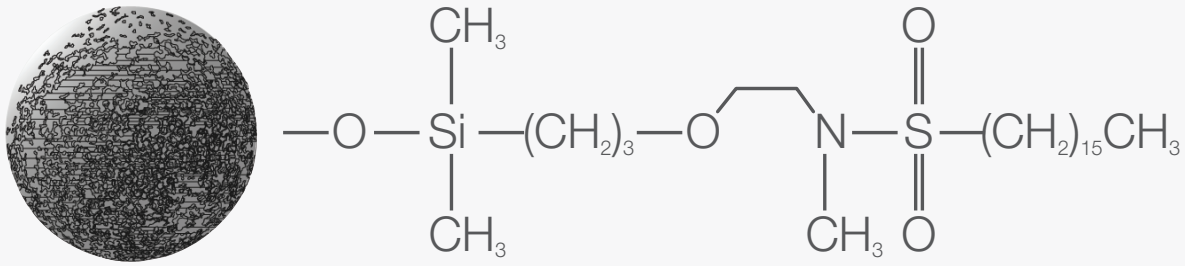
Acclaim PolarAdvantage HPLC columns

For operation at wider chromatographic conditions

Thermo Scientific™ Acclaim™ PolarAdvantage (PA) columns are reversed-phase silica columns with a polar-enhanced stationary phase for operation at wider chromatographic conditions and with a broader application range when compared to conventional reversed-phase columns.

Acclaim PolarAdvantage column bonding



Column features

- Analysis of polar and nonpolar analytes
- Excellent peak shapes for both basic and acidic analytes
- Compatible with 100% aqueous mobile phases
- High reversed-phase capacity
- Unique polar selectivity
- Mass spectrometry (MS) compatible

Separate both polar and nonpolar compounds

Acclaim PA columns are silica-based, reversed-phase columns featuring a polar-enhanced stationary phase. This phase consists of a C16 functional group bonded to the surface of ultrapure silica using a sulfonamide group, coupled to an ether linkage (Figure 1). While providing polarity, hydrophobicity, and selectivity similar to conventional C18 phases, this polar-embedded phase provides excellent peak shapes for both basic and acidic compounds, compatibility with 100% aqueous mobile phases, and hydrolytic stability—overcoming many of the limitations of conventional C8 and C18 reversed-phase columns.

Acclaim PA columns exhibit enhanced retention of polar compounds and the ability to perform analysis of both polar and nonpolar analytes on a single column. By providing for a much larger range of application possibilities, the Acclaim PA column can meet or exceed the requirements for the majority of reversed-phase high performance liquid chromatography (HPLC) separations.

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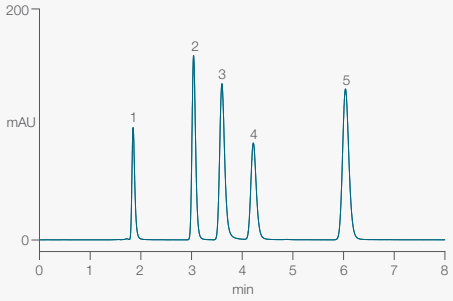
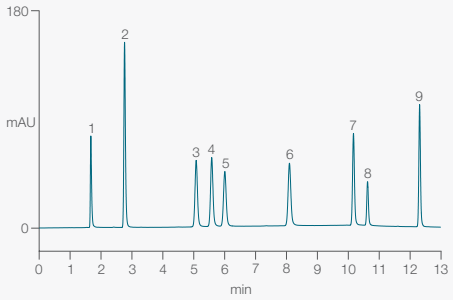
Wide range of applications

For conventional reversed-phase separations, the Acclaim PA column exhibits selectivities similar to standard C8 or C18 phases. As illustrated in Figures 1–8, the Acclaim PA column can be used in a wider range of application areas, including pharmaceuticals, bio and life sciences, food, and environmental analysis. In addition, Figures 1–2 show that efficient separations with good peak shapes are obtained for compounds of life science (nucleic acid bases) and pharmaceutical interest (sulfonamides), using highly aqueous mobile phases.



