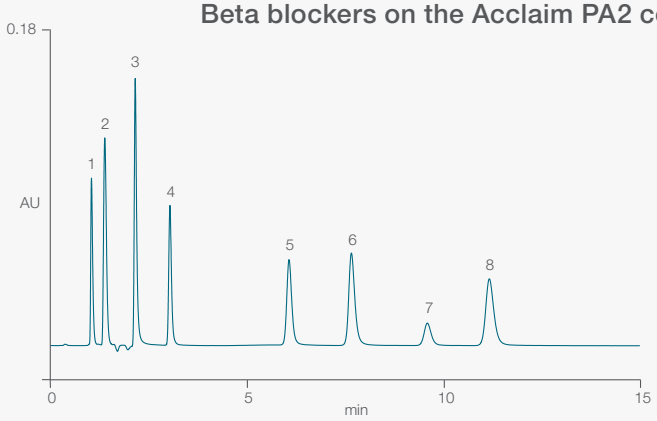
Acclaim PolarAdvantage II (PA2) HPLC columns

A unique and complementary alternative to conventional C18 columns

Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2) columns are silica-based polar-embedded columns for pharmaceutical, environmental, chemical, food, and life science applications.

Beta blockers on the Acclaim PA2 column



Column	Acclaim PA2, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	MeOH/0.2% NH_4OH, pH10, v/v, 60/40
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	(40 ppm each) 1. Maleate 2. Labetalol 3. Metaraminol 4. Atenolol 5. Acebutolol 6. Metoprolol 7. Timolol 8. Oxprenolol

Column features

- Separates polar and nonpolar compounds
- Exceptional hydrolytic stability (pH 1.5–10)
- High polarity for complementary selectivity to C18 columns
- Compatible with 100% aqueous mobile phases
- Good peak shapes for both acidic and basic compounds
- High column efficiency
- Good batch-to-batch reproducibility
- Broad range of applications

Separates polar and nonpolar compounds

The separation of polar and nonpolar compounds in a single run on a reversed-phase column is often a difficult proposition for chromatographers because the polar compounds are eluted either in the void, or unresolved from one another at the beginning of the chromatogram. The challenge with reversed-phase C18 columns, for this type of application, is low retention of polar compounds under the conditions necessary to approximate a separation, dewetting in 100% aqueous mobile phases, and more or less the same selectivity regardless of the column vendor.

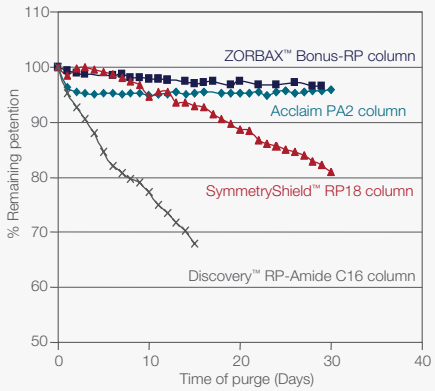
Existing polar-embedded phases offer different selectivity from conventional C18 columns and symmetrical peak shapes for basic compounds, but often at the expense of hydrophobic retention and hydrolytic stability at both low and high pH.

The Acclaim PA2 column is a novel, high-efficiency, silica-based, polar-embedded column, manufactured by bonding a specially designed proprietary amide-embedded ligand to 3 or 5 µm diameter high-purity spherical silica. This column is compatible with 100% aqueous environments over a wide range of pH (see beta blockers in figure above), exhibits high polarity for selectivity complementary to conventional C18 columns, and provides excellent peak shapes and efficiencies for both basic and acidic compounds.

ThermoFisher SCIENTIFIC

Exceptional hydrolytic stability

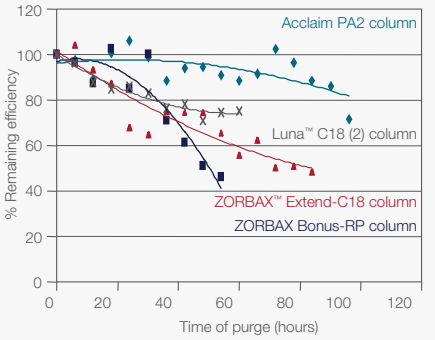
Polar-embedded silica stationary phases provide several advantages over general-purpose C18 phases, including improved peak shapes of basic analytes, compatibility with highly aqueous mobile phases, and unique selectivity characteristics. However, the hydrolytic stability of polar-embedded columns is typically inferior to that of hydrophobic C18 supports, due to lower ligand coverage and the hydrophilic nature of the embedded group. To address the inferior hydrolytic stability issue, Thermo Fisher Scientific scientists have developed a polar-embedded column with enhanced hydrolytic stability that provides selectivity complementary to conventional C18 columns throughout the whole operating pH range (pH 1.5 to 10).



