Acclaim®

Bonded Silica-Based Columns for HPLC

Pharmaceuticals

Environmental

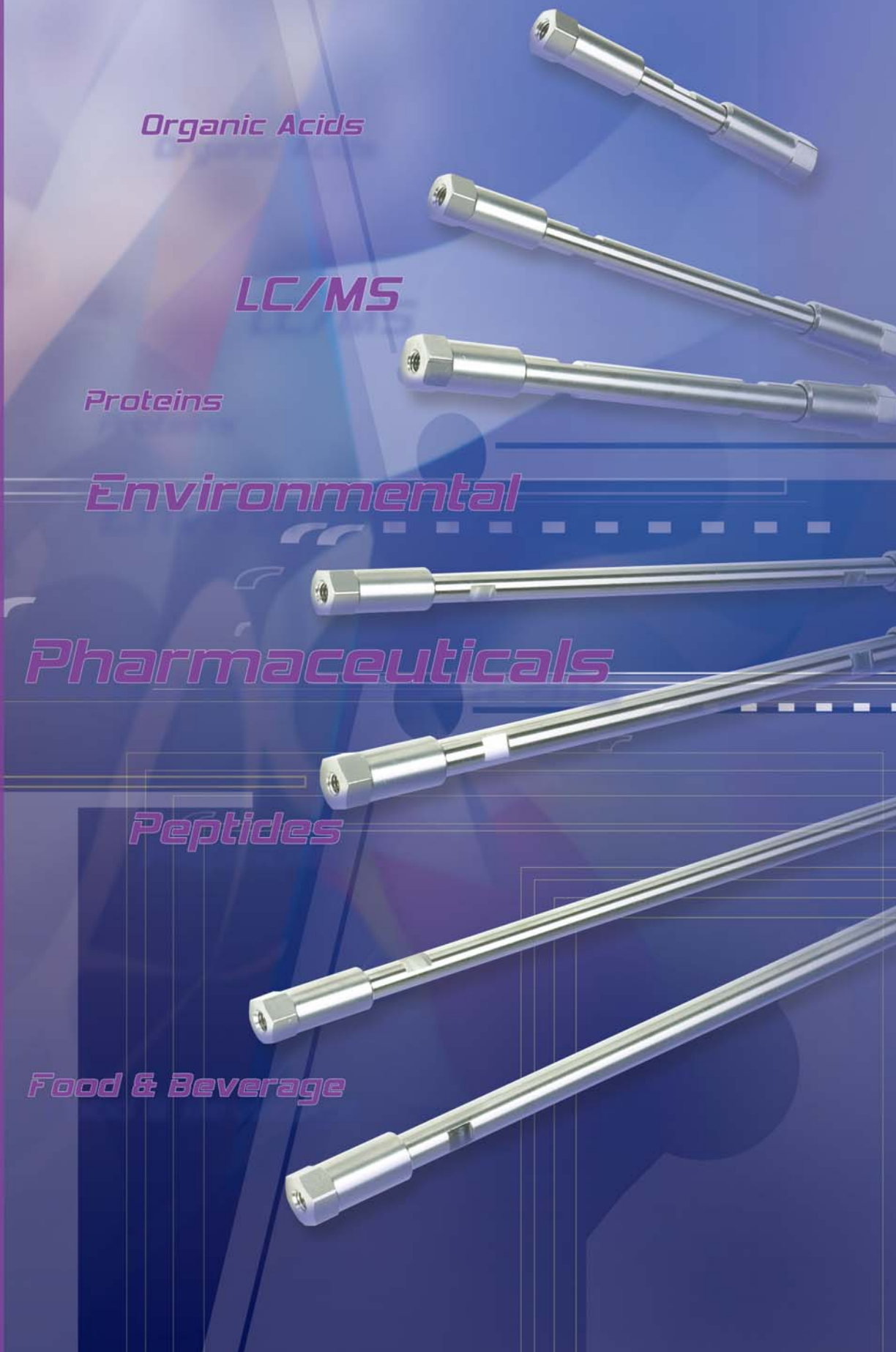
LC/MS

Peptides

Food & Beverage

Proteins

Organic Acids



DIONEX

Ordering and Warranty Information

In the U.S.A.

All Orders Including:

- Credit Card Orders (VISA, American Express, MasterCard)
- Blanket Orders
- Emergency Orders

Call 1-800-Dionex-0 (1-800-346-6390)

or

FAX 1-408-736-4476

Outside the U.S.A.

Please contact your local Dionex representative.

Prices and Payment

Payment Terms are Net 30 Days. Minimum order is $50.00. Prices and specifications are subject to change without notice. Prices prevailing at the time an order is placed will apply. Please verify current pricing and product specifications when placing an order.

Ordering by Credit Card

Dionex accepts payment by MasterCard, VISA, and American Express cards with minimum orders of $50.00. Call 1-800-DIONEX-0 (1-800-346-6390) or your local Dionex office for more information.

Blanket Orders

Dionex will arrange for blanket orders with a specified shipping time. Please call 1-800-DIONEX-0 (1-800-346-6390) for further information.

Shipping and Handling

Orders placed for consumables are typically shipped within two working days. Dionex ships FOB Sunnyvale, CA, for all U.S. orders, unless prior arrangements have been made. For UPS, Federal Express, or any carrier under contract with Dionex, Dionex will prepay the freight charges and add the charges to the invoice. Unless otherwise specified, shipments are made by ground transportation.

Returning Goods

We want our customers to be completely satisfied with products purchased from Dionex. We will replace any unused or defective item if it is returned to us, with authorization, within 90 days of shipment. Please obtain a Return Goods Authorization (RGA) number in advance by contacting your local Dionex office. A restocking fee (up to 25%) will be charged for products returned to Dionex due to customer ordering errors.

Emergency Orders

We are committed to shipping emergency parts to you the same day your order is received, provided the required parts are in stock. A $50.00 handling fee is charged for all emergency shipments. If the emergency order is not shipped within 24 hours, this fee will be waived. To place an emergency order in the U.S., call 1-800-DIONEX-0 (1-800-346-6390) and specify that your order requires emergency one-day service. Or you may fax your order, giving the same information, to 1-408-736-4476. Emergency orders should be placed no later than 1:00 PM Pacific Time.

Column Warranty

A 90-day column warranty is in effect from the day the column or suppressor is shipped from Dionex. After verifying that it was not damaged in transit, install the column in the system and test it by your operating procedure or by making several injections of a similar standard to that used to obtain the test chromatogram shipped with the column. Contact the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest Dionex office (see back cover) if the performance of the column is unsatisfactory.

Inventory Control Stickers

An inventory control code is affixed to the column box to ensure that this product is stored and shipped in accordance with Dionex shelf life guidelines. This process ensures consistent high quality of Dionex column products.

LPN 1668

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA 94088-3603

Contents

Part I

Introduction

Overview of the Acclaim Family

The Acclaim stationary phases differ in their polarity, hydrophobicity, and selectivity, and are designed to perform a wide range of separations. All Acclaim columns are manufactured to ensure consistent performance. The Dionex Acclaim product line features three conventional stationary phases and two specialized phases.

Acclaim 120 C18 and C8

The Acclaim 120 C18 is a classic reversed-phase material, featuring a densely bonded surface with double endcapping. The monomeric type of bonding gives excellent efficiency, full surface coverage, and low silanol activity. The Acclaim 120 C8 is constructed with the same substrate and bonding techniques as the C18, and has the same qualities, but with lower retention and slightly different selectivity.

Acclaim 300 C18

The Acclaim 300 C18 uses a wide-pore silica substrate suitable for peptides and moderately sized proteins, with excellent performance features provided by monomeric bonding and double endcapping.

Acclaim PolarAdvantage

The Acclaim PolarAdvantage (PA) column has a unique polar-embedded phase. This column is compatible with aqueous mobile phases containing no organic solvent modifiers, but also has selectivity similar to C18. The Acclaim PA gives uncompromising performance for basic, acidic, hydrophilic, or hydrophobic analytes in a single run.

Acclaim OA

The Acclaim OA is a specialty column optimized and application-tested for the analysis of hydrophilic organic acids, and provides a complementary technique to the IonPac® anion-exchange and ion-exclusion columns. The Acclaim OA uses a polar-embedded stationary phase that is compatible with aqueous-only mobile phases.

Acclaim Specifications

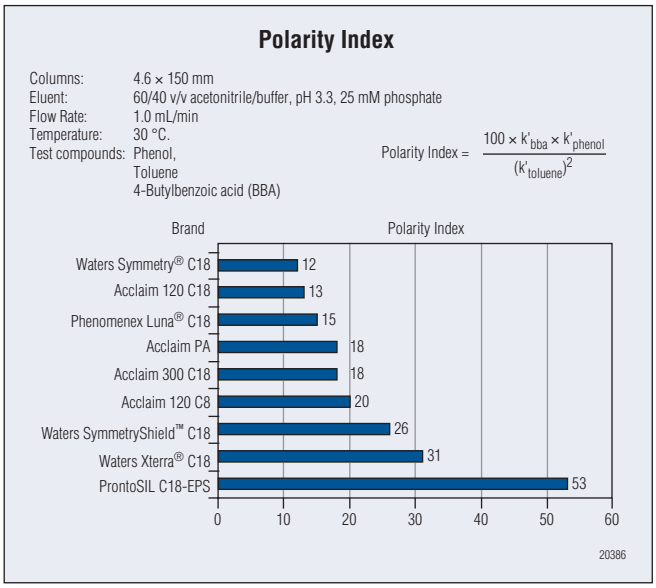
Dionex Phase	Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim PA	Acclaim OA
Bonded Phase	Octadecylsilane	Octylsilane	Octadecylsilane	Sulfamido silane C16	Proprietary
USP type	L1	L7	L1	n.a.	n.a.
Endcap	Yes	Yes	Yes	Yes	Yes
Particle Platform	Silica	Silica	Silica	Silica	Silica
Particle Shape	Spherical	Spherical	Spherical	Spherical	Spherical
Particle Purity	<10 ppm metals	<10 ppm metals	<10 ppm metals	<10 ppm metals	<10 ppm metals
Particle sizes (µm)	3 and 5	3 and 5	3	3 and 5	5
Pore Size (Å)	120	120	300	120	120
Surface Area (m^2/g)	300	300	100	300	300
% C	18	11	7	17	17

Performance Indicators

Acclaim bonded-silica columns are manufactured for outstanding stability and reproducibility for a broad range of selectivities. The Acclaim silica bonding technology developed at Dionex starts with ultrapure spherical silica gel to minimize secondary interactions. The bonding processes are designed to produce very high-density surface coverage, resulting in bonded phases with exceptional efficiency and symmetry for the entire range of analytes, from neutral hydrophobic molecules to basic and acidic molecules. In addition, the Acclaim polar-embedded bonding technology produces phases that extend capability well beyond traditional reversed-phase separations to very polar analytes requiring 100% aqueous conditions for retention. All phases are designed for low-bleed performance for mass spectrometry.

