Acclaim® 120 HPLC Columns

Reversed-phase silica columns for pharmaceutical, nutraceutical, environmental, food, and life science applications in HPLC.

- *High hydrophobicity, low polarity phases*
- *LC/MS compatible*
- *Very high efficiencies*
- *Symmetrical peak shapes*
- *Reproducible column-to-column manufacturing*
- *Ultrapure silica*
- *Stable bonding chemistry*

Unique Bonding Chemistry

The Acclaim 120 line of HPLC columns is a new line of C18 and C8 silica-based, reversed-phase columns manufactured by Dionex using exhaustive bonding and end-capping techniques. Among the many C18 columns on the market, retention behavior of analytes differs significantly due to variation in both the raw silica and the ligand bonding chemistry and density. The Acclaim 120 columns address many of the performance issues encountered with octadecylsilylated silica gel (ODS) columns.

The Acclaim 120 line is comprised of four highly characterized C18 and C8 phases, manufactered using high-purity silica with a 120 Å pore diameter, with very high surface coverage and very low metal content. All the phases 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. All Dionex columns undergo extensive testing to ensure column-to-column reproducibility, and are shipped with certificates of analysis detailing these tests.
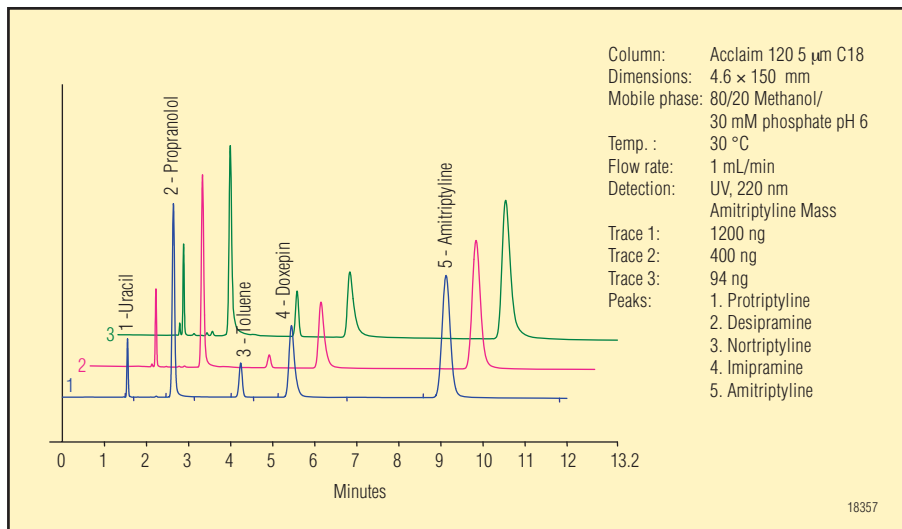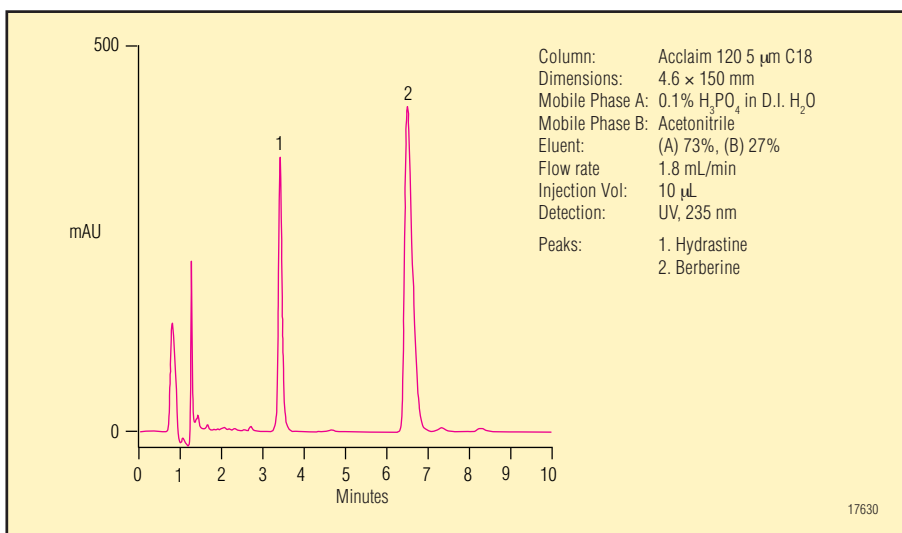
DIONEX

Figure 1 . Dilution of bases.

