Acclaim 120 C18 and C8 HPLC columns

Ideal for pharmaceutical, nutraceutical, environmental, food, and life science applications in HPLC

Reversed-phase silica columns benefits

- High hydrophobicity, low polarity phases
- Liquid chromatography-mass spectrometry (LC-MS) compatible
- Very high efficiencies
- Symmetrical peak shapes
- Reproducible column-to-column manufacturing
- Ultrapure silica
- Stable bonding chemistry

Unique bonding chemistry

Thermo Scientific™ Acclaim™ 120 C18 and C8 HPLC columns are silica-based, reversed-phase columns manufactured using exhaustive bonding and end-capping techniques. These columns are based on high-purity silica which is 120 Å pore diameter, with very high surface coverage and very low metal content. They are LC-MS compatible with very low background and good capacity, ideal for use with high organic mobile phases. The C18 phases exhibit low polarity, high hydrophobicity, and good steric selectivity, which result in high-capacity columns with unique selectivity.

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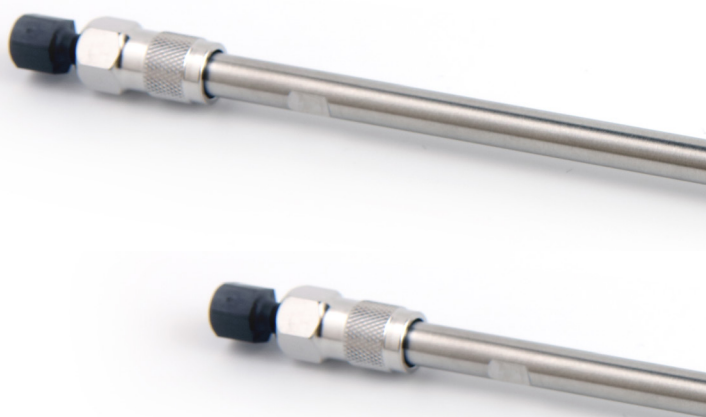
Symmetrical peak shapes

Peak tailing in RP-HPLC is a problem that affects peak resolution and integration. For basic analytes, it results in large part from the ion-exchange interaction of positively charged, basic analytes with negatively charged, exposed silanol groups. Above pH ~4, the surface silanols begin to deprotonate and interact with basic analytes. This effect can be reduced by optimizing the surface coverage of the bonded phase, exhaustive end-capping of the residual silanol groups and minimizing metal contaminants that increase the acidity of the free silanol groups. The tailing on amitriptyline is a very good indicator of the number of exposed silanol groups. As the mass loading of the base onto a column decreases, the performance of a column can be seen more clearly. Figure 1 shows the separation of a group of bases, including amitriptyline, at three analyte concentrations. The toluene is present as a marker. The responses have been normalized to show all three chromatograms with peaks at the same height. Acclaim 120 phases give excellent results, even as the base analyte concentrations approach very low levels.

Columns packed with 2.2 μm particles provide faster separations, as well as high efficiency, excellent performance as seen in Figure 2.

Figure 3 shows the baseline separation of catecholamines using a 5 μm C8 Acclaim 120 stationary phase and an isocratic mobile phase. The high efficiency 5 μm Acclaim 120 columns provide good resolution that can be further enhanced by using the 3 μm particle size. This separation is useful for extracts of brain tissue. The 5-hydroxy precursor of serotonin can also be separated on the same column. The separation can be optimized for serotonin by increasing the concentration of methanol or using one of the shorter formats of the Acclaim 120 columns.
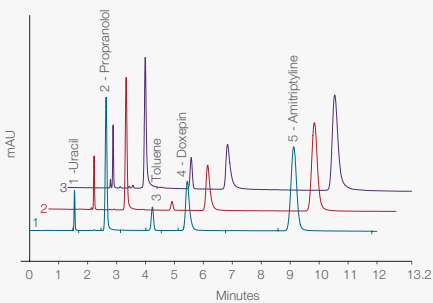