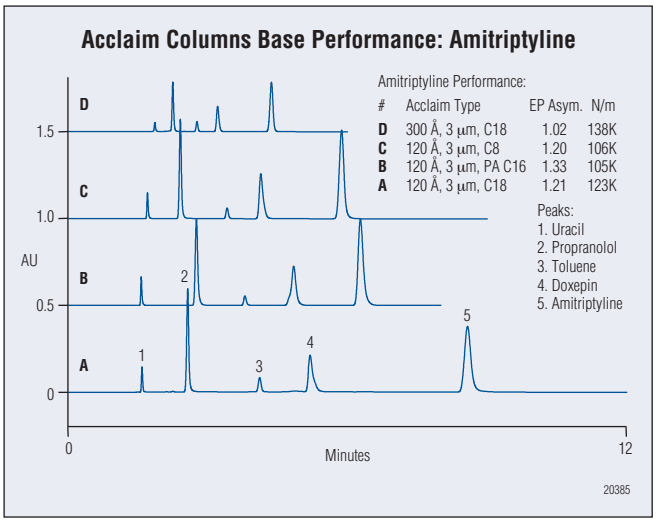
To ensure reproducible performance, all Acclaim products are thoroughly characterized for surface coverage, metal contamination, steric selectivity, column polarity, column hydrophobicity, and low silanol activity for bases.

Polarity Index



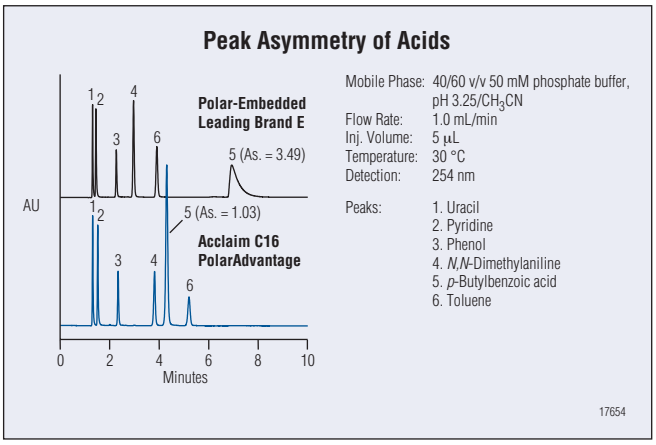
This polarity index figure ranks stationary phases according to relative polarity using the indicated conditions. This ranking can be used to compare differences or similarities in column polarity that affect polar compound selectivity.

Base Assymetry



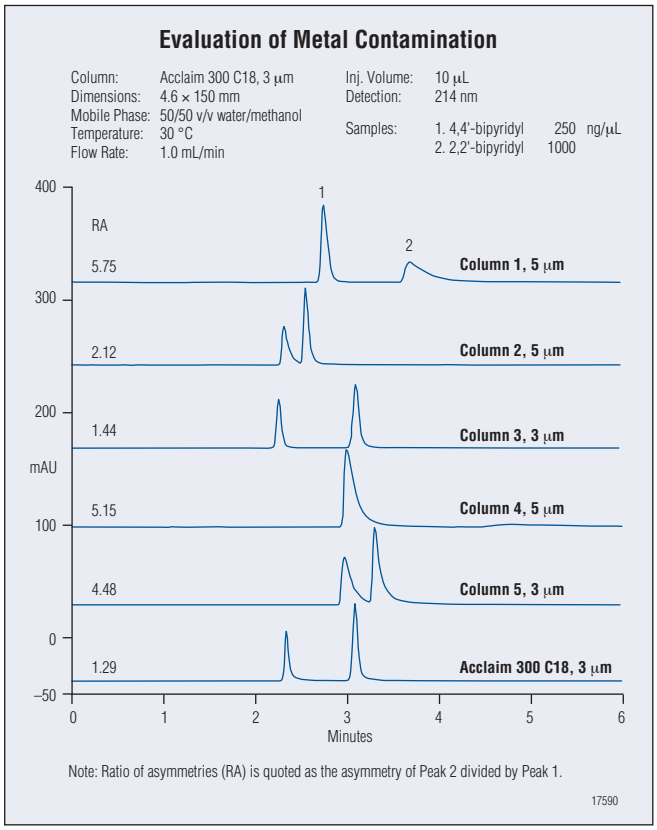
A well-covered surface and vigorous endcapping give superior performance for basic drugs. Amitriptyline is a well-known example of a basic pharmaceutical that interacts strongly with residual silanols to cause asymmetric peaks. Acclaim outperforms ordinary reversed-phase columns

Acid Assymetry



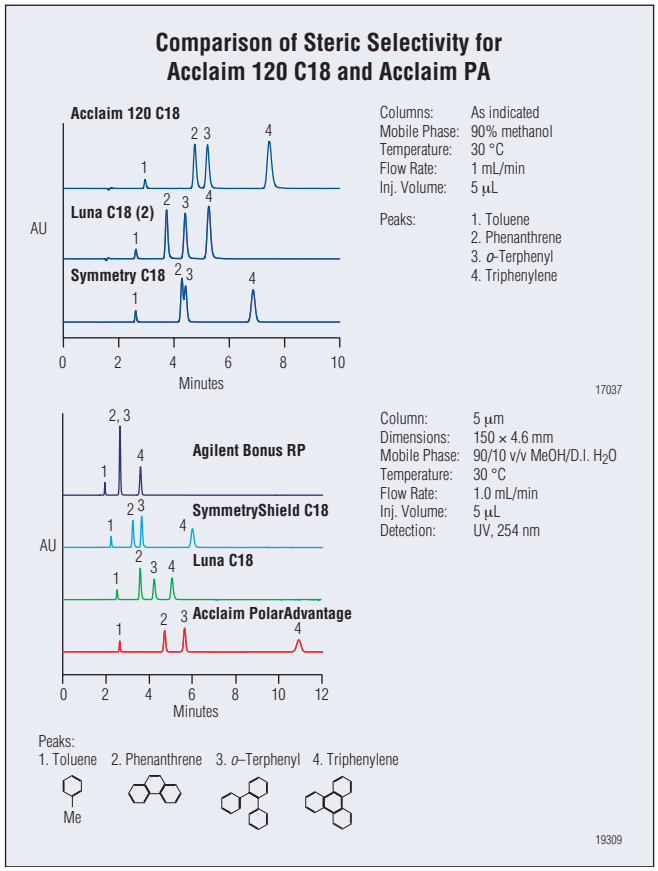
Many polar-embedded columns have good performance for a limited group of compounds. Acclaim PolarAdvantage uses state-of-the-art methods of synthesis to eliminate unwanted secondary interactions. The result is outstanding performance for hydrophilic and hydrophobic acidic, basic, and neutral analytes.

Metal Contamination



Metal contamination can interfere with the separation of many different substances. 2,2′-Bipyridyl is a strong chelating agent, and will reveal any contamination by metal ions. The figure above shows how Acclaim outperforms five competitor columns in this difficult test.

Steric Selectivity

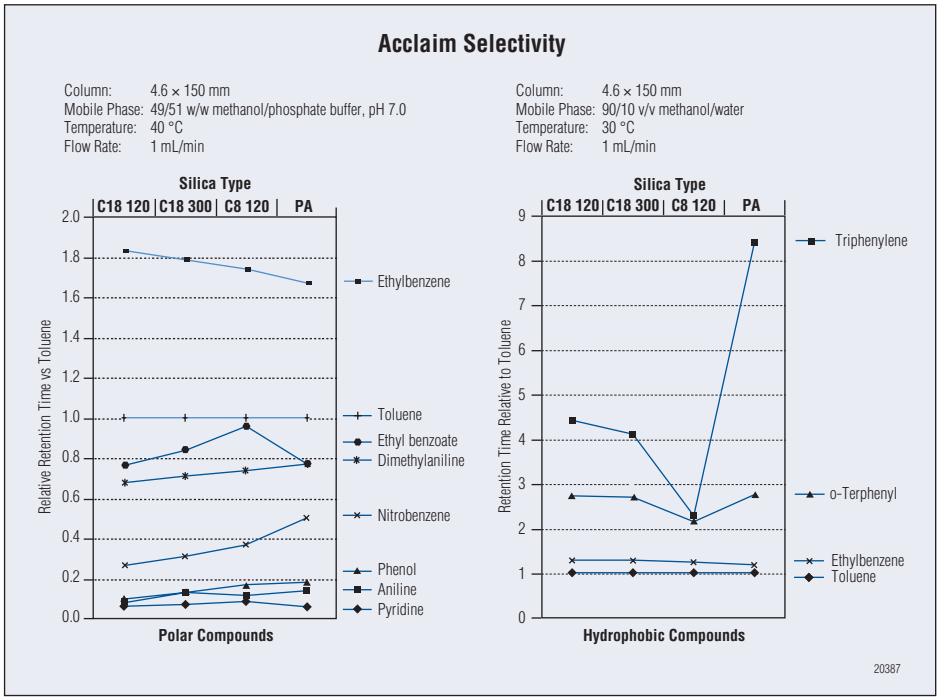


Depending on the bonding chemistry, various C18 columns can have different selectivity to molecular shape. Acclaim may be just what you need for your unique separation challenge.

Selecting an Acclaim Column

Choosing the Best Column Chemistry

When a chromatographer develops a separation, the selectivity of the stationary phase is the first of many factors that must be considered. Selectivity is the result of the differing interactions between each analyte and the stationary phase. Although many factors that influence selectivity can be controlled in the laboratory—such as mobile phase composition, gradient, or temperature—having the right column with optimal selectivity is the essential starting point. Of course, the "right" column depends on the nature of the desired separation. Therefore Dionex offers a selection of different column chemistries.