Column	See chromatogram, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/1% TFA*, pH 1 v/v 50/50
Temperature	50 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Analyte	Toluene

*Trifluoroacetic acid

Figure 1. Accelerated stability test at low pH.



Column	See chromatogram, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	Aged in CH_3CN/20 mM triethylamine, pH 11.5 v/v 50/50 at 60 °C. Tested in CH_3CN/H_2O v/v 70/30 at 60 °C.
Inj. volume	5 µL
Detection	UV, 254 nm
Analyte	Phenanthrene

Figure 2. Accelerated stability test at high pH.

High performance liquid chromatography (HPLC) separations of polar analytes are often run under acidic conditions (<3.0) to reduce tailing of amine-containing compounds. While the acidic conditions improve peak shape, the harsh environment will shorten column life due to cleavage of the bonded phase, resulting in frequent column replacement and instrument downtime. Figure 1 shows an accelerated low-pH degradation test using 1% TFA at pH 1. After 30 days under these conditions, the Acclaim PA2 phase is stable, while the Discovery RP-Amide C16 and the SymmetryShield RP18 columns show significant loss of the bonded phase.

Figure 2 shows a similar accelerated degradation test at high pH, using triethylamine at pH 11.5 to age the column. After 60 h, the Acclaim PA2 column still retains 90% of its retention capacity, whereas the other commercially available columns have dropped to less than 80%, and in some cases less than 50%. Thus, as shown by Figures 1-2, the proprietary bonding of the Acclaim PA2 column is capable of resisting hydrolytic attack by protecting the bonded phase at both low and high pH.

High polarity for selectivity complementary to C18 columns

The ability of a reversed-phase column to retain polar compounds can be estimated based on the polarity of the stationary phase. The more polar the stationary phase, the longer the polar compounds will be retained. Figure 3 shows a comparison of the polarity of a series of commercially available conventional C18 columns and polar-embedded columns. The Thermo Scientific™ Acclaim™ C18 column and the Luna C18 (2) are representative of conventional C18 columns; the other columns are polar-embedded. As expected, the conventional reversed-phase columns exhibit lower polarity than the polar-embedded phases. Included in Figure 3 are three types of Thermo Scientific™ Acclaim™ columns: Thermo Scientific™ Acclaim™ 120 C18 columns, the Thermo Scientific™ Acclaim™ PolarAdvantage (PA) columns, and the Acclaim PolarAdvantage II (PA2) columns. As can be seen from the wide range of polarity encompassed by these columns, they comprise a powerful column portfolio for routine application development.

The polar characteristics of polar-embedded phases often provide significantly better selectivity for mixtures of acids and bases. Generally speaking, you can expect acidic compounds to be retained longer and basic compounds to be retained slightly less on polar-embedded phases than on conventional C18 phases. Figure 4 shows a comparison of the Acclaim C18 column with the Acclaim PA2 column, run under identical conditions, illustrating this trend. The Acclaim PA2 column provides different and complementary selectivity to conventional C18 columns.