Column: Acclaim 120 5 µm C18
Dimensions: 4.6 × 150 mm
Mobile phase: 80/20 Methanol/
30 mM phosphate pH 6
Temp. : 30 °C
Flow rate: 1 mL/min
Detection: UV, 220 nm
Amitriptyline Mass
Trace 1: 1200 ng
Trace 2: 400 ng
Trace 3: 94 ng
Peaks: 1. Protriptyline
2. Desipramine
3. Nortriptyline
4. Imipramine
5. Amitriptyline

18357

Figure 2 . Extract of goldenseal root.

Column: Acclaim 120 5 µm C18
Dimensions: 4.6 × 150 mm
Mobile Phase A: 0.1% H_3PO_4 in D.I. H_2O
Mobile Phase B: Acetonitrile
Eluent: (A) 73%, (B) 27%
Flow rate 1.8 mL/min
Injection Vol: 10 µL
Detection: UV, 235 nm

Peaks: 1. Hydrastine
2. Berberine

17630
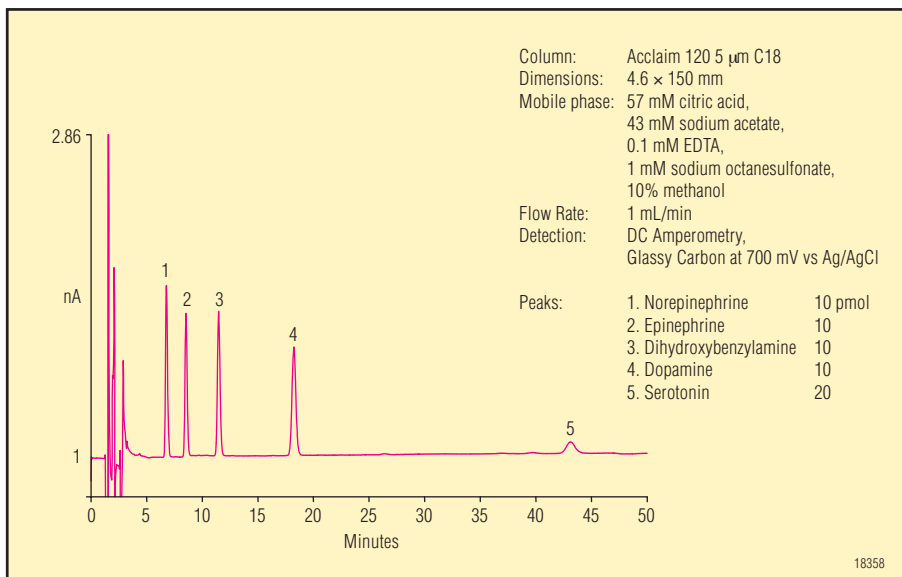
Figure 3. Baseline separation of catecholamines and serotonin.

Column: Acclaim 120 5 µm C18
Dimensions: 4.6 × 150 mm
Mobile phase: 57 mM citric acid,
43 mM sodium acetate,
0.1 mM EDTA,
1 mM sodium octanesulfonate,
10% methanol
Flow Rate: 1 mL/min
Detection: DC Amperometry,
Glassy Carbon at 700 mV vs Ag/AgCl

Peaks: 1. Norepinephrine 10 pmol
2. Epinephrine 10
3. Dihydroxybenzylamine 10
4. Dopamine 10
5. Serotonin 20

18358

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.

Figure 2 shows the separation of components in an extract of Goldenseal root. This application provides a good indication of the purity of the silica. The cleaner the silica, the more symmetrical the berberine peak.

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.

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. To address this issue, some column manufacturers have developed a line of specialty columns specifically for LC-MS applications. The Dionex 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 in less than 4 min. using a 2.1 mm i.d. × 100 mm format column. The hydrophobicity of this phase allows the use of higher solvent levels in the mobile phase for better sensitivity by electrospray mass spectrometry.