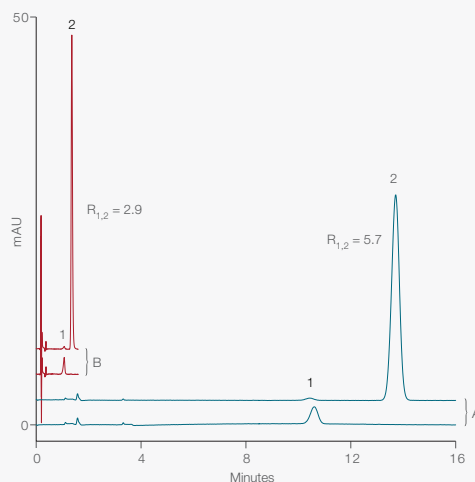| Column	Acclaim 120 C18, 5 μm
Dimension	4.6 × 150 mm
Mobile phase	80/20 Methanol/ 30 mM phosphate pH 6
Eluent	50% v/v water 50% methanol
Temperature	30 °C
Flow rate	1.0 mL/min
Detection	UV, 220 nm
Amitriptyline Mass	Trace 1: 1200 ng Trace 2: 400 ng Trace 3: 94 ng
Peaks	1. Uracil 2. Propranolol 3. Toluene 4. Doxepin 5. Amitriptyline

Figure 1. Dilution of bases.

Column	Acclaim 120 C18 A: 5 μm, 4.6 × 250 mm B: 2.2 μm, 2.1 × 50 mm
Mobile phase	15% (v/v) Acetonitrile + 0.2% Na heptanesulfonate adj. to pH 3.4 with HOAc
Temperature	25 °C
Flow rate	A: 2.00 mL/min B: 0.81 mL/min
Inj. volume	A: 10 μL B: 1.0 μL
Detection	DAD-3000 RS, UV, 280 nm
Peaks	1. Salicylic acid 15 μg/mL 2. Aspirin 500 μg/mL In 99:1 acetonitrile:formic acid
Reference	USP -29 NF-24 page 200

Figure 2. USP assay of aspirin on the A: Acclaim 120 C18, 5 μm and B: Acclaim RSLC 120 C18, 2.2 μm columns.

Column	Acclaim 120 C18, 5 μm
Dimension	4.6 × 150 mm
Mobile phase	43 mM sodium acetate, 0.1 mM EDTA, 1 mM sodium octanesulfonate, 10% methanol
Eluent	50% v/v water 50% methanol
Flow rate	1.0 mL/min
Detection	DC Amperometry, Glassy Carbon at 700 mV vs Ag/AgCl
Peaks	1. Norepinephrine 10 pmol 2. Epinephrine10 pmol 3. Dihydroxybenzylamine 10 pmol 4. Dopamine 10 pmol 5. Serotonin 20 pmol

Figure 3. Baseline separation of catecholamines and serotonin.

LC-MS compatible

More scientists are turning to mass spectrometry detection as the demand for product characterization increases. Historically, many reversed-phase columns have not been compatible with mass spectrometers due to silica bleeding into the mass spectrometer. Acclaim 120 columns use ultrapure silica with a stable bonding procedure to deliver a single line of columns that are LC-MS compatible.

Figure 4 shows the separation of a mixture of pesticides using a 2.1 mm i.d. × 100 mm format column. Triazine pesticides are detected using ESI in positive mode, samples were analyzed in SIM mode to achieve highest sensitivity (1 µg/L detection limit).
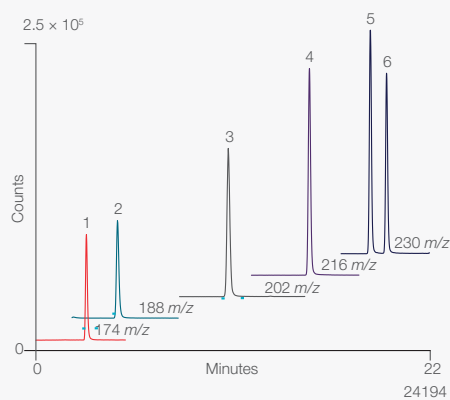
Validated for VANQUISH UHPLC Columns

Thermo Scientific™ Acclaim™ VANQUISH™ C18 UHPLC columns are available to achieve even greater power of separation, speed, and throughput. These columns were developed in conjunction with the Thermo Scientific™ VANQUISH™ UHPLC System to take advantage of the system's extended pressure capabilities and robustness. The result is a robust column that can be operated at higher flow rates and back-pressures in LC and LC-MS analyses.