Column selectivities are interpreted typically in terms of the molecular interactions of the analyte with the stationary phase. Various probe molecules are commonly used to understand these interactions, including hydrophobic interactions, molecular shape, and ion-exchange interactions. Ethylbenzene/toluene selectivity is usually explained as a measure of the hydrophobicity of the column, or the strength of the hydrophobic interaction between analyte and bonded silica, independent of the surface area of the column. Because of their high-density bonding, Acclaim C18 and C8 columns have near maximum hydrophobicity for columns of their type. Triphenylene and *o*-terphenyl are both tricyclic hydrocarbons of similar size but different shapes; the type of bonding chemistry and bonding density influences how these two compounds interact with the stationary phase. Acclaim PA stationary phases have an unusually high selectivity for shape. In addition to hydrophobic retention, phenol, aniline, and pyridine are also retained by dipole-dipole interactions and hydrogen bonding. Polar-embedded stationary phases have stronger interactions of this type than do C18 phases. Selectivity within this group is further affected by the degree of their ion-exchange activity with residual surface silanols at pH 7: the two bases are protonated, but phenol is neutral. Overall, Acclaim columns exhibit low ion-exchange activity under neutral conditions.

Choose the bonded phase with the best selectivity for the desired separation. The first choice is usually C18 because of its excellent efficiency, low silanol activity, and ability to separate many organic molecules. The C8 phase is a good choice where lower retentivity is useful. To resolve highly polar molecules under highly aqueous conditions, or for a slightly different selectivity, Acclaim PA is the best option.

Choosing the Appropriate Pore Size

Select a pore size appropriate to the molecular weight of your sample. Size-exclusion effects can cause unwanted peak broadening. Therefore, select a pore diameter larger than your largest sample molecule. Compared to standard silica, wide-pore silica has less surface area and therefore less retention for small molecules.

MW <15 kDa: use Acclaim 120 or Acclaim PA

MW <150 kDa: use Acclaim 300

Choosing the Optimum Format

Because Acclaim columns are available in many formats, it is important to carefully select the best one for each particular application.

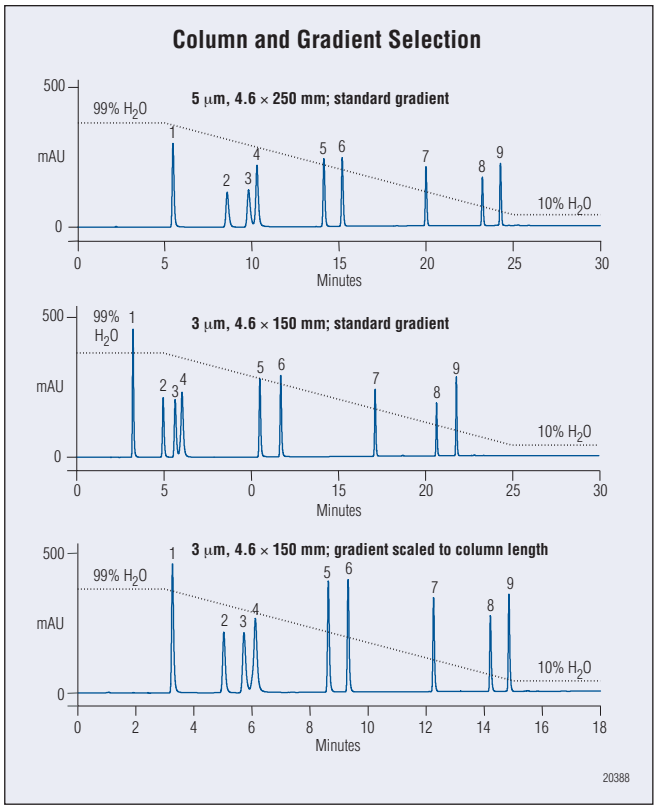
Optimize the analysis time and chromatographic efficiency by evaluating the trade-offs between the particle diameter and column lengths. The 5-µm, 4.6 × 250 mm format is traditionally the first choice for a column, but a 3-µm, 4.6 × 150 mm column delivers similar peak widths in 60% of the time. For a less complex sample, a 50-mm column may provide adequate resolution, while saving time and solvent costs. Improvements in detection limits are an added benefit of the shorter formats. For LC-MS applications, consider an increase in throughput by relaxing the requirements for peak resolution and using a shorter column or larger particle size than would be optimal for UV detection. When using gradient elution, to preserve the relative elution times, scale the gradient time proportionally to the column length. When run time is more important than sharp peaks, optimize the resolution by shortening the column instead of lengthening the gradient. When in doubt, a 3-µm, 150-mm-long column is always a good starting point.

Select a column diameter according to your requirements for sample size, solvent consumption, and sensitivity. As a general rule, the maximum sample load, solvent consumption, and detection limits are proportional to the square of the column diameter. Scale the flow rate by the square of the column diameter to preserve backpressure and retention time: a 2.1-mm column requires 21% of the flow rate of a 4.6-mm column. Given the same mass of sample, the peak area scales according to the inverse square of column diameter; for example, a 1.0-ng sample injected on a 2.1-mm column would yield 4.8 times more area than on a 4.6-mm column. However, to take full advantage of these scaling rules, the HPLC instrument being used must be properly designed and specified for operation with smaller column diameters.

Mobile Phase Recommendations

An understanding of hydrolytic stability and pH ranges for a column is critical to the design of an analytical method. Both alkaline conditions and strong acid conditions degrade silica-based columns over time. The silica substrate resists acids, but is soluble in alkali. The upper pH limit is determined by the rate of dissolution of the silica gel, which is also affected by buffer ions and organic cosolvents. Alkaline buffers will shift pH upon mixing with organic solvents; inorganic anions such as phosphate become more alkaline, whereas organic bases such as TRIS become less alkaline. This effect underlies the common warning that phosphate buffers shorten column life. Acidic conditions, on the other hand, hydrolyze the bonded layer, the rate being proportional to the hydrogen ion concentration. Fortunately, organic modifiers usually provide protection against degradation. The bonding chemistry of the Acclaim C18, C8, and PolarAdvantage is based on monofunctional silanes, and therefore the pH limits (pH 2–8) of the columns are similar. The rate of hydrolysis is slower toward the middle of the pH range. Service life versus quality of separation is a consideration when developing a method.

Acclaim columns are compatible with common mobile phases containing water and a miscible organic solvent. For high UV transparency and low backpressure, the preferred solvents are acetonitrile, methanol, and tetrahydrofuran, in that order. The choice of organic solvent will affect selectivity, and may require



experimentation to obtain the best results. Because retention of many analytes is influenced by the pH of the mobile phase, buffers are often used. Typical buffers range in concentration between 5 and 100 mM; use no more than necessary because high concentrations increase the risk of salt precipitation. Although usually unnecessary, Acclaim columns are compatible with base deactivating additives (e.g., diethylamine).

Use the highest practical quality of water, solvents, and buffer components; HPLC-grade materials have low UV absorbance and are submicron filtered by the manufacturer. To prevent fouling of your Acclaim column, use mobile phases that have been filtered through a 0.5-µm or smaller filter.

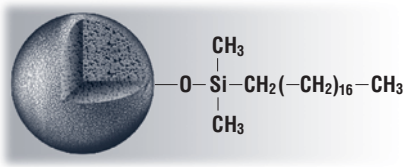
Reliability and Durability

Dionex understands that column quality and reliability are essential to a successful analysis. Acclaim columns are thoroughly tested individually, so the chromatographer can have full confidence in each column. Manufacturing starts with an ultrapure silica substrate, using carefully selected lots with narrow ranges of physical parameters. By design, the bonding processes are clean and reproducible. Each batch of bonded silica receives a full suite of validation tests appropriate to its intended use. The bonded silica is packed in precision-polished 316 stainless steel hardware using highly reliable packing processes. Each packed column is tested to ensure the same optimal performance. The quality assurance reports for silica lot validation and column performance explain the test protocols, list the specifications, and show the actual chromatograms (see Appendix for details).

PART TWO

Chapter 1: Acclaim 120 C18

The Acclaim 120 C18 series columns feature a densely bonded monolayer of octadecyldimethylsiloxane on a highly pure, spherical, silica substrate with 120-Å-pore structure.



The Acclaim 120 C18 columns are the classic reversed-phase columns. These columns are recommended for general-purpose reversed-phase applications where high surface coverage, low silanol activity, and excellent efficiency are required. The Acclaim 120 C18 columns feature:

- Highly efficient, symmetrical peaks for difficult basic and chelating analytes
- Ultrapure silica substrate
- Optimized surface pretreatment, proprietary high-density bonding process, and double endcapping
- Reliability designed into the manufacturing process and assured by thorough and appropriate testing
- High hydrophobicity and low polarity yield high selectivity for hydrophobic substances
- LC/MS compatible
- Wide range of applications in pharmaceutical, environmental, food testing, and product-quality testing for small molecules

Recommended Ranges of Operation

This bonded phase is stable in the range of pH 2–8. The silica substrate is mechanically strong enough to tolerate high flow rates and pressures up to 4000 psi. To maintain wetting of the hydrophobic surface, the recommended range for organic composition is 20–100%. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, high concentrations of halide ions at pH <2.5 should be avoided.

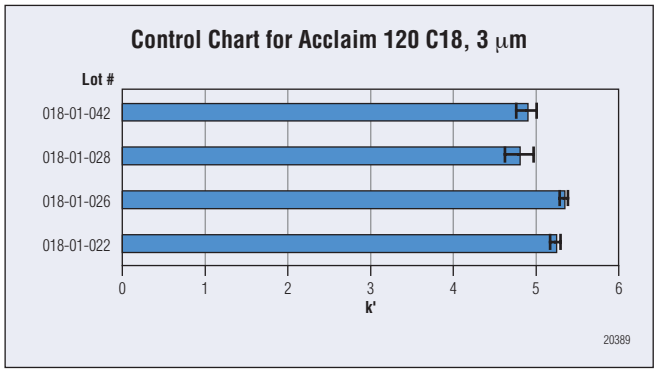
Physical Specifications	
Bonding	C18 monomeric
USP code	L1
Endcapping	Double
% C	17.5–18.9
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameter	2.9–3.2 or 4.2–4.5 µm

Performance Specifications

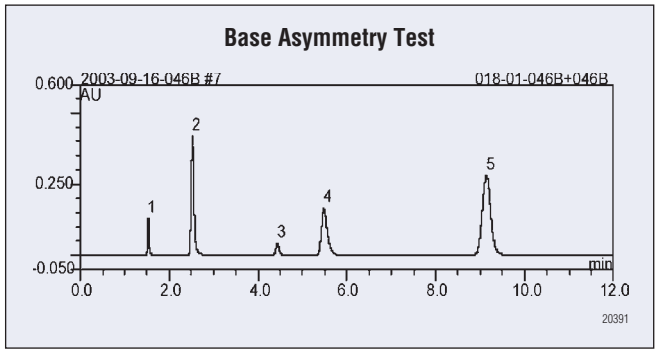
Each lot of bonded silica is tested for percent carbon, polar selectivity, metal activity, base asymmetry, and steric selectivity. A lot test certificate ships with every analytical column that describes each test in detail, the specification ranges, and actual test results. These tests are also described in the appendix. Acclaim columns are individually tested to ensure the consistency of the packing process. The quality assurance report shows the retention time, efficiency, and asymmetry for phenanthrene under test conditions.

