Acclaim 300 HPLC columns

Ideal for protein, peptide, and macromolecule separations

Thermo Scientific™ Acclaim™ 300 HPLC columns are reversed-phase silica columns for protein, peptide, and macromolecule separations in the biopharmaceutical industry. Column features include:

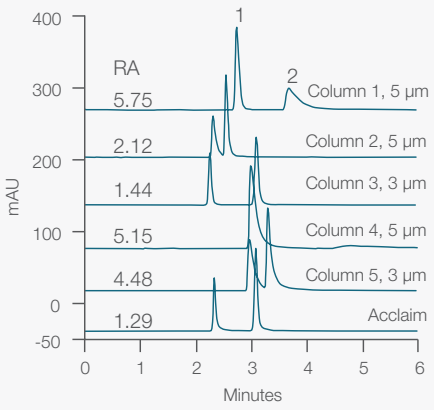
- ultrapure silica
- stable bonding chemistry
- liquid chromatography-mass spectrometry (LC-MS) compatible
- very high chromatographic efficiencies
- symmetrical peak shapes
- reproducible column-to-column manufacturing

Unique bonding chemistry

Thermo Scientific Acclaim 300 C18 columns are designed for the separation of proteins, peptides, and other biological macromolecules. This line of columns extends the same unique bonding chemistry developed for the Acclaim 120 columns to a wide-pore silica substrate. The Acclaim 300 columns consist of 3 µm diameter silica particles with 300 Å diameter pores, bonded with a highly characterized C18 phase. The unique bonding chemistry results in a high-density, highly uniform phase coverage with extensive end-capping. The use of a 3 µm silica particle accelerates the diffusion of the mobile phase into the stationary phase, resulting in fast, high-resolution separations. Compared to 5 µm column packings, it is possible to increase the flow rate of the mobile phase and run shallower gradients on shorter columns to achieve the desired separation in a shorter time period.

Symmetrical peak shapes

Residual metals in the silica substrate can lead to poor peak shape, especially for basic compounds. Figure 1 compares results for the evaluation of metal content in some competitive 300 Å columns with an Acclaim 300 column. The ratio of asymmetries (RA) is the ratio of the asymmetry factor for Peak 2 to the asymmetry factor for Peak 1. The closer this value is to unity (a value of 1), the lower the metal contamination level in the silica particles. The Acclaim 300 column shows the smallest value for the RA. Lower metal levels in the silica lead to better separations.



Column	Acclaim 300 Å, 3 µm, C18
Dimension	4.6 × 150 mm
Eluent	50% v/v water 50% methanol
Temperature	30 °C
Flow rate	1.0 mL/min
Inj. volume 1	0 µL
Detection	214 nm
Sample	1. 4,4'-bipyridyl 250 ng/µL 2. 2,2'-bipyridyl 1000 ng/µL

Ratio of asymmetries (RA) is quoted as the asymmetry of Peak 2 divided by Peak 1

Figure 1. Evaluation of metal contamination

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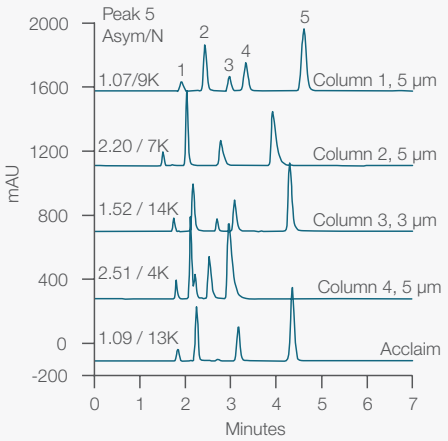
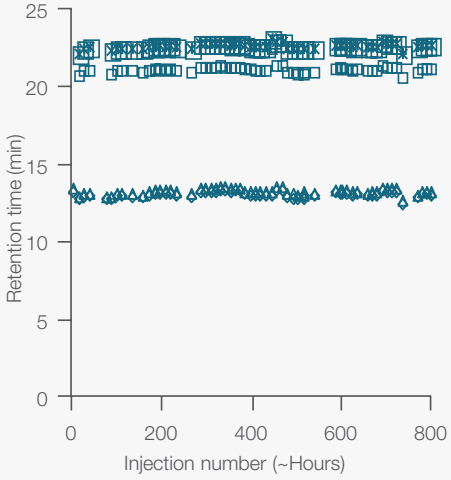
Another important parameter, residual silanol content, can be assessed by measuring the asymmetry of basic compounds. Above pH ~4, surface silanols begin to deprotonate and interact with basic analytes, giving rise to poor peak shape. Figure 2 compares the chromatographic analysis of a test mixture obtained on several competitive products to that obtained on an Acclaim 300 column. Note that the Acclaim 300 column asymmetry value is near one, and also that the highest efficiency (N) for Peak 5, amitriptyline, is obtained. This figure illustrates that peak asymmetry is maintained without a loss of peak efficiency. These data confirm that the high-density bonding chemistry developed for the Acclaim products has either covered the majority of the surface silanol groups, or rendered them inaccessible by active compounds. The combination of high-purity silica and high-density bonding gives the Acclaim 300 columns the ability to perform separations of basic compounds with unprecedented peak symmetry.