Column	Acclaim 120 C18, 3 µm
Dimension	120 Å, 2.1 × 100 mm
Eluent	A: 40 mmol/L formic acid in water B: Acetonitrile
Gradient	95% A and 5% B isocratic, in 6 min to 65% B, hold for 12 min, in 0.5 min to 5% B, hold for 9.5 min
Flow rate	0.25 mL/min
Inj. volume	10 µL
Temperature	30 °C
Detection	MSQ, ESI+, needle voltage: 3.5 kV; probe temperature: 400 °C; cone voltage: 50 V; dwell time: 0.5 s
Peaks	1. Atrazine-desisopropyl SIM1: 173.9–174.3 (0–5 min) 2. Atrazine-desethyl SIM2: 187.9–188.3 (2–8 min) 3. Simazine SIM3: 202.0–202.4 (8–15 min) 4. Atrazine SIM4: 216.1–216.5 (12–18 min) 5,6. Terbuthylazine/Propazine SIM5: 230.1–230.5 (17–22 min)

Figure 4. SIM chromatograms of different pesticides.

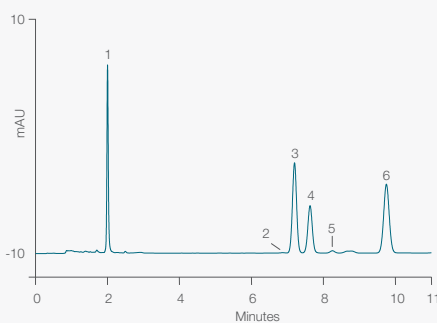| Column	Acclaim 120 C18, 3 µm
Dimension	4.6 × 150 mm
Eluent	95/5 v/v acetonitrile/methanol
Flow rate	2.0 mL/min
Inj. volume	5 µL
Temperature	30 °C
Detection	UV, 280 nm
Peaks	1. All-trans retinol (Vitamin A) 0.12 mg/mL 2. δ-Tocopherol (impurity) – 3. Ergocalciferol (Vitamin D_2) 0.12 mg/mL 4. Cholecalciferol (Vitamin D_3) 0.06 mg/mL 5. β-Tocopherol (impurity) – 6. α-Tocopherol (Vitamin E) 1.3 mg/mL

Figure 5. Fat soluble vitamins.

Food applications

Acclaim 120 columns are rugged and reliable, ideally suited to applications, such as food products, that are subjected to regulatory control. The high efficiencies of these columns—combined with their high capacity—produce sharp, narrow peaks that are readily quantified, as illustrated by the separation of fat-soluble vitamins A, E, D2, and D3 from the beta- and delta-tocopherol impurities in Figure 5. The high efficiencies of these columns allow not only for better resolution between difficult peak pairs, but also for good sensitivity of the trace components.

Pharmaceuticals

Acclaim 120 columns are also well suited to pharmaceutical applications. With the tight specifications applied to the entire column manufacturing process, from the physical characteristics of the raw silica to the bonding chemistry and column testing, these columns provide good peak shapes and reproducible chromatography from one column to the next. Figure 7 and 8 show separations of benzodiazepines and antihistamines. Note the enhanced peak resolution of the antihistamine compounds in Figure 8, which allows for identification of minor contaminant peaks.
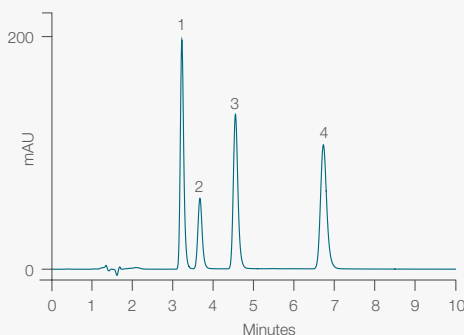

Column	Acclaim 120 C18, 5 μm
Dimension	4.6 × 150 mm
Mobile phase	60% acetonitrile
Flow rate	1.0 mL/min
Detection	UV, 235 nm
Peaks	1. Oxazepam
	2. Clonazepam
	3. Temazepam
	4. Diazepam

Figure 7. Benzodiazepines

Column	Acclaim 120 C18, 5 μm
Dimension	4.6 × 150 mm
Mobile phase	A: 50 mM sodium acetate
	B: Methanol
Flow rate	1.0 mL/min
Isocratic	(A) 20%, (B) 80%
Temperature	25 °C
Detection	UV, 249 nm
Peaks	1. Thenyldiamine HCl
	2. Phenothiazine
	3. Promethazine HCl
	4. Pyrrobutamine phosphate
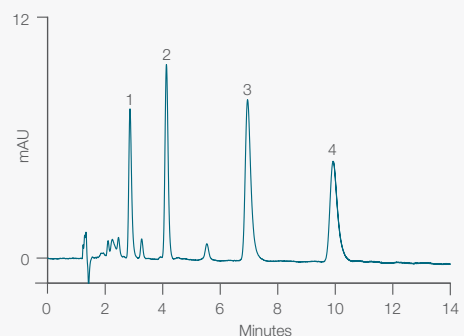
Figure 8. Antihistamines