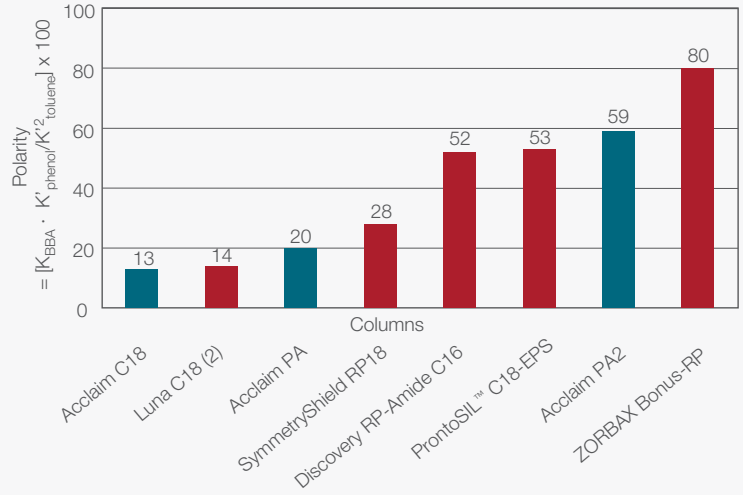
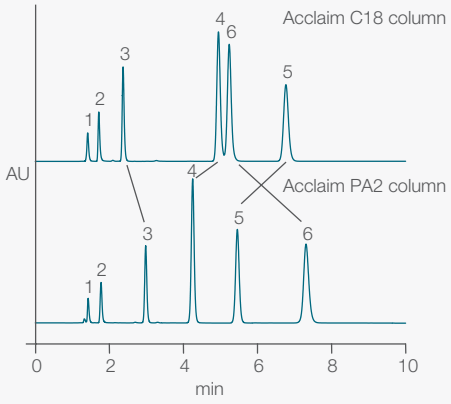


Figure 3. Polarity comparison.



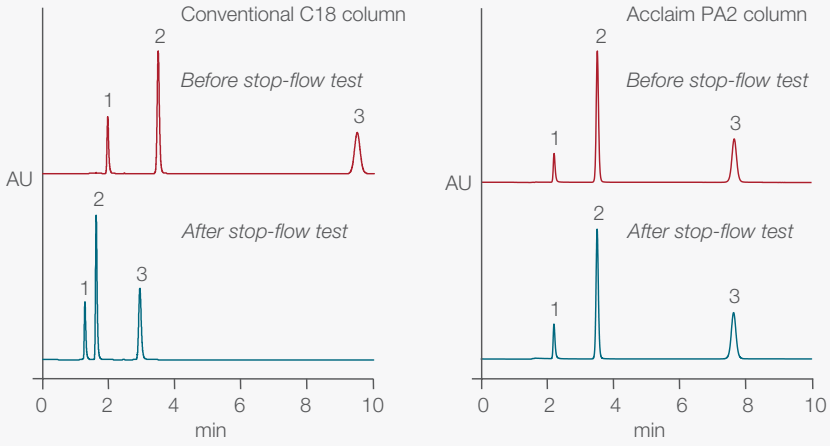
Column	See chromatograms, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/25 mM potassium phosphate buffer, pH 3.3 v/v 60/40
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 206 nm
Peaks	1. Uracil 7.5 µg/mL
	2. Pyridine 50 µg/mL
	3. Phenol 50 µg/mL
	4. N,N-Dimethylaniline 50 µg/mL
	5. Toluene 60 µg/mL
	6. p-Butylbenzoic acid 50 µg/mL

Figure 4. Unique selectivity.

Compatibility with 100% aqueous mobile phases

Chromatographers have learned, often the hard way, that conventional reversed-phase columns are not compatible with 100% aqueous mobile phases. For most conventional C18 columns, 95% aqueous is the limit before the phase collapses and retention is lost. Unfortunately, for many highly polar, water-soluble organic compounds, retention on a reversed-phase column requires the use of little or no organic modifier.

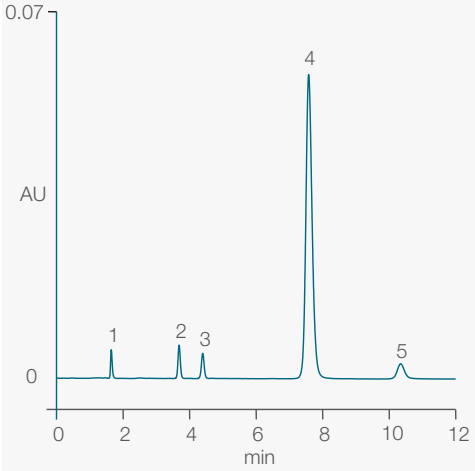
The carefully-designed bonding technology of the Acclaim PA2 column incorporates hydrophilic functional groups between the hydrophobic alkyl chain and the silica surface that allows the surface to remain wetted, even in 100% aqueous mobile phase conditions. As shown in Figure 5, the conventional C18 column rapidly loses its ability to retain, following exposure to a pure aqueous buffer. In comparison, the Acclaim PA2 column is highly compatible with this aqueous environment, providing consistent and reliable retention times for even very hydrophilic nucleic acid bases such as cytosine, uracil, and thymine.