Food Applications

The Dionex Acclaim 120 is a rugged and reliable product line, 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, D_2, and D_3 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.
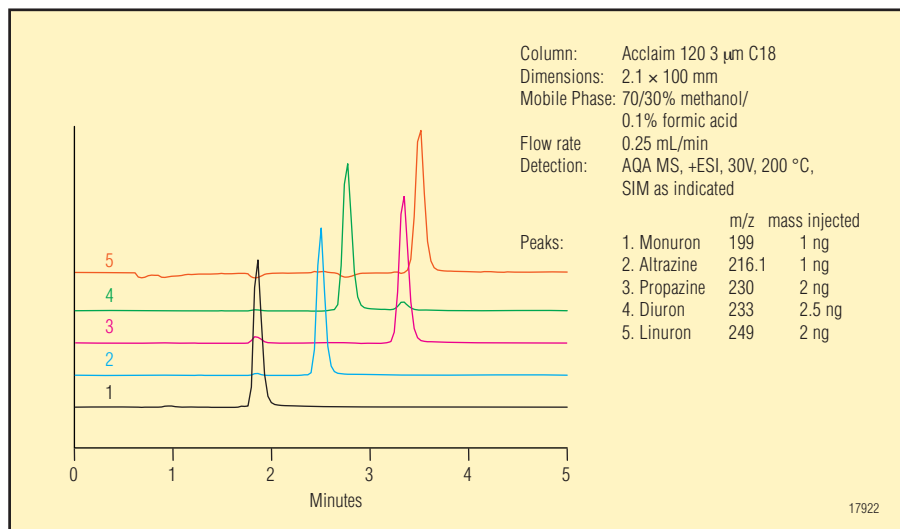

Column: Acclaim 120 3 µm C18
Dimensions: 2.1 × 100 mm
Mobile Phase: 70/30% methanol/
               0.1% formic acid
Flow rate   0.25 mL/min
Detection: AQA MS, +ESI, 30V, 200 °C,
               SIM as indicated

		m/z	mass injected
Peaks:	1. Monuron	199	1 ng
	2. Altrazine	216.1	1 ng
	3. Propazine	230	2 ng
	4. Diuron	233	2.5 ng
	5. Linuron	249	2 ng

17922

Figure 4 . LC-MS of pesticides.

Column: Acclaim 120 3 µm C18
Dimensions: 4.6 × 150 mm
Eluent: 95/5 v/v acetonitrile/methanol
Flow Rate: 2.0 mL/min
Inj. Vol.:   5 µL
Temp.:   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
        4. Cholecalciferol (Vitamin D_3) 0.06
        5. β-Tocopherol (impurity)   –
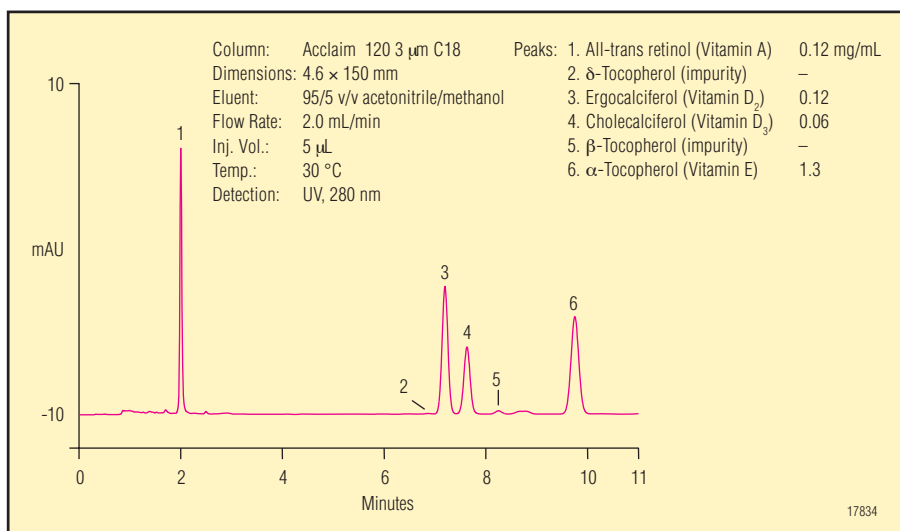   6. α-Tocopherol (Vitamin E)   1.3

17834

Figure 5. Fat soluble vitamins.