Biopharmaceutical

Peptide mapping is frequently used to study the primary structure of proteins. This can be a challenging separation since a high number of peptides need to be baseline resolved. The Acclaim 120 C18 column provided excellent peak capacity and reproducibility for peptide separations as is shown in Figure 9.
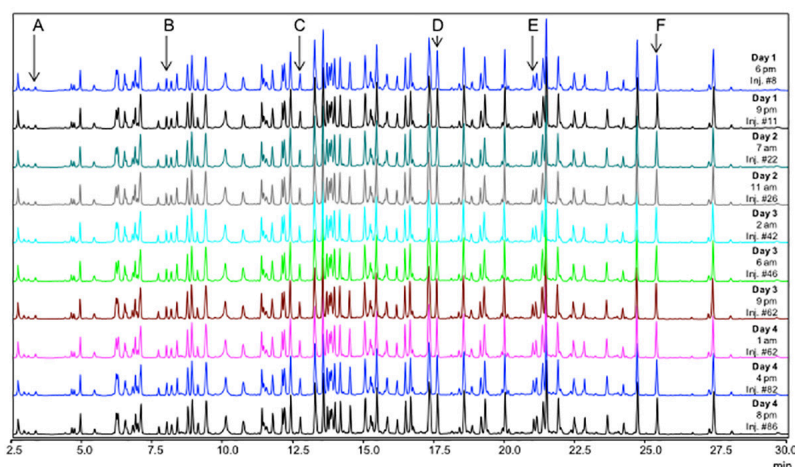
Column	Acclaim 120 C18, 2.2 μm
Dimension	2.1 × 250 mm
Mobile phase	A: 0.05% TFA in water
	B: 0.04% TFA in 8/2 acetonitrile/water (v/v)
Gradient	0–30 min: 4–55% B, 30–31 min: 55-100% B, 31–35 min: 100% B, 35–36 min: 100–4% B, 36–56 min: 4% B
Flow rate	0.3 mL/min
Inj. volume	2 μL
Temperature	50 °C; still air mode
Detection	214 nm
	Data collection rate: 10 Hz
	Response time 0.4 s
Flow cell	10 mm LightPipe

Figure 9. Ten randomly chosen chromatograms for the peptide mapping of a tryptic BSA digest. In total, 86 injections were performed covering a time frame of 80 hours without any user interference.

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Natural products

With the increased interest in natural products in recent years, the identification of nutraceutical marker compounds has received much attention in HPLC method development. This importance is reflected by the publication of a series of HPLC methods by the Institute for Nutraceutical Advancement (INA). The Acclaim columns are packed with a highly characterized bonded phase that interacts with polar compounds, such as those shown in Figure 10, in a predictable and reproducible manner. These columns provide excellent peak shape and good resolution for a wide variety of natural products. Figure 11 shows the separation of isoflavones in red clover. Isoflavones are important as potential immune-enhancers and anticancer agents.
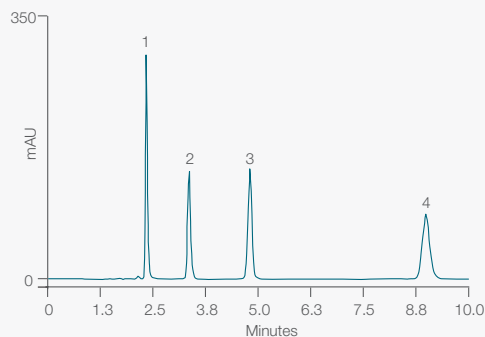

Figure 10. Caffeine analogs

Column	Acclaim 120 C18, 5 µm
Dimension	4.6 × 150 mm
Mobile phase	A: 50 mM sodium acetate
	B: Acetonitrile
	C: Methanol
Composition	(A) 6%, (B) 8%, (C) 6%
Flow rate	1.0 mL/min
Detection	UV, 274 nm
Peaks	1. 7-Methylxanthine
	2. Theobromine
	3. Theophylline
	4. Caffeine
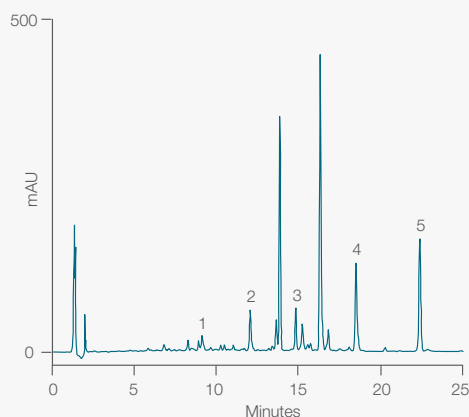
Figure 11. Isoflavones in red clover.