Column	See chromatograms, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	2.5 mM methanesulfonic
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL

Detection	UV, 254 nm
Peaks	1. Cytosine 2. Uracil 3. Thymine

Figure 5. Dewetting test in 100% aqueous eluent.



Column	Acclaim PA2, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	MeOH/20 mM potassium phosphate buffer, pH 7.0 v/v 80/20
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Sample	NIST SRM870 (column performance test mix for liquid chromatography)

Peaks	Concentration (µg/mL)	Asymmetry (EP)
1. Uracil	1.4	1.31
2. Toluene	70	1.05
3. Ethylbenzene	85	1.04
4. Amitriptyline	140	1.10
5. Quinizarin	4.7	1.10

Figure 6. Excellent peak shape for basic compounds.

Good chromatographic performance for both acidic and basic compounds

At pH 7, amitriptyline is a sensitive probe for measuring silanol activity. At this pH, free silanols are negatively charged and the amitriptyline molecule is protonated (pK_a = 9.3). The protonated base interacts with ionized silanols by means of an ion-exchange mechanism; the degree of tailing in this case is a direct measure of silanol activity. NIST 870 specifies both the chromatographic conditions and the nature of the test mixture for evaluating silanol activity on reversed phase silica columns. As shown in Figure 6, the Acclaim PA2 column provides a highly symmetrical peak for amitriptyline under this well-accepted testing protocol. Regardless, for particularly difficult samples, comprising highly basic compounds and requiring a highly aqueous mobile phase, acidic conditions will produce the best results on the Acclaim PA2 column.

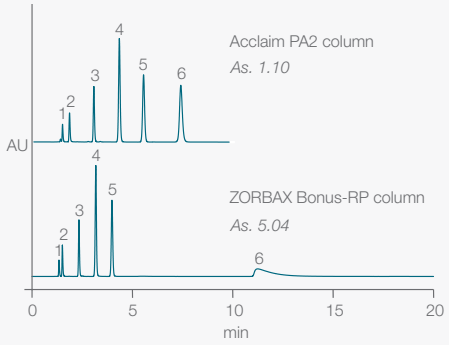
Figure 7 shows that the Acclaim PA2 column provides excellent peak shapes for acidic, basic, and neutral compounds within a single run in a phosphate buffer at pH 3.2. In comparison, the ZORBAX Bonus-RP column, featured as a hydrolytically stable polar-embedded phase, shows poor peak shape for p-butylbenzoic acid, despite the fact that neutral and basic compounds are eluted with symmetrical peak shapes.

High column efficiency

The combination of advanced bonding technology and optimal packing procedures results in superior column efficiencies for Acclaim PA2 columns. As illustrated in Figure 8, the Acclaim PA2 column provides higher efficiencies than other reversed-phase columns designed for high-pH applications, including the XTerra MS C18 column.

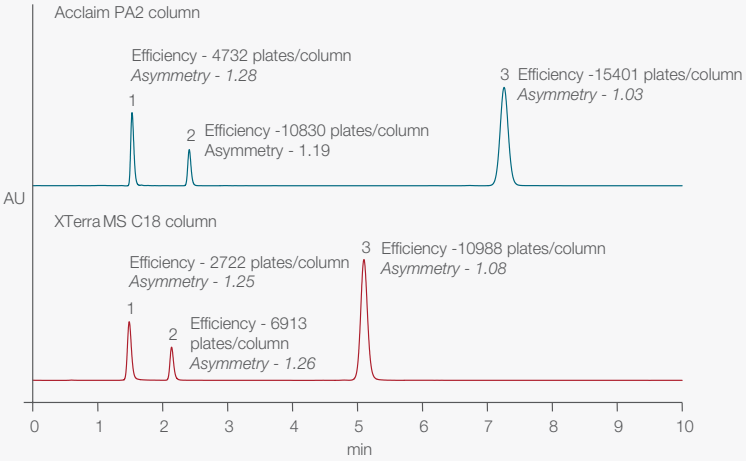
Good batch-to-batch reproducibility

Each Acclaim PA2 column is manufactured to stringent specifications to ensure column-to-column reproducibility (Figure 9). Each column is shipped with a lot validation sheet showing the test results and specifications for the lot of bonded silica packed into the column and an individual test chromatogram validating performance. All columns are individually tested for capacity and efficiency, and closely monitored for metal contamination.