Performance Specifications		
Lot	Polar selectivity ratio	0.12–0.16
	Base asymmetry	0.98–1.50 (5 µm) 0.98–1.60 (3 µm)
	Metal activity ratio	0.90–1.30
	Steric selectivity ratio	1.58–1.65
Column	Efficiency (N/m)	90000 (5 µm) 120000 (3 µm)
	Asymmetry (EP)	0.98–1.20
	Retention time	8.41–9.29 (5 µm) 8.31–9.19 (3 µm)

Chromatographic specifications for Acclaim 120 C18 4.6 × 150 mm columns.



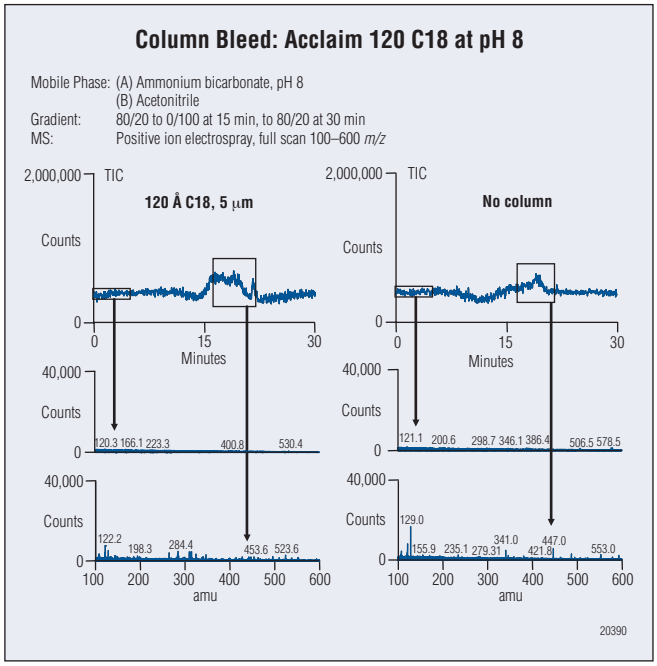
Control chart showing k' (phenanthrene) for four lots of 3 µm C18. The data show the average and standard deviation for all columns shipped from that lot.



Chromatogram of the base asymmetry test taken from the certificate for lot 018-01-046B.

LC/MS Compatibility

Acclaim 120 C18 columns are LC/MS compatible. LC/MS analysis shows very low bleed for this column. Out of the box, this column is ready to use after only a few minutes of conditioning with solvent. The same low bleed is attained over the entire pH range of 2–8. The baseline obtained from an Acclaim column is very similar to that obtained from an empty capillary tube.



Ordering Information: Acclaim 120 C18

Standard particle sizes are nominally 3 and 5 µm. Analytical columns are available in 2.1- and 4.6-mm diameters; standard lengths are 50, 100, and 150 mm for 3- and 5-µm particles and 250 mm for 5-µm particles. Capillary formats are available in 75 and 300 µm, 1.0-mm and custom diameters, and in 50-, 150-, or 250-mm lengths. Micro precolumn and nano precolumn cartridges are available in several formats. Guard columns in both 2.0- and 4.3-mm sizes packed with 5-µm particles are recommended to protect both the 3 and 5 µm analytical columns.

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.

Acclaim 120 C18 Analytical Columns

3 µm	75 µm × 50 mm (NAN75)	162238
3 µm	75 µm × 150 mm (NAN75)	162239
3 µm	300 µm × 50 mm (FUS)	162236
3 µm	300 µm × 150 mm (FUS)	162237
3 µm	1 × 50 mm (MIC)	162234
3 µm	1 × 150 mm (MIC)	162235
3 µm	2.1 × 50 mm	059128
3 µm	2.1 × 100 mm	059129
3 µm	2.1 × 150 mm	059130
3 µm	4.6 × 50 mm	059131
3 µm	4.6 × 100 mm	059132
3 µm	4.6 × 150 mm	059133
5 µm	75 µm × 50 mm (NAN75)	162456
5 µm	75 µm × 150 mm (NAN75)	162457
5 µm	75 µm × 250 mm (NAN75)	162458
5 µm	300 µm × 50 mm (FUS)	162453
5 µm	300 µm × 150 mm (FUS)	162454
5 µm	300 µm × 250 mm (FUS)	162455
5 µm	1 × 50 mm (MIC)	162450
5 µm	1 × 150 mm (MIC)	162451
5 µm	1 × 250 mm (MIC)	162452
5 µm	2.1 × 50 mm	059142

Acclaim 120 C18 Analytical Columns

5 µm	2.1 × 100 mm	059143
5 µm	2.1 × 150 mm	059144
5 µm	2.1 × 250 mm	059145
5 µm	4.6 × 50 mm	059146
5 µm	4.6 × 100 mm	059147
5 µm	4.6 × 150 mm	059148
5 µm	4.6 × 250 mm	059149

Acclaim 120 C18 Guard Columns

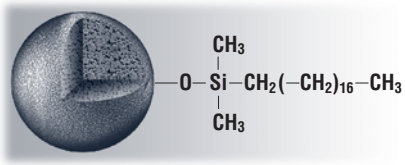
5 µm	2.0 × 10 mm	059447
5 µm	4.3 × 10 mm	059446
	Biocompatible cartridge holder for 2.0 × 10 and 4.3 × 10 mm guards	059456
	Guard to analytical column coupler	059457
	Holder and coupler kit	059526

Acclaim 120 C18 Micro and Nano Precolumns

5 µm	300 µm × 1 mm (pack of 5)	162306
5 µm	300 µm × 5 mm (pack of 5)	162236
5 µm	500 µm × 5 mm (pack of 5)	162324
5 µm	500 µm × 15 mm (pack of 5)	162325
5 µm	800 µm × 5 mm (pack of 5)	162323
5 µm	1 × 5 mm (pack of 5)	162321
5 µm	1 × 15 mm (pack of 5)	162322

Chapter 2: Acclaim 120 C8

The Acclaim 120 C8 series columns feature a densely bonded monolayer of octyldimethylsiloxane on a highly pure, spherical silica substrate with a 120-Å-pore structure.



The Acclaim 120 C8 series employs the same bonding chemistry and substrate as C18, and therefore features the same high standards of efficiency, coverage, and silanol activity. The Acclaim 120 C8 features:

- Highly efficient, symmetrical peaks with difficult basic and chelating analytes
- Ultrapure silica substrate
- Optimized surface pretreatment, proprietary high-density bonding process, and vigorous endcapping
- Reliability designed into the manufacturing process and assured by thorough and appropriate testing
- Less hydrophobic, less retentive than C18
- LC/MS compatible
- Excellent performance for basic pharmaceuticals and environmental samples

Recommended Ranges of Operation

The bonded phase is stable in the range of pH 2–8. The silica substrate is mechanically strong enough to tolerate high flow rates and pressures up to 4000 psi. To maintain wetting of the hydrophobic surface, the recommended range for organic composition is 20–100%. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, avoid high concentrations of halide ions at pH <2.5.

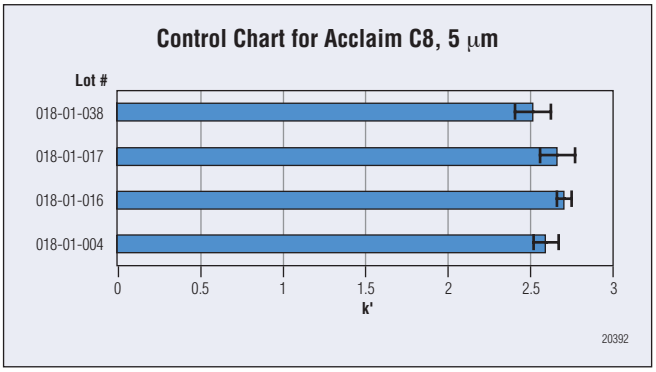
Physical Specifications	
Bonding	C8 monomeric
USP code	L7
Endcapping	Yes
% C	10.1–11.8
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameter	2.9–3.2 or 4.2–4.5 µm

Performance Specifications

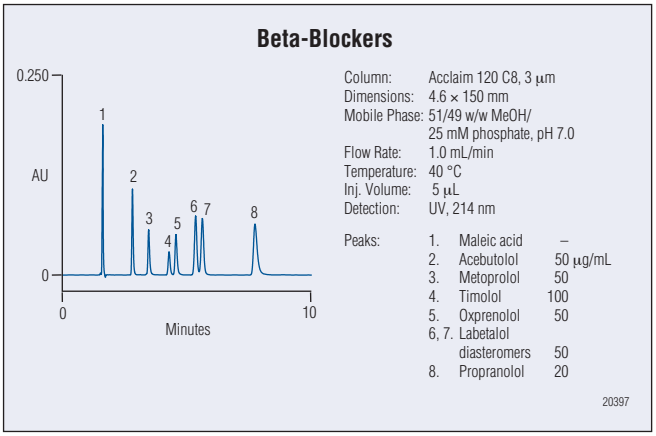
Each lot of bonded silica is tested for percent carbon, polar selectivity, metal activity, and base asymmetry. A lot test certificate is shipped with every analytical column; the certificate provides a detailed description of each test, the specification ranges, and actual test results. These tests are also described in the appendix. Acclaim columns are individually tested to ensure the consistency of the packing process. The quality assurance report shows the retention time, efficiency, and asymmetry for phenanthrene under standard conditions.

Performance Specifications		
Lot	Polar selectivity ratio	0.22–0.26
	Base asymmetry	0.98–1.50
	Metal activity ratio	0.90–1.30
Column	Efficiency (N/m)	90000 (5 µm) 120000 (3 µm)
	Asymmetry (EP)	0.98–1.20
	Retention time	5.39–5.95 (5 µm) 5.34–5.90 (3 µm)

Chromatographic specifications for Acclaim 120 C8 4.6 × 150 mm columns.