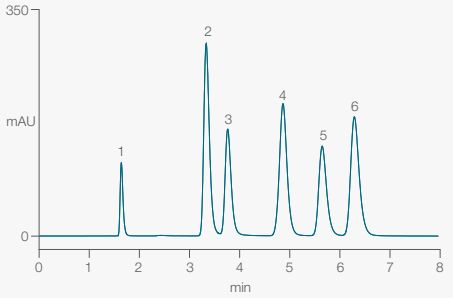
Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	30 mM phosphate, pH 3.5
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Cytosine 50 µg/mL 2. Uracil 50 µg/mL 3. Adenine 50 µg/mL 4. Guanine 50 µg/mL 5. Thymine 100 µg/mL

Figure 1. Separation of nucleic acid bases.



Column	Acclaim PA, 3 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: 90/10 v/v MeOH/20 mM phosphate, pH 2.7, B:10/90 v/v MeOH/20 mM phosphate, pH 2.7
Gradient	15–30% A at 5 min, to 75% at 10 min, to 85% at 12 min
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 254 nm
Peaks	1. Sulfanilic acid 6 µg/mL 2. Sulfanilamide 4 µg/mL 3. Sulfadiazine 10 µg/mL 4. Sulfathiazole 10 µg/mL 5. Sulfamerazine 10 µg/mL 6. Sulfamethazine 10 µg/mL 7. Sulfamethoxazole 14 µg/mL 8. Sulfisoxazole12 µg/mL 9. Sulfadimethoxine 18 µg/mL

Figure 2. Separation of sulfa drugs.

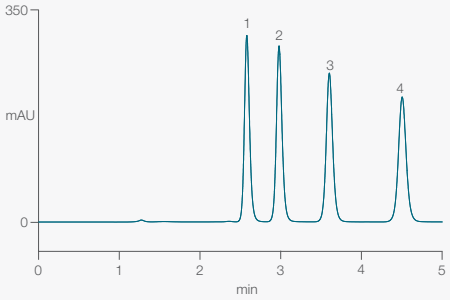


Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	80/20 v/v MeOH/30 mM phosphate, pH 6.0
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 220 nm
Peaks	1. Uracil 10 µg/mL 2. Protriptyline 50 µg/mL 3. Nortriptyline 25 µg/mL 4. Doxepin 50 µg/mL 5. Imipramine 40 µg/mL 6. Amitriptyline 50 µg/mL

Figure 3. Separation of antidepressants.

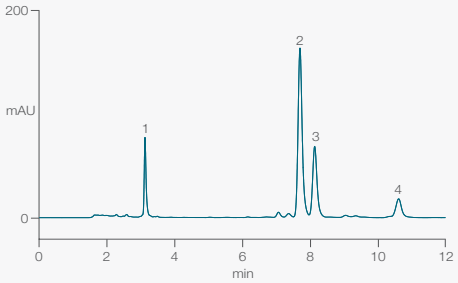
Separate polar and nonpolar analytes on a single column

Separation of antidepressants (Figure 3), nonpolar parabens (Figure 4), and fat-soluble vitamins (Figure 5) represent more conventional reversed-phase separations, whereas the addition of water-soluble vitamins (Figure 6) illustrates the versatility of this unique column.



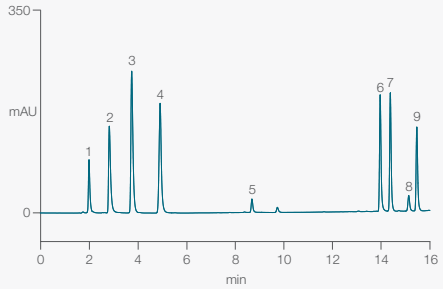
Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	60/40 v/v CH_3CN/D.I. H_2O
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	2 µL
Detection	UV, 254 nm
Peaks	1. Methylparaben 250 µg/mL 2. Ethylparaben 250 µg/mL 3. Propylparaben 250 µg/mL 4. Butylparaben 250 µg/mL

Figure 4. Separation of parabens.



Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	97/3 v/v CH_2CN/D.I. H_2O
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	10 µL
Detection	UV, 264 nm
Peaks	1. All-trans Retinol (Vitamin A) 120 µg/mL 2. Ergocalciferol (Vitamin D_2) 120 µg/mL 3. Cholecalciferol (Vitamin D_3) 60 µg/mL 4. α-Tocopherol (Vitamin E) 1300 µg/mL

Figure 5. Separation of fat-soluble vitamins A, D_2, D_3, and E.