Column	Acclaim 120 C18, 5 µm
Dimension	4.6 × 150 mm
Mobile phase	A: 0.1% H_3PO_4 in D.I. H_2O
	B: Acetonitrile
Gradient	10% B to 76% B over 35 min
Flow rate	1.2 mL/min
Detection	UV, 260 nm
Peaks	1. Genistin
	2. Ononin
	3. Sissotrin
	4. Formononotin
	5. Biochanin A

Manufacturing reproducibility

Scientists working on products for human consumption, whether they are pharmaceuticals, food products, or nutraceuticals, are under a great deal of pressure to produce fast and accurate results. Whether these scientists are working under the International Conference on Harmonization (ICH) guidelines or fall under the jurisdiction of the Food & Drud Adminitration (FDA), it is important that their analytical methods are rugged and reliable. To meet the needs of these scientists, each Acclaim 120 column is manufactured according 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 detailing the extensive testing to which it has been subjected. The lot validation includes a chromatography-based metals test and tests for polar selectivity, hydrophobic steric selectivity, and base asymmetry. Production columns are tested for capacity and efficiency, and closely monitored for metal contamination.

Thermo Scientific™ Acclaim™ Guard Cartridges are packed with the same validated, 5 µm, bonded silicas that are used to pack the analytical columns. By using analytical-quality bonded phase in the guard cartridges, selectivity changes due to mismatched guards and columns are minimized.

Specifications	C18	C8
USP listing	L1	L7
Starting material	Ultrapure silica	Ultrapure silica
Particle size	2.2 µm, 3 µm, 5 µm	2.2 µm, 3 µm, 5 µm
Particle shape	Spherical	Spherical
Particle size distribution (40/90)	1.2	1.2
Total carbon content (%)	18.0	11.2
Carbon surface coverage (µmol/m²)	3.2	3.7
End-capped	Yes	Yes
Metal impurity (ppm) Na, Fe, Al	<10	<10
Pore volume (mL/g)	0.9	0.9
Average pore diameter (Å)	120	120
Surface area (m²/g)	300	300
pH range	2–8	2–8
Temperature	<60 °C	<60 °C

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Acclaim columns ordering information

Column	Particle size (µm)	Format	Length (mm)	ID	Acclaim 120 C18 part number	Acclaim 120 C8 part number
Acclaim	2.2	RSLC column	30	2.1	071400	-
			50	2.1	068981	072615
			75	3.0	075697	-
			100	2.1	068982	072616
			100	3.0	071604	072620
			150	2.1	071399	072617
			250	2.1	074812	074811
		Validated for VANQUISH UHPLC Systems	150	2.1	071399-V	-
			250	2.1	074812-V	-
	3.0	HPLC column	33	3.0	066272	-
			50	2.1	059128	059122
			50	4.6	059131	-
			75	3.0	066273	-
			100	2.1	059129	059123
			100	3.0	076186	-
			100	4.6	059132	059126
			150	2.1	059130	059124
			150	3.0	063691	068970
			150	4.6	059133	059127
			250	2.1	076187	-
			250	3.0	070077	-
	5.0	HPLC column	50	2.1	059142	059134
			50	4.6	059146	059138
			100	2.1	059143	
			100	4.6	059147	059139
			150	2.1	059144	059136
			150	4.6	059148	059140
			250	2.1	059145	
			250	4.6	059149	059141
	5.0	Guard cartridge	10	2.1	069689	069688
			10	3.0	071981	071979
			10	4.6	069695	069696

Acclaim Guard Holder ordering information

Guard Holder	Part number
Thermo Scientific™ Acclaim™ Guard Cartridge Holder V-2	069580
Thermo Scientific™ Acclaim™ Guard Cartridge Holder-Coupler Kit V-2	069707
Thermo Scientific™ Acclaim™ Guartd Cartridge Coupler V-2	074188

Expect reproducible results with sample prep, columns and vials

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