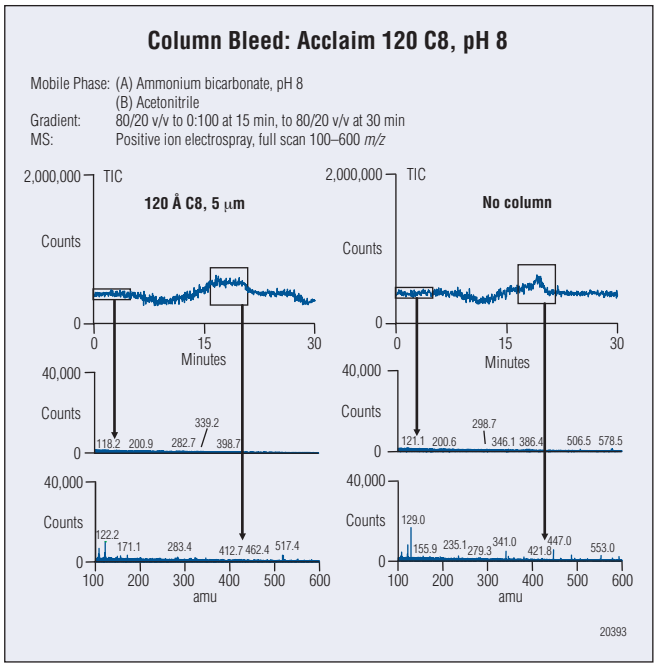
Control chart showing k' (phenanthrene) for four lots of 5-µm C8. The data show the average and standard deviation for all columns shipped from that lot.



Acclaim 120 C8 has excellent performance for basic drugs such as these beta-blockers. Peaks are efficient and symmetrical.

LC/MS Compatibility

Acclaim 120 C8 columns are LC/MS compatible. LC/MS analysis shows very low bleed for this column. Out of the box, this column is ready to use after only a few minutes of conditioning with solvent. The same low bleed is attained over the entire pH range of 2–8. The baseline obtained for an Acclaim column is very similar to that obtained for an empty capillary tube.



Ordering Information: Acclaim 120 C8

Standard particle sizes are nominally 3 and 5 µm. Analytical columns are available in 2.1- and 4.6-mm diameters; standard lengths are 50, 100, and 150 mm for 3- and 5-µm particles plus 250 mm for 5-µm particles. Capillary formats are available in 75 and 300 µm, 1.0-mm and custom diameters, and in 50-, 150-, or 250-mm lengths. Micro precolumn and nano precolumn cartridges are available in several formats. Guard cartridges in both 2.0- and 4.3-mm diameters packed with 5-µm particles are recommended to protect both the 3 µm and 5 µm analytical columns.

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.

Acclaim 120 C8 Analytical Columns		
3 µm	75 µm × 50 mm (NAN75)	162208
3 µm	75 µm × 150 mm (NAN75)	162209
3 µm	300 µm × 50 mm (FUS)	162206
3 µm	300 µm × 150 mm (FUS)	162207
3 µm	1 × 50 mm (MIC)	162204
3 µm	1 × 150 mm (MIC)	162205
3 µm	2.1 × 50 mm	059122
3 µm	2.1 × 100 mm	059123
3 µm	2.1 × 150 mm	059124
3 µm	4.6 × 50 mm	059125
3 µm	4.6 × 100 mm	059126
3 µm	4.6 × 150 mm	059127
5 µm	75 µm × 50 mm (NAN75)	162216
5 µm	75 µm × 150 mm (NAN75)	162217
5 µm	75 µm × 250 mm (NAN75)	162218
5 µm	300 µm × 50 mm (FUS)	162213
5 µm	300 µm × 150 mm (FUS)	162214
5 µm	300 µm × 250 mm (FUS)	162215
5 µm	1 × 50 mm (MIC)	162210
5 µm	1 × 150 mm (MIC)	162211
5 µm	1 × 250 mm (MIC)	162212
5 µm	2.1 × 50 mm	059134
5 µm	2.1 × 100 mm	059135

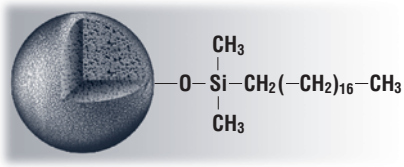
Acclaim 120 C8 Analytical Columns		
5 µm	2.1 × 150 mm	059136
5 µm	2.1 × 250 mm	059137
5 µm	4.6 × 50 mm	059138
5 µm	4.6 × 100 mm	059139
5 µm	4.6 × 150 mm	059140
5 µm	4.6 × 250 mm	059141

Acclaim 120 C8 Guard Columns		
5 µm	2.0 × 10 mm	059449
5 µm	4.3 × 10 mm	059448
Biocompatible cartridge holder for 2.0 × 10 and 4.3 × 10 mm guards		059456
Guard to analytical column coupler		059457
Holder and coupler kit		059526

Acclaim 120 C8 Micro and Nano Precolumns		
5 µm	300 µm × 1 mm (pack of 5)	162304
5 µm	300 µm × 5 mm (pack of 5)	162266
5 µm	500 µm × 5 mm (pack of 5)	162264
5 µm	500 µm × 15 mm (pack of 5)	162265
5 µm	800 µm × 5 mm (pack of 5)	162263
5 µm	1 × 5 mm (pack of 5)	162261
5 µm	1 × 15 mm (pack of 5)	162262

Chapter 3: Acclaim 300 C18

The Acclaim 300 C18 series columns feature a densely bonded monolayer of octadecyldimethylsiloxane on a highly pure, spherical silica substrate with a wider, 300-Å-pore structure.



The Acclaim 300 series columns are designed for peptide mapping and separation of small proteins up to 150 kDa. The Acclaim 300 is also useful for general-purpose, reversed-phase chromatography of small molecules. The Acclaim 300 C18 columns feature:

- Technology designed for high-resolution peptide mapping applications and protein separations
- High-efficiency 3-µm spherical silica substrate
- The same high-performance bonding chemistry as the Acclaim 120 series, but using a silica substrate with larger 300-Å pores and lower surface area
- Application tested for suitability in peptide mapping
- Secondary interactions that are minimized for repeatable results day to day and column to column
- Recommended for reversed-phase separation of peptides and proteins <150 kDa

The unique bonding chemistry results in a high-density, highly uniform phase coverage with extensive endcapping. 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, a given separation can be achieved in a shorter run time by increasing the flow rate of the mobile phase and running shallower gradients on shorter columns.

Recommended Ranges of Operation

The bonded phase is stable in the range of pH 2–8. The silica substrate is mechanically strong enough to tolerate high flow rates and pressures up to 4000 psi. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, avoid high concentrations of halide ions at pH <2.5. To maintain wetting of the hydrophobic surface, the recommended range for organic composition is 5–100%.

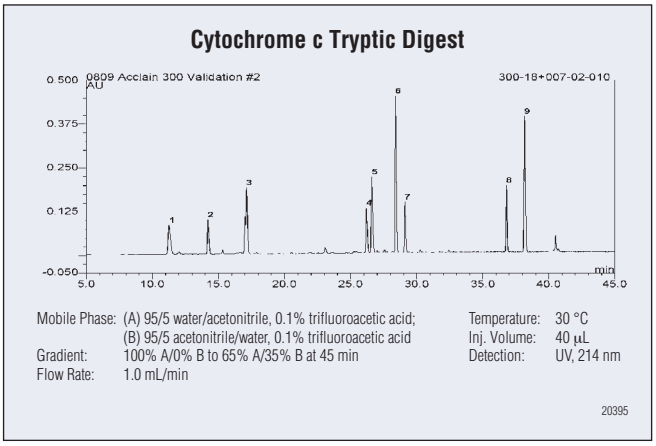
Physical Specifications	
Bonding	C18 monomeric
USP code	L1
Endcapping	Double
% C	6.7–7.5
Pore size	260–300 Å
Surface area	90–100 m^2/g
Particle diameters	2.9–3.2 µm

Performance Specifications

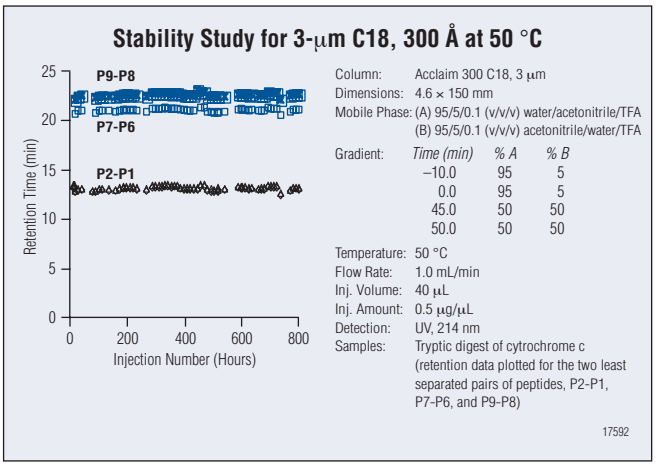
Each lot of bonded silica is tested for percent carbon, metal contamination, base asymmetry, and peptide selectivity. The lot test certificate shipped with every analytical column describes each test in detail, the specification ranges, and actual test results. These tests are also described in the appendix. Acclaim columns are individually tested to ensure the consistency of the packing process. The quality assurance report shows the retention time, efficiency, and asymmetry for phenanthrene under standard conditions.

Performance Specifications		
Lot	Base asymmetry	0.98–1.30
	Metal activity ratio	0.80–1.30
	Cytochrome c tryptic digest peptide map	P2-P1: 2.8–3.1 min P7-P6: 0.6–1.0 P9-P8: 1.1–1.5
Column	Efficiency (N/m)	115000
	Asymmetry (EP)	0.98–1.20
	Retention time	4.30–4.80

Chromatographic specifications for Acclaim 300 C18 4.6 × 150 mm columns.