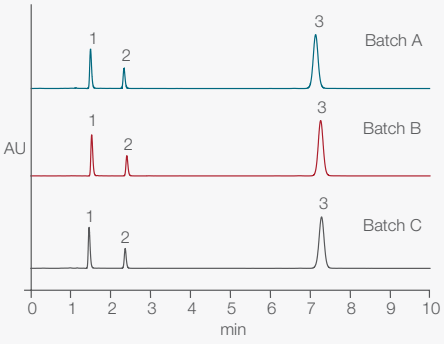
Column	See chromatograms, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/25 mM potassium phosphate buffer, pH 3.3 v/v 60/40
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 206 nm
Peaks	1. Uracil 7.5 µg/mL
	2. Pyridine 50 µg/mL
	3. Phenol 50 µg/mL
	4. N,N-Dimethylaniline 50 µg/mL
	5. Toluene 60 µg/mL
	6. p-Butylbenzoic acid 50 µg/mL

Figure 7. Excellent peak shape for both basic and acidic compounds.



Column	See chromatograms, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Uracil
	2. Dimethylphthalate
	3. Phenanthrene

Figure 8. High column efficiency.



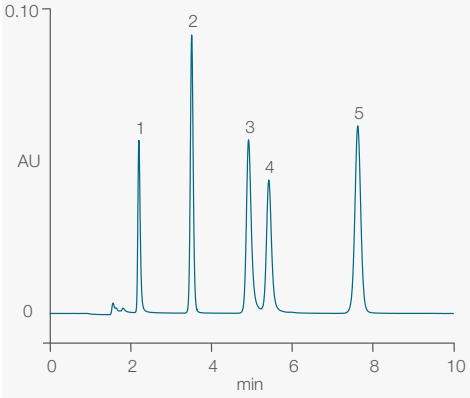
Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/D.I. H_2O, v/v, 70/30
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Uracil
	2. Dimethylphthalate
	3. Phenanthrene

Figure 9. Batch-to-batch reproducibility.

Broad range of applications

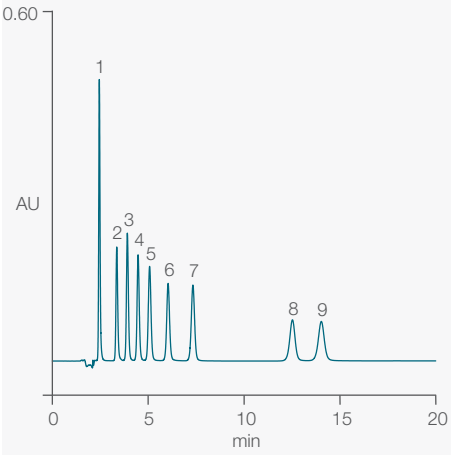
The Acclaim PA2 columns can be used for a wide range of applications, as illustrated in Figures 10–20. Figures 10, 11, and 12 show separations for nucleic acid bases, sulfa drugs, and water-soluble vitamins, respectively, in highly aqueous conditions with excellent resolution and peak efficiencies.

Separations of fat-soluble vitamins and parabens (Figures 13 and 14) represent more conventional reversed-phase applications that are readily achieved on the Acclaim PA2. Figures 15 and 16 demonstrate the potential of separating basic drugs, such as antidepressant drugs and beta blockers, at elevated pH to achieve optimum retention and peak shapes.



