine

Figure 12. Separation of selected amino acids in HILIC mode.

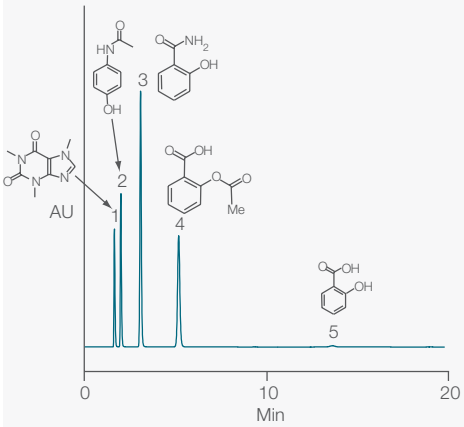
Examples of applications

The Acclaim Mixed-Mode WAX-1 column can be used either as a primary column for a broad range of samples commonly analyzed in the pharmaceutical, food and beverage, and chemical industries, or a confirmation column to complement conventional RP columns by providing orthogonal selectivity. Figure 13 shows a pharmaceutical sample separation of a basic active ingredient and its counter-ion simultaneously determined on an Acclaim Mixed-Mode WAX-1 column. Figures 14–16 demonstrate the separations of ingredients in three over-the-counter medicines that contain basic, neutral, and acidic ingredients. Figures 17 and 18 show efficient analysis of common ingredients in soft drinks.



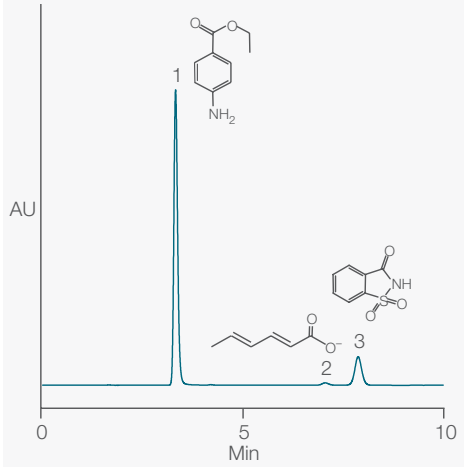
Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	30/70 v/v acetonitrile/phosphate buffer, pH 6.0 (50 mM overall)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2.5 µL
Detection	UV, 220 nm
Sample	0.5 mg/mL of trimipramine maleate
Peaks	1. Trimipramine 2. Maleate

Figure 13. Simultaneous determination of basic drug and its counter ion.



Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	40/60 v/v Acetonitrile/buffer (6.8 g potassium monophosphate and 0.5 g pyrophosphate in 1000 g D.I. H2O, pH is adjusted to 6.0 with NaOH
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	1 µL
Detection	UV, 220 nm
Sample prep	1. Grind 500 mg tablet into fine powder. 2. Weigh 400 mg of the powder and dissolve with 40 g of D.I. water. 3. After sonicating for 5 min, the filter suspension with 0.45 µm membrane filter.4. Dilute the filtrate fourfold with D.I. water
Peaks	1. Caffeine 2. Acetaminophen 3. Salicylamide 4. Acetyl salicylic acid (Aspirin) 5. Salicylic acid

Figure 14. Analysis of a pain relief medicine.

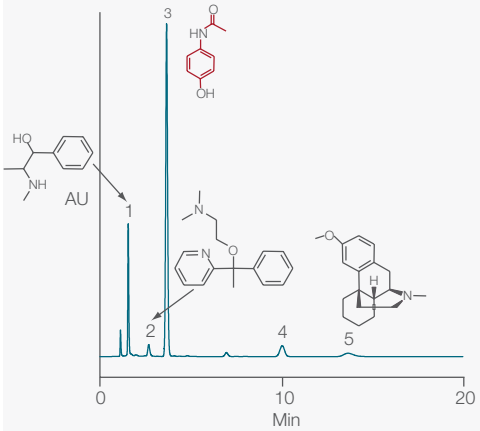


Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	40/30/30 v/v/v Acetonitrile/ D.I. H_2O/buffer (13.6 g potassium monophosphate and 1 g pyrophosphate in 1000 g D.I. H_2O, pH is adjusted to 6.15 with NaOH and HCl)
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2.5 µL
Detection	UV, 210 nm
Peaks	1. Benzocaine 2. Sorbate 3. Saccharin

Figure 15. Analysis of a toothache pain reliever.