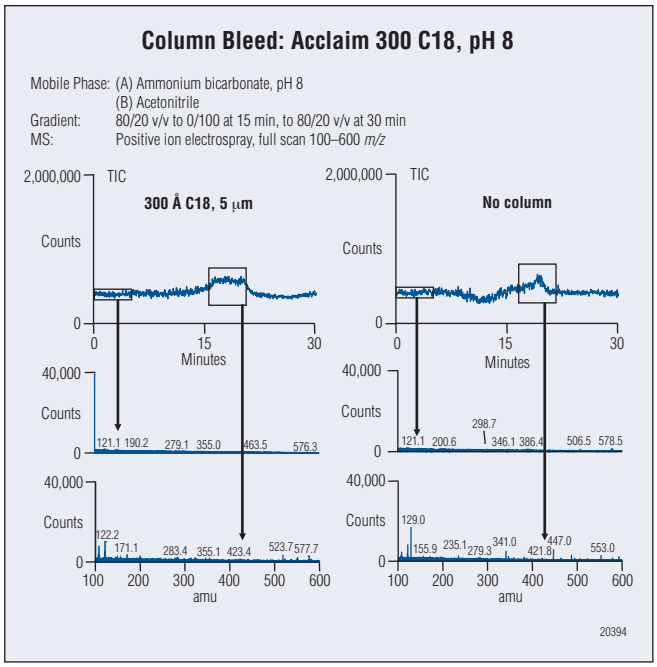


Chromatogram of cytochrome c tryptic digest taken from the certificate for lot #007-02-010. Dionex monitors and controls the retention time difference between three pairs of peptides: P2-P1, P7-P6, and P9-P8.



LC/MS Compatibility

Acclaim 300 C18 columns are LC/MS compatible. LC/MS testing shows very low bleed for this column. Out of the box, this column is ready to use after only a few minutes of conditioning with solvent. Low bleed is attained over the entire pH range of 2–8. The baseline obtained for an Acclaim column is very similar to that obtained for an empty capillary tube.



Ordering Information: Acclaim 300 C18

The standard particle size for the Acclaim 300 C18 series columns is nominally 3 µm. Analytical columns are available in 2.1- and 4.6-mm diameters; standard lengths are 50 and 150 mm. Guard cartridges in both 2.0- and 4.3-mm sizes packed with 3-µm particles are recommended to protect your analytical columns. Capillary formats are available in 75 and 300 µm, 1.0-mm and custom diameters, and in 50- or 150-mm lengths.

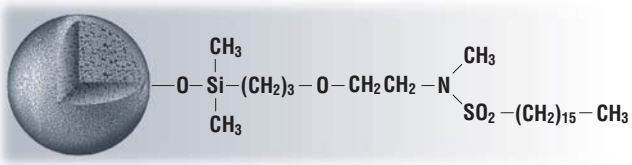
To order in the U.S., call (800) 346-6390 or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim 300 C18 Analytical Columns		
3 µm	75 µm × 50 mm (NAN75)	162223
3 µm	75 µm × 150 mm (NAN75)	162224
3 µm	300 µm × 50 mm (FUS)	162221
3 µm	300 µm × 150 mm (FUS)	162222
3 µm	1 × 50 mm (MIC)	162219
3 µm	1 × 150 mm (MIC)	162220
3 µm	2.1 × 50 mm	060263
3 µm	2.1 × 150 mm	060264
3 µm	4.6 × 50 mm	060265
3 µm	4.6 × 150 mm	060266

Acclaim 300 C18 Guard Columns		
3 µm	2.0 × 10 mm	060395
3 µm	4.3 × 10 mm	060393
Biocompatible cartridge holder for 2.0 × 10 and 4.3 × 10 mm guards		059456
Guard to analytical column coupler		059457
Holder and coupler kit		059526

Chapter 4: Acclaim PolarAdvantage (PA)

The Acclaim PA column has a patented, state-of-the-art surface chemistry that renders it hydrophilic enough to be compatible with solvent-free mobile phases. The ether and sulfonamide linkages are more hydrolytically stable than the amides used in many polar-embedded phases. The methods of synthesis minimize both residual silanols and amines, thus making Acclaim PA suitable for acidic, basic, or neutral analytes.



Acclaim PA columns are reversed-phase silica columns with a polar-enhanced stationary phase for operation over a wider range of chromatographic conditions and with a broader application range when compared to conventional reversed-phase columns. Acclaim PA has selectivity similar to C18 for many analytes of low polarity, with the added advantage of compatibility with aqueous-only mobile phases. Some classes of compounds (for example, nitroaromatics) show significantly different selectivity patterns on this bonded phase. The high-density bonding provides good retention of hydrophilic analytes. Above 90% organic solvent composition of the mobile phase, this column starts to show some normal-phase HPLC characteristics. The Acclaim PA benefits include:

- Compatibility with solvent-free applications without any compromise to performance for acids and bases
- Novel polar-embedded surface layer
- Ability to work with 0–100% aqueous or 0–100% organic solvent mobile phases
- Resolves hydrophilic compounds
- High selectivity for hydrophobic compounds
- Different selectivity than C18 makes PA useful as a confirmation column
- Wide range of applications in pharmaceutical, environmental, food testing, and product-quality testing

Physical Specifications	
Bonding	C16 monomeric, polar-embedded
USP code	n.a
Endcapping	Double
% C	16.0–18.0
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameters	2.9–3.2 µm or 4.2–4.5 µm

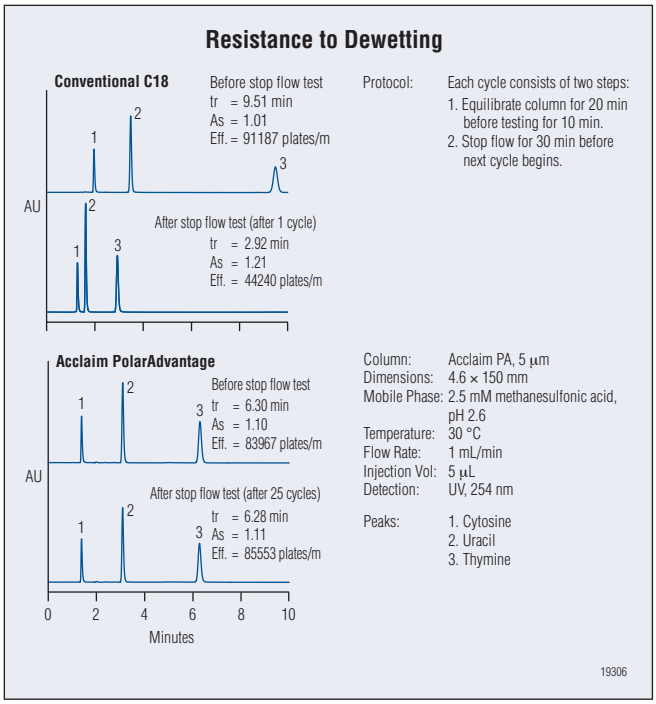
Recommended Ranges of Operation

The bonded phase is conservatively rated for a pH range of 2–8, and may be extended under suitable conditions. The silica substrate is mechanically strong enough to tolerate high flow rates and pressures up to 4000 psi. The surface remains wetted from 0–100% organic solvent in the mobile phase, even with strong aqueous buffers. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, avoid high concentrations of halide ions at pH <2.5.

Resistance to Dewetting

The surface of a conventional C18 phase is very hydrophobic. With highly aqueous mobile phases, the partial pressure of dissolved gases can expel the liquid from the pores of the stationary phase. This process is called dewetting and is deleterious to acceptable chromatographic performance. The mildly hydrophilic Acclaim PA column allows aqueous-only mobile phases to maintain excellent contact with the stationary phase surface.

While the onset of dewetting is somewhat unpredictable, stopping the flow of mobile phase through the column can initiate the process. The accompanying figure shows the effect of repeatedly stopping the flow through a C18 and a PA column. The C18 column dewets in a single cycle, but the PA remains wetted through many cycles.



Hydrolytic Stability

Under favorable conditions, Acclaim PA can exhibit very good hydrolytic stability, even below pH 2, as shown in the figure below. All bonded silica is vulnerable to hydrolysis. Therefore, the basic question the chromatographer asks is "For my analysis, what is the service lifetime of my column?" Unfortunately, there is no generally agreed-upon means to answer this question. Dionex conservatively rates the Acclaim PA at pH 2–8 for general use. Conditions recommended for optimum column life include: (1) pH within the recommended range, (2) organic modifier in the mobile phase, (3) temperatures near ambient, and (4) organic buffer salts (e.g., TRIS) for pH >7. Under these conditions, one can expect a satisfactory life span from this column.

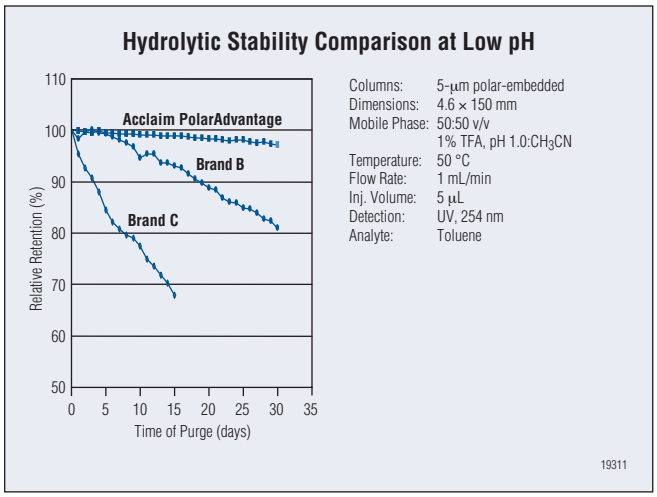


Chart of retention vs time at 50% acetonitrile, 0.5% TFA for polar-embedded columns.

Performance Specifications

Each lot of bonded silica is tested for percent carbon, polar selectivity, metal activity, base asymmetry, and acid asymmetry. A lot validation certificate is packaged with every analytical column; the certificate describes in detail each test, the specification ranges, and actual test results. These tests are also described in the appendix. Acclaim columns are individually tested to ensure the consistency of the packing process. The quality assurance report shows the retention time, efficiency, and asymmetry for phenanthrene under standard test conditions.

Performance Specifications		
Lot	Polar selectivity ratio	0.14–0.18
	Base asymmetry	0.98–1.50 (5 µm) 0.98–1.60 (3 µm)
	Metal activity ratio	0.80–1.90
	Acid asymmetry	0.98–1.65
Column	Efficiency (N/m)	90000 (5 µm) 120000 (3 µm)
	Asymmetry (EP)	0.98–1.20
	Retention time	7.70–8.40 (5 µm) 7.70–8.40 (3 µm)

Chromatographic specifications for Acclaim PA, 4.6 × 150 mm.

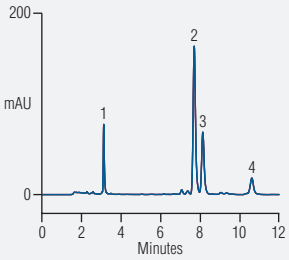
LC/MS Compatibility

Acclaim PA may be used for LC/MS applications, with some restrictions related to column bleed. The polar-embedded group, although chemically stable, is readily ionized in the mass spectrometer source. For best results and lowest background, operate below 50% organic solvent and between pH 3 and pH 7. A new column should be conditioned for one hour with 100% acetonitrile. Store the column in 100% organic solvent between uses, and flush the column to waste with organic solvent before each use.