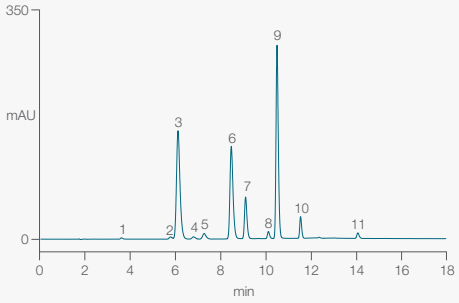
Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: CH_3CN, B: 25 mM KH_2PO_4, pH 3.5
Gradient	0–20% A at 15 min, 60% A at 17–20 min
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 210 nm
Peaks	1. Thiamine HCl 20 µg/mL 2. Nicotinic acid 20 µg/mL 3. Pyridoxine 20 µg/mL 4. Niacinamide 20 µg/mL 5. Pantothenic acid 20 µg/mL 6. Folic acid 20 µg/mL 7. Vitamin B12 20 8. Biotin 20 µg/mL 9. Riboflavin 20 µg/mL

Figure 6. Separation of water-soluble vitamins.

Versatile operating conditions

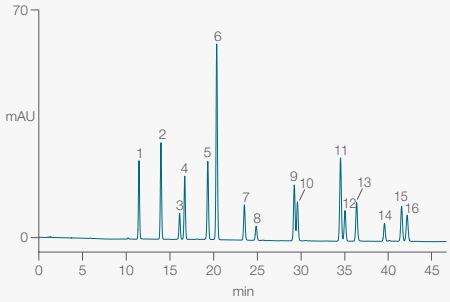
Standard environmental applications can also be performed (Figures 7-8) without significant changes to analyte elution order, when compared to conventional C18 phases.

The range of chromatographic conditions that can be used with the Acclaim PA column, combined with the wide polarity range of target analytes, has resulted in an extremely versatile column that can be used for method development, problem solving, and other analytical areas where conventional reversed-phase columns have limitations.



Column	Acclaim PA, 3 µm
Dimensions	4.6 x 150 mm
Mobile phase	A: 2.5 mM MSA* in MeOH, B: 2.5 mM MSA in D.I. H_2O
Gradient	Hold A/B (60:40) for 4.5 min, A/B (60:40) to A/B (95:5) in 6 min, Hold A/B (95:5) for 7.5 min
Temperature	25 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 285 nm
Peaks	1. Phenol 2. 2-Chlorophenol 3. 4-Nitrophenol 4. 2-Nitrophenol 5. 2,4-Dimethylphenol 6. 4-Chloro-3-methylphenol 7. 2,4-Dichlorophenol 8. 2,4-Dinitrophenol 9. 2-Methyl-4,6-dichlorophenol 10. 2,4,6-Trichlorophenol 11. Pentachlorophenol
	*Methanesulfonic acid

Figure 7. Separation of phenols (EPA 604 method mix).



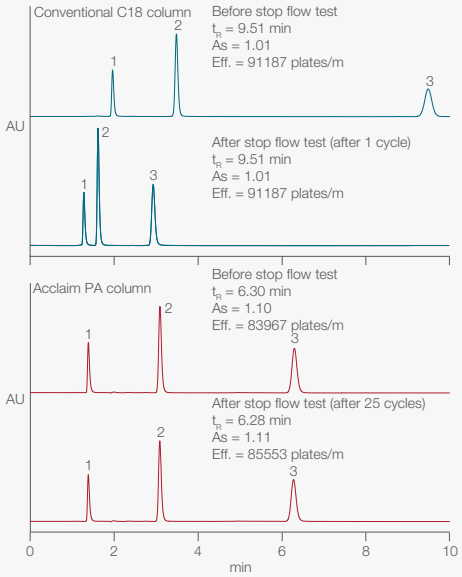
Column	Acclaim PA, 5 µm
Dimensions	4.6 x 250 mm
Mobile phase	A: CH_3CN, B: D.I. H_2O
Gradient	Hold A/B (50:50) for 5 min, A/B (50:50) to A/B (85:15) in 35 min, Hold A/B (85:15) for 5 min
Temperature	25 °C
Flow rate	2 mL/min
Inj. volume	10 µL
Detection	UV, 254 nm
Peaks	1. Naphthalene 2. Acenaphthylene 3. Acenaphthene 4. Fluorene 5. Phenanthrene 6. Anthracene 7. Fluoranthene 8. Pyrene 9. Benzo[a] anthracene 10. Chrysene 11. Benzo[b] fluoranthene 12. Benzo[k] fluoranthene 13. Benzo[a] pyrene 14. Dibenz[a,h] anthracene 15. Benzo[g,h,i] perylene 16. Indeno[1,2,3-cd] pyrene