Column	Acclaim 300 Å, 3 µm, C18
Dimension	4.6 × 150 mm
Eluent	80/20 v/v methanol/20 mM Na_3PO_4, pH 6.0
Temperature	30 °C
Flow rate	1.0 mL/min
Inj. volume 1	10 µL
Detection	214 nm
Sample	1. Uracil 0.04 mg/mL 2. Propranolol 0.06 mg/mL 3. Toluene 0.08 µL/mL 4. Doxepin 0.08 mg/mL 5. Amitriptyline 0.24 mg/mL

Figure 2. Base asymmetry to gauge residual silanol



Column	Acclaim 300 Å, 3 µm, C18				
Eluents	4.6 × 150 mm				
Mobile phase	A. 95% v/v water 5% acetonitrile 0.1% TFA; B. 95% v/v acetonitrile 5% water 0.1% TFA				
Gradient		–10	0	45	50
Time (min)	%A	95	95	50	50
	%B	5	5	50	50
Temperature	50 °C				
Flow rate	1.0 mL/min				
Inj. volume	40 µL				
Inj. amt	0.5 µg/µL				
Detection	214 nm				
Sample	Tryptic digest of cytochrome C (retention data plotted for the two least separated pairs of peptides)				

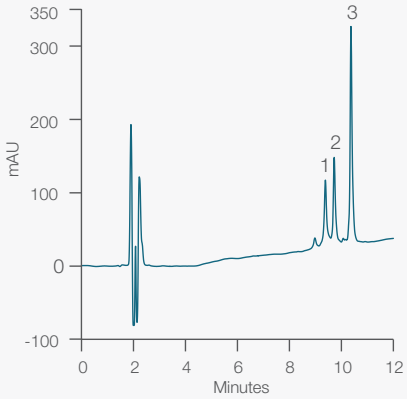
Figure 3. Column stability testing

Column stability

Some column manufacturers report results of column stability studies in terms of column volumes passed through the column, but they perform these tests on short columns (5 cm) with small actual column volumes. The large number of column volumes reported from that testing look impressive but are not necessarily meaningful, because most real separations are performed on longer columns. Figure 3 shows the results of stability testing done on a 4.6 × 150 mm Acclaim 300 column. Gradient separations of a tryptic digest of cytochrome c were performed on a single Acclaim 300 column (pH of eluent = 2.0). The retention time of three of the peptides are shown in this figure. As can be seen, even after 800 injections, the retention time of the peptides is relatively constant. You can be assured that the Acclaim 300 columns continue to perform just the same after hundreds of injections, as they did when first installed.

High resolution

Figure 4 shows the separation of three cytochrome c isozymes that differ by only a few amino acid residues. This separation demonstrates the high efficiency the Acclaim 300 columns deliver. These columns meet the needs of chemists for the separation of peptides and complete proteins. High chromatographic efficiency is assured because each column is individually tested, and the customer receives the test chromatogram demonstrating the column's performance.