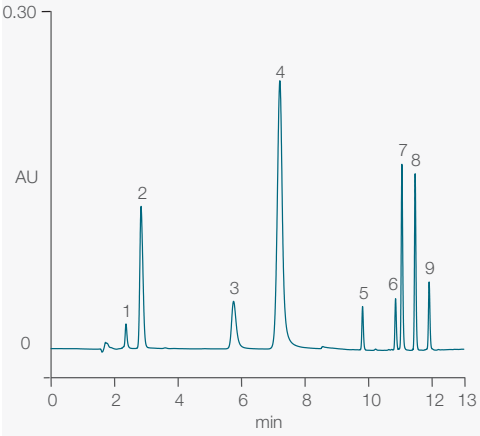
Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	25 mM potassium phosphate buffer, pH 3.3
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Cytosine 25 µg/mL 2. Uracil 25 µg/mL 3. Adenine 25 µg/mL 4. Guanine 25 µg/mL 5. Thymine 50 µg/mL

Figure 10. Nucleic acid bases.



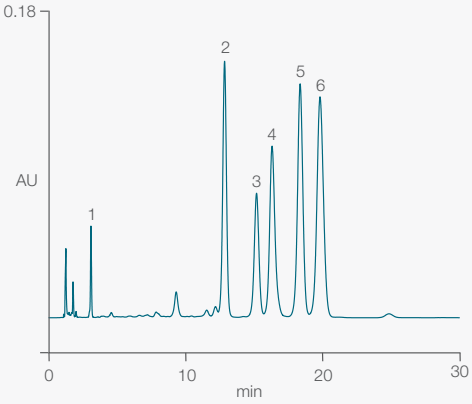
Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/0.1 M NH_4OAc, pH 5.4, v/v, 25/75
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 265 nm
Peaks	Peaks: (50 ppm each) 1. Sulfanilamide 2. Sulfathiazole 3. Sulfamerazine 4. Sulfamethazine 5. Sulfisoxazole 6. Sulfapyridazine 7. Sulfamethoxazole 8. Sulfadimethoxine 9. Sulfaquinoxaline sodium

Figure 11. Sulfa drugs.



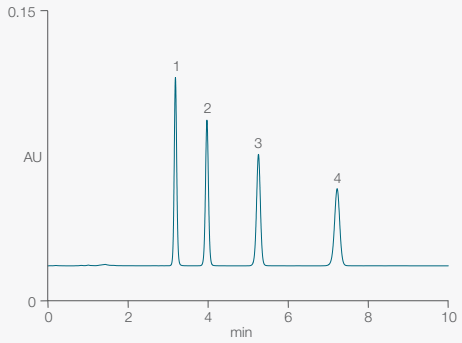
Column	Acclaim PA2, 5 µm
Dimensions	4.6 × 150 mm
Mobile phase	A: CH3CN25/75, B: 25 mM phosphate buffer, pH 3.31
Gradient	Hold B for 5 min B to A/B (40/60) in 7 min hen to A/B (75/25) in 1 min Hold A/B (75/25) for 4 min
Temperature	30 °C
Flow rate	1 m L/min
Inj. volume	50 µL
Detection	UV, 210 nm
Peaks	Peaks: (50 ppm each) 1. Thiamine 2.5 µg/mL 2. Ascorbic acid 60 µg/mL 3. Pyridoxine 2.5 µg/mL 4. Niacinamide 20 µg/mL 5. Pantothenic acid 10 µg/mL 6. Vitamin B12 1.25 µg/mL 7. Folic acid 5 µg/mL 8. Riboflavin 5 µg/mL 9. Biotin 12.5 µg/mL

Figure 12. Water-soluble vitamins.



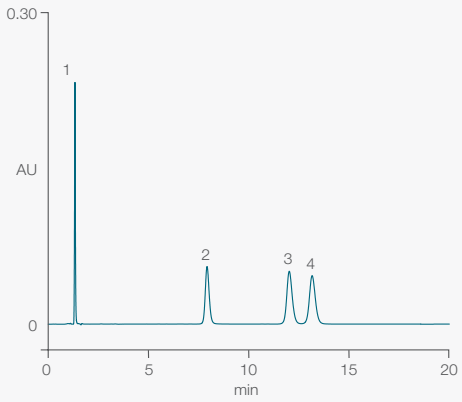
Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/0.1% MSA, v/v,94/6
Temperature	30 °C
Flow rate	1.5 mL/min
Inj. volume	20 µL
Detection	UV, 210 nm
Peaks	(150 ppm each) 1. Vitamin A acetate 2. Vitamin E acetate 3. Vitamin D2 4. Vitamin D3 5. Vitamin K1 6. Vitamin E

Figure 13. Fat-soluble vitamins.