Figure 8. Separation of PAH polyaromatic hydrocarbons (EPA 610 method mix).

Resistant to dewetting

Because of their high solubility, many polar analytes are adequately retained on a reversed-phase column only when the organic content of the eluent is very low (<5%). The stationary phase of a conventional C8 or C18 reversed-phase column has a highly hydrophobic surface. The attraction between this surface and the aqueous eluent can become so weak that the surface tension of the liquid pulls aqueous mobile phase out of the pores of the silica particles. This phenomenon is best described as dewetting, although it is commonly referred to as phase collapse. Dewetting leads to unexpected loss of analyte retention, reduced efficiency, and changes in peak-shape. The patent-pending technology of the Acclaim PA column incorporates a region of hydrophilic functional groups between the hydrophobic C16 chain and silica surface. This technology allows the surface to remain wetted even in 100% aqueous mobile phase conditions.

The onset of dewetting is unpredictable, but stopping the flow of eluent through the column is known to initiate this effect. Figure 9 shows the effect of repeatedly stopping the flow for 30 min between injections. The Acclaim PA column is immune to a loss of analyte retention, whereas the conventional C18 column dewets in only one cycle.



Column	Acclaim PA, 5 µm
Dimensions	4.6 x 150 mm
Mobile phase	2.5 mM MSA*, pH 2.6
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Cytosine 2. Uracil 3. Thymine
Protocol	Each cycle consists of two steps: 1) Equilibrate column for 20 min before testing for 10 min. 2) Stop flow for 30 min before next cycle begins.
	*Methanesulfonic acid

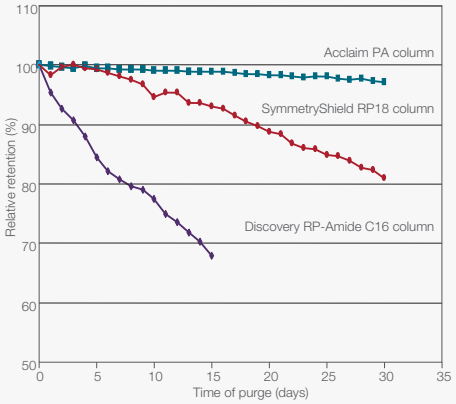
Figure 9. Resistance to dewetting.

Stable at low pH

HPLC separations of polar analytes are often run under acidic conditions to reduce tailing of amine-containing compounds. Separations under these conditions can shorten column life due to cleavage of the bonded phase. This cleavage results in frequent column replacement and instrument downtime. The proprietary bonding of the Acclaim PA column resists hydrolytic attack by protecting the bonded phase at low pH values. Many MS applications require mobile phases containing modifiers such as formic acid, TFA, or acetic acid. Low pH stability makes the Acclaim PA column an excellent choice for these types of analyses.

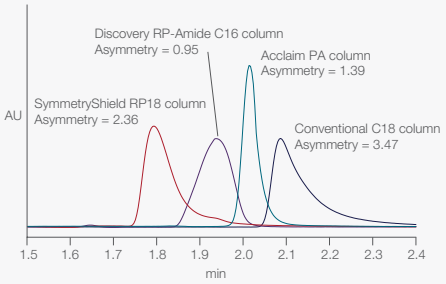
Improved peak shape

Interaction with residual silanol groups on the surface of the silica particle can result in peak tailing, which can affect peak resolution and integration. This effect can be reduced by optimizing the surface coverage of the bonded phase, exhaustive end-capping of the residual silanol groups, and minimization of metal contaminants that increase the acidity of the free silanol groups. The tailing of pyridine is a very good indicator of the number of exposed silanol groups on the silica surfaces. Figure 11 compares the performance of the Acclaim PA column with leading competitive columns for pyridine peak asymmetry. Exhaustive end-capping of the Acclaim PA phases, combined with the embedded polar functional group, provide improved peak shapes for both basic and acidic analytes at low- and mid-range pH values (Figure 12). Figure 12 also illustrates that while providing a much broader range of application possibilities, the Acclaim PA column maintains selectivities very similar to conventional C18 phases when compared to other polar-embedded column types.