Column	Acclaim 300				
Eluents	4.6 × 150 mm				
Mobile phase	A. 95% v/v Water 5% acetonitrile 0.1% TFA; B. 95% v/v acetonitrile 5% Water 0.1%				
Gradient		–5	0	15	20
Time (min)	%A	80	80	45	45
	%B	20	20	55	55
Temperature	30 °C				
Inj. volume	10 µL (1 µg/µL)				
Detection	214 nm				
Sample	1. Yeast cytochrome c 2. Equine cytochrome c 3. Bovine cytochrome c				

Figure 4. Separation of cytochrome c species variants

Figures 5 shows the analysis of a yeast cytochrome c tryptic digest, complete in less than 30 min. Figure 6 compares the separation of the same bovine cytochrome c tryptic digest at three different pH values. Although changes in retention are expected and can be used to optimize the separation, the stability of these columns over a wide pH range is evident. These examples demonstrate the high-resolution capability of the Acclaim 300 columns.

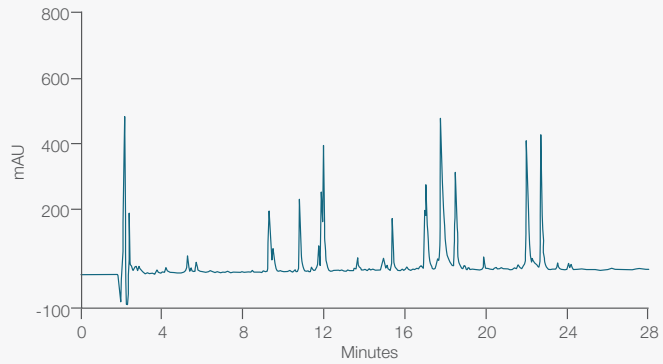


Figure 5. Analysis of Saccharomyces cervisae, cytochrome c tryptic digest

Flexibility in method development

Biopharmaceutical separations are very challenging, and flexibility is often needed to achieve the desired chromatography. For example, trifluoroacetic acid (TFA) is widely used for peptide and protein separations. However, other ion-pairing agents or buffers can give better resolution, peak shape, or unique selectivity than TFA. The unique bonding chemistry developed for the Acclaim family of columns allows the use of TFA levels as low as 0.01%, while maintaining good peak shape and chromatographic efficiency. This attribute can be important for LC-MS applications because TFA concentrations of 0.1% or higher have been shown to reduce analyte sensitivity when using electrospray (ESI) interfaces in LC-MS.

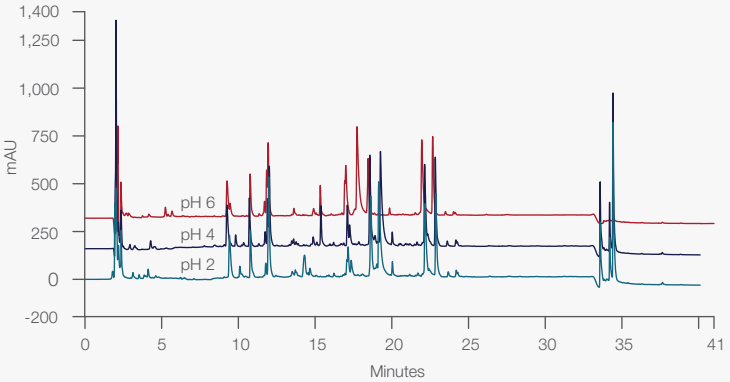


Figure 6. Comparison of bovine cytochrome c tryptic digests at pH 2, 4, and 6

Manufacturing reproducibility

Regardless of the area of endeavor, scientists are under a great deal of pressure to provide fast and accurate results. It is essential that the methods and columns used in these areas are rugged and reliable. To meet the exacting needs of our customers, each Acclaim 300 column is manufactured to stringent specifications to ensure column-to-column reproducibility. 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. The lot validation includes a chromatography-based metals test and tests for polar selectivity, hydrophobic steric selectivity, and base asymmetry. Production columns are individually tested for capacity and efficiency, and closely monitored for metals contamination.

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Specifications	
Column chemistry	C18 alkyl
Particle size	3 µm
Surface area	100 m^2/g
Pore size	300 Å
pH range	2.5 to 7.5
Temperature limit	60 °C
Operating pressure (Max)	6000 psi

Ordering information

Column	Particle size (µm)	Format	Length (mm)	2.1 ID	3.0 ID	4.6 ID
Acclaim 300	3.0	Analytical column	50	060263	-	060265
			150	060264	063684	060266
		Guard cartridges	10	069690	-	069697

Acclaim Guard Holder ordering information

Guard Holder	Part number
Thermo Scientific™ Acclaim™ SST Guard Cartridge Holder	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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