Wide Range of Applications

Because the Acclaim PA column exhibits similar selectivity and hydrophobicity to many commercial C18 columns, it can potentially be used for most applications developed on C18 columns, but with the additional benefits of improved peak shape for basic compounds and 100% aqueous compatibility. The Acclaim PA is useful for the separation of fat-soluble vitamins by conventional reverse-phase conditions, as well as the separation of water-soluble vitamins using a gradient starting with a 100% aqueous mobile phase for high retention of polar analytes, followed by an acetonitrile gradient.

Separation of Fat-Soluble Vitamins A, D_2, D_3, and E Using the Acclaim PA

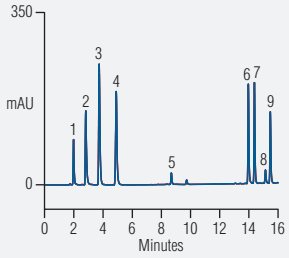


Column:	Acclaim PA, µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	97/3 v/v CH_2CN/D.I. H_2O
Temperature:	30 C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	UV, 264 nm

Peaks:		
	1. All-trans Retinol (Vitamin A)	120 µg/mL
	2. Ergocalciferol (Vitamin D_2)	120
	3. Cholecalciferol (Vitamin D_3)	60
	4. α-Tocopherol (Vitamin E)	1300

19302

Separation of Water-Soluble Vitamins



Column:	Acclaim PA, 3 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) CH_3CN
	(B) 25 mM KH_2PO_4, pH 3.5
Temperature:	30 °C
Gradient:	0–20% A at 15 min, 60% A at 17–20 min
Flow Rate:	1 mL/min
Inj. Volume.	5 µL
Detection:	UV, 210 nm

Peaks:		
	1. Thiamine HCl	20 µg/mL
	2. Nicotinic acid	20
	3. Pyridoxine	20
	4. Niacinamide	20
	5. Pantothenic acid	20
	6. Folic acid	20
	7. Vitamin B_{12}	20
	8. Biotin	20
	9. Riboflavin	20

19303

Ordering Information: Acclaim PolarAdvantage (PA)

Standard particle sizes are nominally 3 and 5 µm. Analytical columns are stocked in 2.1- and 4.6-mm-diameter widths and a range of lengths. Guard cartridges in both 2.0- and 4.3-mm sizes, packed with 5-µm particles, are recommended to protect both the 3 and 5 µm analytical columns. Capillary formats are available in 75 and 300 µm, 1.0-mm and custom diameters, and in 50-, 150-, or 250-mm lengths. Micro precolumn and nano precolumn cartridges are available in several formats. Other geometries can be made to order. Inquire with your Dionex sales representative.

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.

Acclaim PA Analytical Columns		
3 µm	75 µm × 50 mm (NAN75)	162244
3 µm	75 µm × 150 mm (NAN75)	162245
3 µm	300 µm × 50 mm (FUS)	162242
3 µm	300 µm × 150 mm (FUS)	162243
3 µm	1 × 50 mm (MIC)	162240
3 µm	1 × 150 mm (MIC)	162241
3 µm	2.1 × 100 mm	061316
3 µm	2.1 × 150 mm	061317
3 µm	4.6 × 150 mm	056318
5 µm	75 µm × 50 mm (NAN75)	162252
5 µm	75 µm × 150 mm (NAN75)	162253
5 µm	75 µm × 250 mm (NAN75)	162254
5 µm	300 µm × 50 mm (FUS)	162249
5 µm	300 µm × 150 mm (FUS)	162250
5 µm	300 µm × 250 mm (FUS)	162251
5 µm	1 × 50 mm (MIC)	162246
5 µm	1 × 150 mm (MIC)	162247
5 µm	1 × 250 mm (MIC)	162248
5 µm	4.6 × 50 mm	061319

Acclaim PA Analytical Columns		
5 µm	4.6 × 150 mm	061320
5 µm	4.6 × 250 mm	061321

Acclaim PA Guard Columns		
5 µm	2.0 × 10 mm	061331
5 µm	4.3 × 10 mm	061332
Biocompatible cartridge holder for 2.0 × 10 and 4.3 × 10 mm guards		059456
Guard to analytical column coupler		059457
Holder and coupler kit		059526

Acclaim PA Cartridge Formats		
5 µm	300 µm × 1 mm (pack of 5)	162310
5 µm	300 µm × 5 mm (pack of 5)	162302
5 µm	500 µm × 5 mm (pack of 5)	162336
5 µm	500 µm × 15 mm (pack of 5)	162337
5 µm	800 µm × 5 mm (pack of 5)	162335
5 µm	1 × 5 mm (pack of 5)	162333
5 µm	1 × 15 mm (pack of 5)	162334

Chapter 5: Acclaim OA for Organic Acids

The Acclaim OA columns are a special bonded-silica stationary phase packed in metal-free PEEK column bodies.

Acclaim OA organic acid columns are reversed-phase silica columns, designed for the separation of aliphatic and aromatic organic acids at low pH with UV-Visible detection. The Acclaim OA uses a patented polar-embedded stationary phase that allows a broad range of operating conditions, including 100% aqueous mobile phases. The Acclaim OA column benefits include:

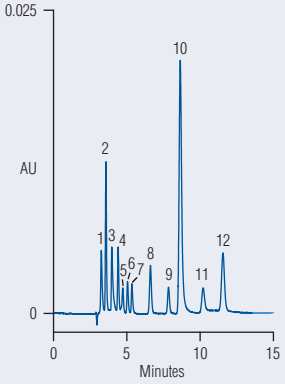
- Determination of hydrophilic organic acids in foods and beverages
- Determination of aliphatic and aromatic organic acids in pharmaceuticals, chemical intermediates, and environmental samples
- Use-tested to guarantee consistent organic acid separations
- Compatible with 100% aqueous mobile phases
- Excellent peak shapes for organic acids
- High reversed-phase capacity with retention similar to standard C18 phases

Physical Specifications	
Bonding	Proprietary
USP code	n.a
Endcapping	Double
% C	17
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameter	4.2–4.5 µm

Guaranteed Performance for Organic Acids

Acclaim OA performance is guaranteed. Acclaim OA columns undergo extensive testing to ensure column-to-column reproducibility, and are shipped with quality assurance reports detailing these tests. The Acclaim OA columns are use-tested for two specific applications—isocratic and gradient. These operating conditions provide a good starting point for methods development.

Isocratic Separation of Hydrophilic Organic Acids



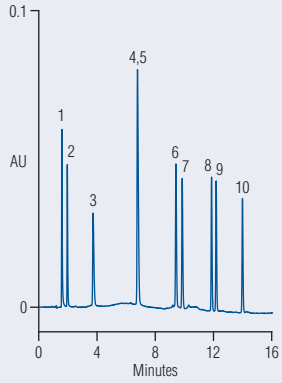
Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 100 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks:		
1.	Oxalic acid	15 mg/L (ppm)
2.	Tartaric acid	120
3.	Formic acid	180
4.	Malic acid	120
5.	*iso*-Citric acid	120
6.	Lactic acid	180
7.	Acetic acid	120
8.	Citric acid	120
9.	Succinic acid	120
10.	Fumaric acid	7
11.	*cis*-Aconitic acid	**
12.	*trans*-Aconitic acid	**

** 7 ppm total for *cis* and *trans* isomers

20011

Gradient Separation of C1–C7 Aliphatic Organic Acids



Column: Acclaim OA, 5µm
Dimensions: 4 × 150 mm
Mobile Phase: (A) CH_3CN
(B) 2.5 mM methanesulfonic acid
Gradient: Hold B for 1 min
B to A:B (45:55) in 11 min
Hold A:B (45:55) for 4 min
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 15 µL
Detection: UV, 210 nm

Peaks:		
1.	Formic acid	10 mmol/L
2.	Acetic acid	10
3.	Propionic acid	10
4.	Butyric acid	10
5.	Isobutyric acid	10
6.	Isovaleric acid	10
7.	*n*-Valeric acid	10
8.	Isocaproic acid	10
9.	*n*-Caproic acid	10
10.	Heptanoic acid	10

20017

Performance Specifications

Each lot of bonded silica is tested for percent carbon, metal activity, and application suitability. A lot validation certificate is sent with every analytical column that describes in detail each test, the specification ranges, and actual test results. These tests are also described in the appendix. Acclaim columns are individually tested to ensure the consistency of the packing process. The quality assurance report shows the retention time, efficiency, and asymmetry for succinic acid under standard conditions.

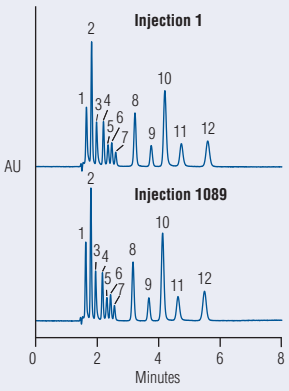
Performance Specifications		
Lot	Metal activity ratio	0.80–1.50
	Gradient organic acids chromatogram	Resolution (caproic-isocaproic) 2.80
Column	Efficiency (N/m)	72000
	Asymmetry (EP)	0.95–1.15
	Retention time	4.70–5.30
	Resolution (Lactic-Acetic)	1.30–1.50

Chromatographic specifications for Acclaim OA 4.6 × 150 mm.

Recommended Ranges of Operation

The bonded phase is stable over the range of pH 2–8. The rugged PEEK column hardware withstands up to 4000 psi. The columns and hardware are compatible with 100% methanol or acetonitrile. Above 50% organic solvent, the column hardware pressure rating is 2000 psi. PEEK is not compatible with tetrahydrofuran at concentrations greater than 20%.

Ruggedness Test at pH 2.68



Column: Acclaim OA, 5µm
Dimensions: 4 × 150 mm
Mobile Phase: 40 mM Na_2SO_4, pH (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks:

1.	Oxalic acid	15 mg/L (ppm)
2.	Tartaric acid	120
3.	Formic acid	180
4.	Malic acid	120
5.	*iso*-Citric acid	120
6.	Lactic acid	180
7.	Acetic acid	120
8.	Citric acid	120
9.	Succinic acid	120
10.	Fumaric acid	3
11.	*cis*-Aconitic acid**	
12.	*trans*-Aconitic acid**	

* *3 ppm total for *cis* and *trans* isomers

20012

Ordering Information: Acclaim OA

The standard particle size is nominally 5 µm. Analytical columns are available in 4.0-mm diameters; standard lengths are 150 and 250 mm. Guard cartridges in 4.3 × 10 mm are recommended to protect your analytical columns. Other geometries in PEEK or stainless steel are available; inquire with your Dionex sales representative.