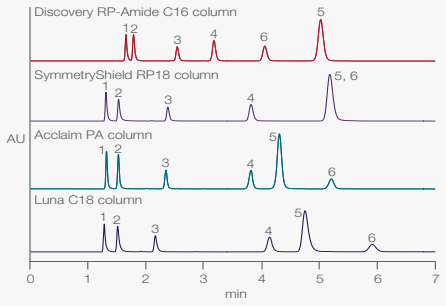
Column	See chromatogram, 5 µm
Dimensions	4.6 x 150 mm
Eluent	50/50 v/v 1% TFA pH 1.0/CH_3CN
Temperature	50 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Analyte	Toluene

Figure 10. Hydrolytic stability comparison at low pH.



Column	See chromatogram, 5 µm
Dimensions	4.6 x 150 mm
Eluent	50/50 v/v CH_3CN/D.I. H_2O
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 226 nm
Analyte	Pyridine
Concentration	85 µg/mL

Figure 11. Low silanol activity.



Column	See chromatogram, 5 µm
Dimensions	4.6 x 150 mm
Eluent	40/60 v/v 50 mM phosphate pH 3.2/CH_3CN
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Uracil 2. Pyridine 3. Phenol 4. *N,N*–Dimethylaniline 5. *p*–Butylbenzoic acid 6. Toluene

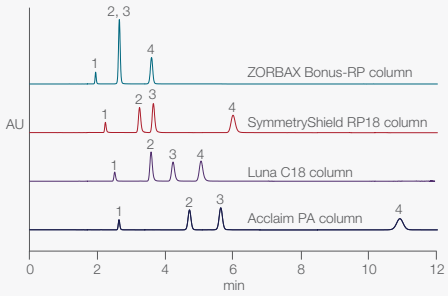
Figure 12. Comparison of selectivity.

Enhanced steric selectivity and resolution

The Acclaim PA can provide enhanced resolution of closely related compounds, resulting in additional ability to improve separations, compared to conventional reversed-phases and other types of polar-embedded phases (Figure 13). This ability can be very beneficial when analyzing very complex mixtures, resolving closely eluting compounds, or detecting low-level contaminants.

Reproducible manufacturing

To meet the exacting needs of our customers, each Acclaim PA column is manufactured to stringent specifications to ensure column-to-column reproducibility and reliability. Each column is shipped with a lot validation showing the test results and specifications for the lot of bonded silica packed into the column, and an individual test chromatogram validating performance.



Column	See chromatogram, 5 µm
Dimensions	4.6 x 150 mm
Eluent	90/10 v/v MeOH/D.I. H_2O
Temperature	30 °C
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	UV, 254 nm
Peaks	1. Toluene
	2. Phenanthrene
	3. o-Terphenyl
	4. Triphenylene

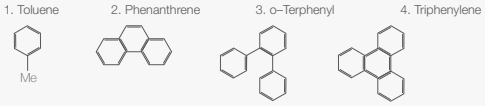


Figure 14. Comparison of steric selectivity.

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Acclaim PA column specifications

Specifications	
Phase	C16
Particle sizes	2.2 µm, 3.0 µm and 5.0 µm
Pore size	120 Å
Surface area	300 m^2/g
Carbon load	16–18%
pH range	2-8

Ordering information

Column	Format	Particle size (µm)	Length (mm)	2.1 ID (mm)	3.0 ID (mm)	4.6 ID (mm)
Acclaim PA	Analytical	2.2	50	072622	-	-
			100	072623	-	-
			150	072634	-	-
		3.0	50	063174	-	-
			100	061316	-	-
			150	061317	063693	061318
			250	-	070079	-
		5.0	150	-	-	061320
			250	-	-	061321
	Guard Cartridge (2/pk)	5.0	10	069691	071983	069698

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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