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.

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 Dionex Acclaim columns are packed with a highly characterized bonded phase that interacts with polar compounds, such as those shown in Figure 9, in a predictable and reproducible manner. These columns provide excellent peak shape and good resolution for a wide variety of natural products. Figure 10 shows the separation of isoflavones in red clover. Isoflavones are important as potential immune-enhancers and anticancer agents.
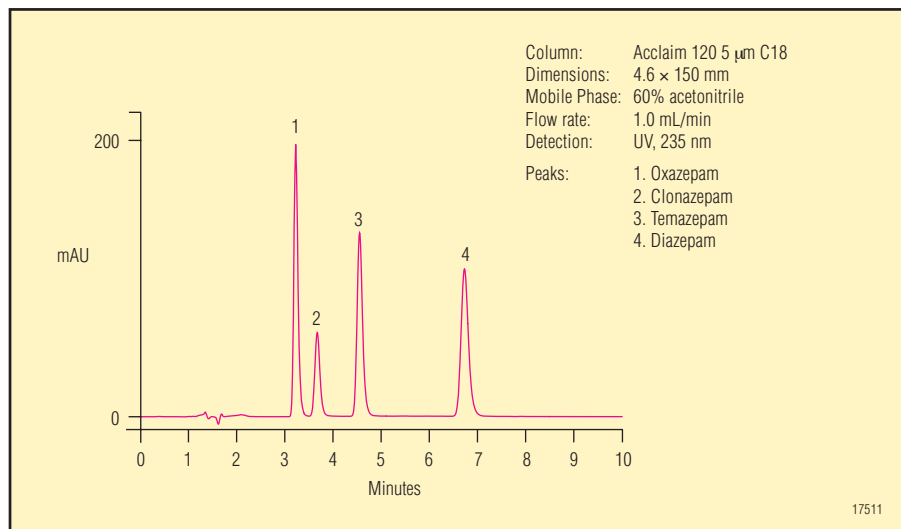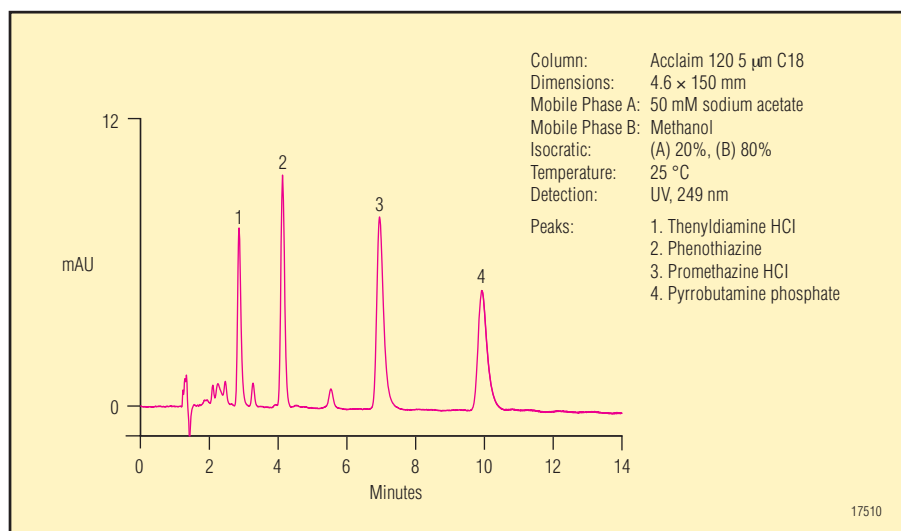

Column:	Acclaim 120 5 μm C18
Dimensions:	4.6 × 150 mm
Mobile Phase:	60% acetonitrile
Flow rate:	1.0 mL/min
Detection:	UV, 235 nm

Peaks:
1. Oxazepam
2. Clonazepam
3. Temazepam
4. Diazepam

17511

Figure 7. Benzodiazepines.