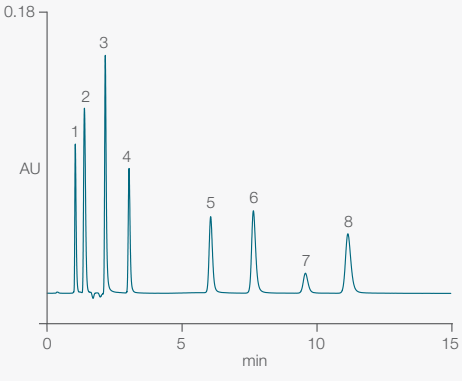
Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	CH_3CN/H_2O v/v 60/40
Temperature	30 °C
Flow rate	1.0 mL/min
Inj. volume	2 µL
Detection	UV, 254 nm
Peaks	(0.1 mg/mL each) 1. Methylparaben 2. Ethylparaben 3. Propylparaben 4. Butylparaben

Figure 14. Parabens.



Column	Acclaim PA2, 5 µm		
Dimensions	4.6 × 150 mm		
Mobile phase	MeOH/20 mM potassium phosphate buffer, pH 7.0 v/v 80/20		
Temperature	30 °C		
Flow rate	1 mL/min		
Inj. volume	5 µL		
Detection	UV, 254 nm		
Sample	NIST SRM870 (column performance test mix for liquid chromatography)		
Peaks		Concentration (µg/mL)	Asymmetry (EP)
	1. Uracil	10	1.08
	2. Doxepin	100	1.14
	3. Imipramine	80	1.15
	4. Amitriptyline	100	1.11

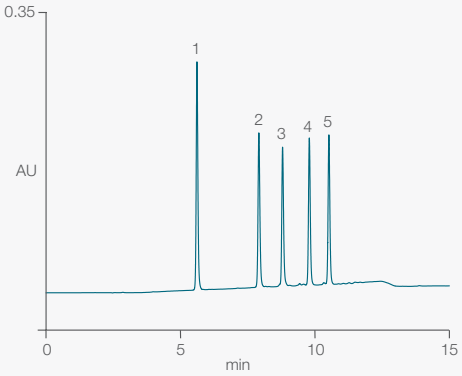
Figure 15. Antidepressants.



Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	MeOH/0.2% NH_4OH, pH10, v/v, 60/40
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	(40 ppm each) 1. Maleate 2. Labetalol 3. Metaraminol 4. Atenolol 5. Acebutolol 6. Metoprolol 7. Timolol 8. Oxprenolo 8. Oxprenolol

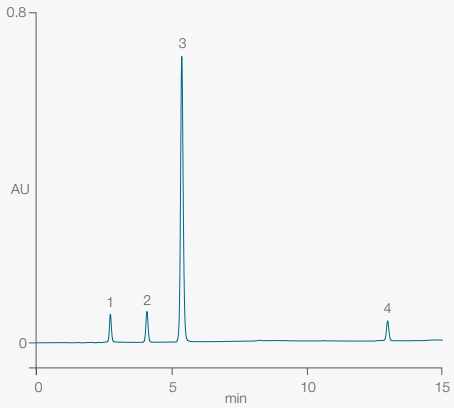
Figure 16. Beta blockers.

The versatility of the Acclaim PA2 column is clearly illustrated in the next series of chromatograms. Acidic mobile phases are used in Figures 17 and 18 for the separation of peptides and cough syrup ingredients, respectively. Due to the nature of the cough syrup ingredients, highly aqueous conditions are necessary.



Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	Acetonitrile/0.1% methanesulfonic acid from 1/99 to 55/45 (v/v) in 15 min
Temperature	30 °C
Flow rate	1.0 mL/min
Inj. Volume	25 µL
Detection	UV, 220 nm
Peaks	1. Gly-Tyr 2. Val-Tyr-Val 3. Met enkephalin 4. Leu enkephalin 5. Angiotensin II

Figure 17. Peptide mix.