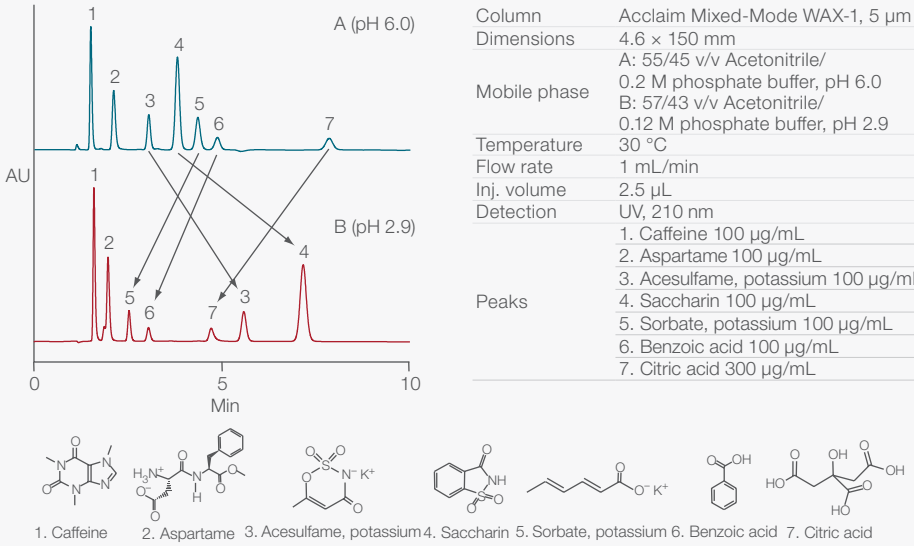
Manufacturing standards ensure reproducibility

Each Acclaim Mixed-Mode WAX-1 column is manufactured to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a quality assurance report, covering column asymmetry and efficiency.



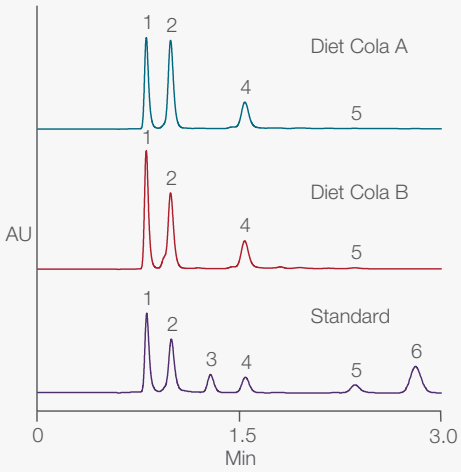
Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	10/90 v/v Acetonitrile/phosphate buffer (13.6 g potassium monophosphate and 1 g pyrophosphate in 1000 g D.I. H_2O, pH is adjusted to 6.15 with NaOH and HCl)
Temperature	30 °C
Flow rate	1.5 mL/min
Inj. volume	2.5 µL
Detection	UV, 210 nm
Peaks	1. Pseudoephedrine 2. Doxylamine 3. Acetaminophen 4. Citrate 5. Dextromethorphan

Figure 16. Separation of active ingredients in a cough syrup.



Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 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
Inj. volume	2.5 µL
Detection	UV, 210 nm
Peaks	1. Caffeine 100 µg/mL 2. Aspartame 100 µg/mL 3. Acesulfame, potassium 100 µg/mL 4. Saccharin 100 µg/mL 5. Sorbate, potassium 100 µg/mL 6. Benzoic acid 100 µg/mL 7. Citric acid 300 µg/mL

Figure 17. Analysis of soft drinks.



Column	Acclaim Mixed-Mode WAX-1, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	57/43 v/v Acetonitrile/ 120 mM phosphate buffer, pH 2.9
Temperature	30 °C
Flow rate	2 mL/min
Inj. volume	2.5 µL
Detection	UV, 210 nm
Peaks	1. Caffeine 2. Aspartame 3. Sorbate 4. Benzoate 5. Citrate 6. Acesulfame

Figure 18. Rapid analysis of soft drinks.

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Acclaim Mixed-Mode WAX-1 column specifications

Specifications	
Column chemistry	Alkyl amine
Base silica	Spherical, high-purity
Particle sizes	3 µm and 5 µm
Pore size	120 Å
Surface area	300 m^2/g

Ordering information

Column	Format	Particle size (µm)	Length (mm)	ID (mm)	Part number
Mixed-Mode WAX-1	HPLC column	3.0	50	3.0	071908
			150	2.1	070089
				3.0	070088
		5.0	150	2.1	067084
				4.6	064984
			250	4.6	064985
	Guard cartridge (2/pk)	5.0	10	2.1	069686
				3.0	071909
				4.6	069704

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

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