Column:	Acclaim 120 5 μm C18
Dimensions:	4.6 × 150 mm
Mobile Phase A:	50 mM sodium acetate
Mobile Phase B:	Methanol
Isocratic:	(A) 20%, (B) 80%
Temperature:	25 °C
Detection:	UV, 249 nm

Peaks:
1. Thenyldiamine HCl
2. Phenothiazine
3. Promethazine HCl
4. Pyrrobutamine phosphate

17510

Figure 8. Antihistamines.

Column:	Acclaim 120 5 μm C18
Dimensions:	4.6 × 150 mm
Mobile Phase A:	50 mM sodium acetate
Mobile Phase B:	Acetonitrile
Mobile Phase 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
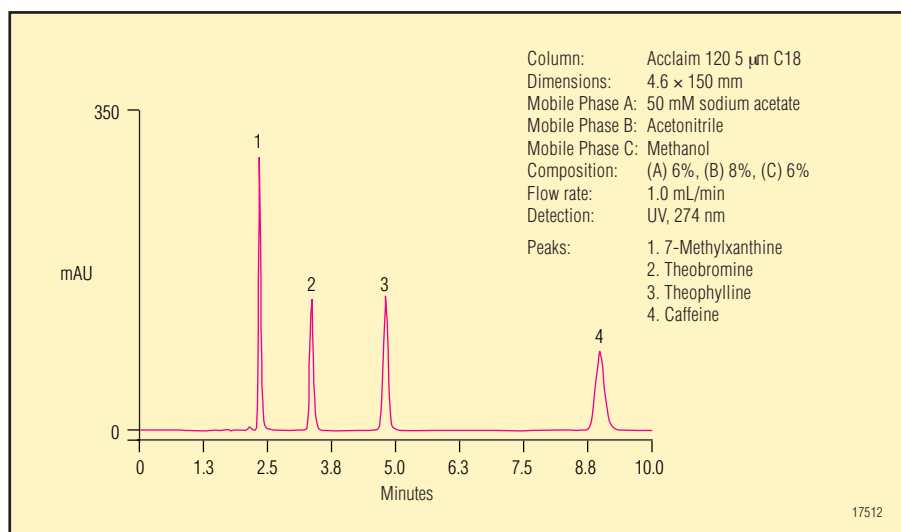3. Theophylline
4. Caffeine

17512

Figure 9. Caffeine analogs.

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 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.

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.
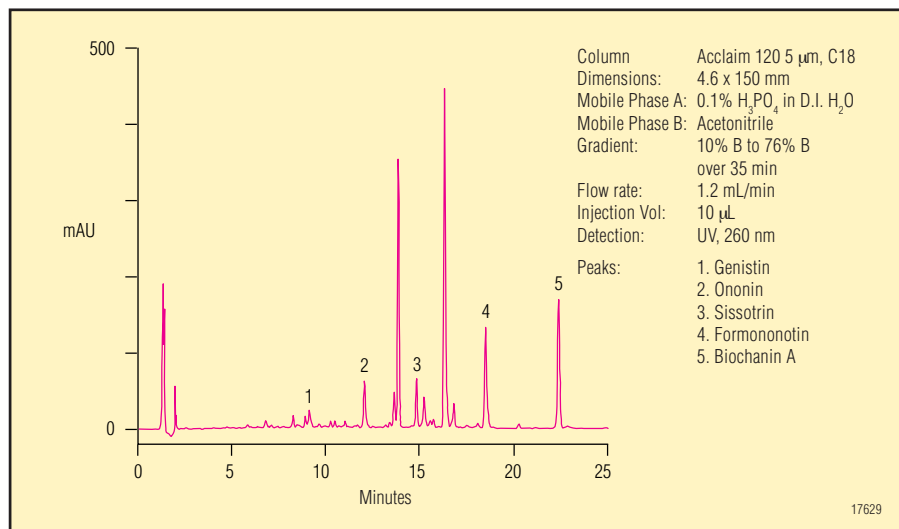

Column	Acclaim 120 5 μm, C18
Dimensions:	4.6 x 150 mm
Mobile Phase A:	0.1% H_3PO_4 in D.I. H_2O
Mobile Phase B:	Acetonitrile
Gradient:	10% B to 76% B over 35 min
Flow rate:	1.2 mL/min
Injection Vol:	10 μL
Detection:	UV, 260 nm
Peaks:	1. Genistin
	2. Ononin
	3. Sissotrin
	4. Formononotin
	5. Biochanin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

17629

Figure 10. Isoflavones in red clover.