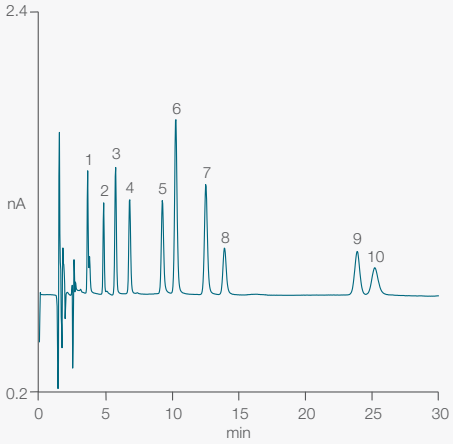


Column	Acclaim PA2, 5µm
Dimensions	4.6 × 150 mm
Mobile phase	Acetonitrile/50 mM phosphate buffer, pH 1.9 from 10/90 to 40/60 (v/v) in 15 min
Temperature	30 °C
Flow rate	1.0 mL/min
Inj. Volume	20 µL
Detection	UV, 210 nm
Peaks	1. Doxylamine 2. Pseudo-ephedrine 3. Acetaminophen 4. Dextromethorphan

Figure 18. Active ingredients in cough syrup.

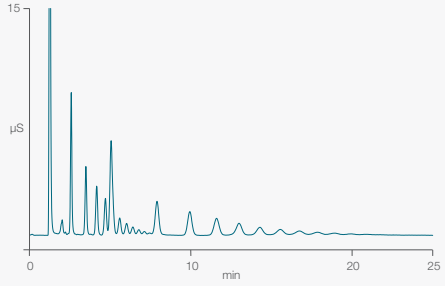
In Figure 19, the Acclaim PA2 column is used for ion-pair chromatography for the baseline separation of nine catecholamines and their metabolites commonly found in urine.

In Figure 20, the Acclaim PA2 column is used in an entirely different approach to separate anionic surfactants using a borate buffer and suppressed conductivity detection.



Column	Acclaim PA2, 3 µm
Dimensions	2.1 × 150 mm
Mobile phase	Buffer (11.98 g citric acid, 3.53 g NaOAc, 37.2 mg EDTA, 10 mL of 0.1 M methanesulfonic acid)/ MeOH v/v 90/10
Temperature	30 °C
Flow rate	0.2 mL/min
Inj. Volume	2 µL
Detection	dc amperometry [GC electrode, 800 mV]
Peaks	(mg/mL each) 1. VMA 2. HMPG 3. NE 4. E 5. DHBA 6. NMN 7. MN 8. DA 9. HV 10. HIAA

Figure 19. Catecholamines in urine.



Column	Acclaim PA2, 5 µm
Dimensions	2.1 × 150 mm
Mobile phase	Acetonitrile/25 mM borate buffer v/v 40/60
Temperature	30 °C
Flow rate	1 mL/min
Inj. Volume	20 µL (1000 ppm)
Detection	Suppressed conductivity detection (Thermo Scientific™ Dionex™ AMMS-III Suppressor)
Sample	POE(30) Ammonium lauryl sulfate

Figure 20. POE(30) Ammonium lauryl sulfate.

Acclaim PA2 column specifications

Specifications	
Phase	Polar-embedded
Particle size	2.2 µm, 3 µm and 5 µm
Pore size	120 Å
Surface area	300 m^2/g
pH range	1.5–10

thermo scientific

Ordering information

Column	Format	Particle size (µm)	Length (mm)	2.1 ID (mm)	3.0 ID (mm)	4.6 ID (mm)
Acclaim PA2	Analytical	2.2	30	071402	-	-
			50	068989	071608	-
			100	068990	071607	-
			150	071401	-	-
			250	074814		
		3.0	33	-	066276	-
			50	077999	068973	063189
			75	-	066277	-
			100	077998	078000	078001
			150	063187	063705	063191
			250	077997	070080	-
		5.0	150	-	-	063197
			250	-	-	063199
	Guard Cartridge (2/pk)	5.0	10	069693	071985	069699

Column	Particle size (µm)	Length (mm)	ID (mm)	Part number
Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2)	2.2	150	2.1	071401-V
Acclaim PolarAdvantage II (PA2) Analytical	2.2	250	2.1	074814-V

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**

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