SPECIFICATIONS		
	C18	**C8**
USP Listing	L1	L7
Starting Material	Ultrapure silica	Ultrapure silica
Particle Size	3 μm, 5 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 ($\mu mol/m^2$)	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 (m^2/g)	300	300
pH range	2–8	2–8
Temperature	<60 °C	<60 °C

Ordering Information

To order in the U.S., call (800) 346-6390, or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

PART NUMBERS	
Analytical Columns	**Part Number**
Acclaim 120, C8, 3 µm Analytical (2.1 × 50 mm)	059122
Acclaim 120, C8, 3 µm Analytical (2.1 × 100 mm)	059123
Acclaim 120, C8, 3 µm Analytical (2.1 × 150 mm)	059124
Acclaim 120, C8, 3 µm Analytical (4.6 × 50 mm)	059125
Acclaim 120, C8, 3 µm Analytical (4.6 × 100 mm)	059126
Acclaim 120, C8, 3 µm Analytical (4.6 × 150 mm)	059127
Acclaim 120, C18, 3 µm Analytical (2.1 × 50 mm)	059128
Acclaim 120, C18, 3 µm Analytical (2.1 × 100 mm)	059129
Acclaim 120, C18, 3 µm Analytical (2.1 × 150 mm)	059130
Acclaim 120, C18, 3 µm Analytical (4.6 × 50 mm)	059131
Acclaim 120, C18, 3 µm Analytical (4.6 × 100 mm)	059132
Acclaim 120, C18, 3 µm Analytical (4.6 × 150 mm)	059133
Acclaim 120, C8, 5 µm Analytical (2.1 × 50 mm)	059134
Acclaim 120, C8, 5 µm Analytical (2.1 × 100 mm)	059135
Acclaim 120, C8, 5 µm Analytical (2.1 × 150 mm)	059136
Acclaim 120, C8, 5 µm Analytical (2.1 × 250 mm)	059137
Acclaim 120, C8, 5 µm Analytical (4.6 × 50 mm)	059138
Acclaim 120, C8, 5 µm Analytical (4.6 × 100 mm)	059139
Acclaim 120, C8, 5 µm Analytical (4.6 × 150 mm)	059140
Acclaim 120, C8, 5 µm Analytical (4.6 × 250 mm)	059141
Acclaim 120, C18, 5 µm Analytical (2.1 × 50 mm)	059142
Acclaim 120, C18, 5 µm Analytical (2.1 × 100 mm)	059143
Acclaim 120, C18, 5 µm Analytical (2.1 × 150 mm)	059144
Acclaim 120, C18, 5 µm Analytical (2.1 × 250 mm)	059145
Acclaim 120, C18, 5 µm Analytical (4.6 × 50 mm)	059146
Acclaim 120, C18, 5 µm Analytical (4.6 × 100 mm)	059147
Acclaim 120, C18, 5 µm Analytical (4.6 × 150 mm)	059148
Acclaim 120, C18, 5 µm Analytical (4.6 × 250 mm)	059149
Guard Columns	**Part Number**
5-µm guard cartridges are used with both 3-µm and 5-µm analytical columns.	
Acclaim 120, C8, 5 µm Guard Cartridges (2 × 10 mm), 2 ea.	059449
Acclaim 120, C8, 5 µm Guard Cartridges (4.3 × 10 mm), 2 ea.	059448
Acclaim 120, C18, 5 µm Guard Cartridges (2 × 10 mm), 2 ea.	059447
Acclaim 120, C18, 5 µm Guard Cartridges (4.3 × 10 mm), 2 ea.	059446
Acclaim Guard Kit (holder and coupler)	059526
Acclaim SST Guard Cartridge Holder	059456
Guard to Analytical Column Coupler	059457

DIONEX

www.dionex.com ON THE WEB

D-store

Acclaim is a registered trademark of Dionex Corporation.