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.

Acclaim OA Analytical Columns

5 µm 4.0 × 150 mm 062903
5 µm 4.0 × 250 mm 062902

Acclaim QA Guard Columns

5 µm 4.3 × 10 mm 062925
Biocompatible cartridge holder for 2.1 × 10 mm and 4.3 × 10 mm guards 059456
Guard to analytical column coupler 059457
Holder and coupler kit 059526

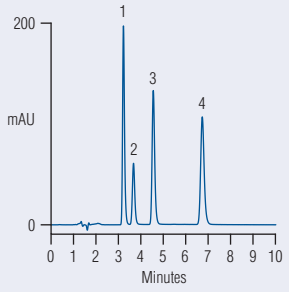
PART THREE

Applications

Contents

Pharmaceutical

Separation of Four Benzodiazepine Drugs on Acclaim 120 C18



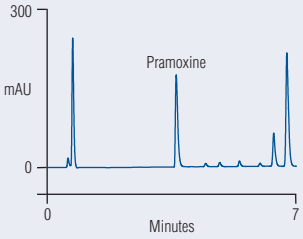
Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 60% acetonitrile
Flow Rate: 1.0 mL/min
Detection: UV, 235 nm

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

17511

Assay for Pramoxine in Topical Anesthetic Ointment on Acclaim 120 C18 with SPE Cleanup on OnGuard II RP

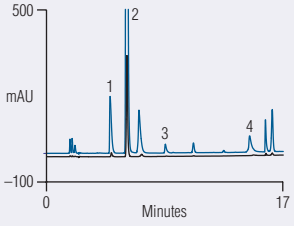
Sample Prep:
1. Disperse 0.5 g ointment in 8.5 mL MeOH
2. Add 0.5 mL conc. NH_4OH and make to 10 mL with MeOH
3. Apply 1.0 mL to a conditioned OnGuard II RP 1-cc cartridge and expel with 1 mL air
4. Elute twice with 2.0 mL of 2% acetic acid in acetonitrile and expel with 1 mL air.
5. Combine and make to 5 mL



Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 50 mm
Mobile Phase: (A) MeOH
(B) 10 mM H_3PO_4, adjusted to pH 3.1 with NH_4OH
Gradient: 25%A until 0.7 min, ramp to 85% A at 6 min, end at 7 min
Flow Rate: 1.0 mL/min
Temperature: 30 °C
Detection: UV, 226 nm
Inj. Volume: 5 µL

20396

Determination of Four Active Ingredients in a Multisymptom Cold Remedy Using Acclaim PA



Column: Acclaim PA, 3 µm
Dimensions: 4.6 × 150 mm
Injection: 10 µL
Mobile Phase: (A) Acetonitrile
(B) 10 mM phosphoric acid adjusted to pH 3.1 with ammonium hydroxide
Temperature: 30 °C
Detection: UV, 210 nm

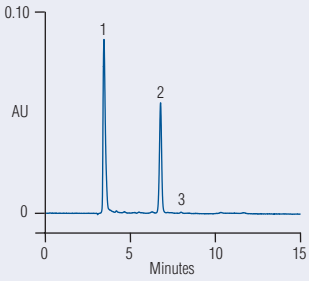
Gradient :

Time	*% A*	*% B*	*Flow*
0	8	92	1.0
2	8	92	
10	20	80	
17	50	50	

Peak #	Compound	Label Concentration	% Recovery
1	Pseudoephedrine HCl	2.0 mg/mL	96
2	Acetaminophen	33	100
3	Doxylamine succinate	0.41	90
4	Dextromethorphan HBr	1.0	95

20362

Bromide and Organic Acids in Cough Syrup Using the Acclaim OA



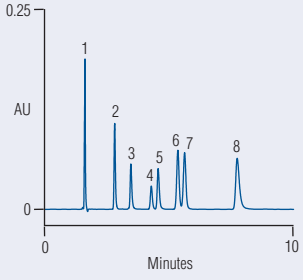
Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample Prep: OnGuard II P
Sample: NyQuil®

Peaks:
1. Bromide (Dextromethorphan HBr)
2. Citrate (Inactive ingredient)
3. Succinate (Doxylamine succinate)

NyQuil is a registered trademark of Proctor and Gamble.

20025

Isocratic Separation of Six Cardiac Antiarrhythmic Drugs (Beta-Blockers) on Acclaim 120 C8

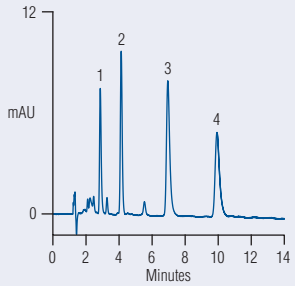


Column: Acclaim 120 C8, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 51/49 w/w MeOH/25 mM phosphate, pH 7.0
Flow Rate: 1.0 mL/min
Temperature: 40 °C
Inj. Volume: 5 µL
Detection: UV, 214 nm

Peaks:		
1.	Maleic acid	–
2.	Acebutolol	50 µg/mL
3.	Metoprolol	50
4.	Timolol	100
5.	Oxprenolol	50
6, 7.	Labetalol diasteromers	50
8.	Propranolol	20

20397

Isocratic Resolution of Antihistamines and Their Impurities on Acclaim 120 C18

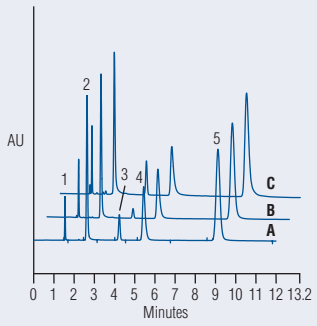


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 50 mM sodium acetate
(B) Methanol
Isocratic: (A) 20%, (B) 80%
Temperature: 25 °C
Detection: UV, 249 nm

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

17510

Separation of Basic Drugs on Acclaim 120 C18



Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 80/20 methanol/ 30 mM phosphate, pH 6
Temperature : 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 220 nm

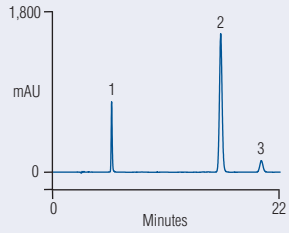
Amitriptyline Mass:
Trace A: 1200 ng
Trace B: 400 ng (normalized peak height)
Trace C: 94 ng (normalized peak height)

Peaks:
1. Uracil
2. Propranolol
3. Toluene
4. Doxepin
5. Amitriptyline

Note: The high performance of this column is maintained as the sample is diluted.

18357

Active Ingredients in Sunscreen Lotion on Acclaim 120 C18 Using ASE Extraction

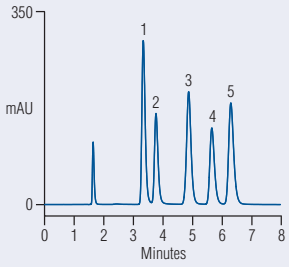


Extraction Conditions:
ASE 200 with 11 mL extraction cell
50/50 MeOH/CH_2Cl_2
Column: Acclaim 120 C18 column
Mobile Phase: Isocratic 85/14/0.75 methanol/water/HOAc
Inj. Volume: 20 µL
Detection: UV, 310 nm

Peaks:
1. Benzophenone-3
2. Octyl methoxycinnamate
3. Octyl salicylate

20398

Separation of Five Antidepressants on Acclaim PA

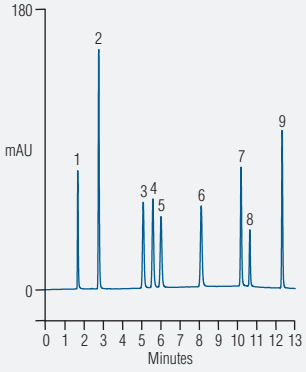


Column: Acclaim PA, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 80/20 v/v MeOH/ 30 mM phosphate, pH 6.0
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 220 nm

Peaks:		
1. Protriptyline	50
2. Nortriptyline	25
3. Doxepin	50
4. Imipramine	40
5. Amitriptyline	50

19299

Gradient Resolution of Sulfa Drugs on Acclaim PA

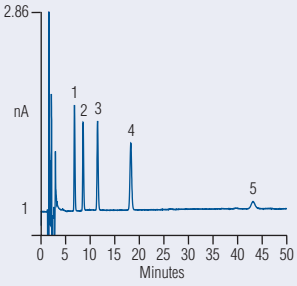


Column: Acclaim PA, 3 µm
Dimensions 4.6 × 150 mm
Mobile Phase: (A) 90/10 methanol/ 20 mM phosphate, pH 2.7
(B) 10/90 methanol/ 20 mM phosphate, pH 2.7
Inj. Volume: 10 µL
Gradient: (A) 15–30% at 5 min, to 75% at 10 min, to 85% at 12 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Detection: UV, 254 nm

Peaks:	
1. Sulfanilic acid	6 µg
2. Sulfanilamide	4
3. Sulfadiazine	10
4. Sulfathiazole	10
5. Sulfamerazine	10
6. Sulfamethazine	10
7. Sulfamethoxazole	14
8. Sulfisoxazole	12
9. Sulfadimethoxine	18

19300

Baseline Separation of Catecholamines and Serotonin at Physiological Concentrations on Acclaim 120 C18 with Electrochemical Detection

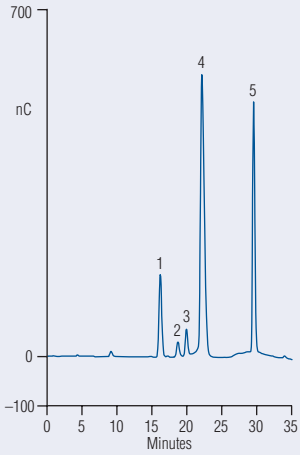


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 57 mM citric acid, 43 mM sodium acetate, 0.1 mM EDTA, 1 mM sodium octanesulfonate, 10% methanol
Flow Rate: 1 mL/min
Detection: dc amperometry, Glassy Carbon at 700 mV, Ag/AgCl Referemce electrode

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

18358

Spectinomycin and Lincomycin on Acclaim PA with Electrochemical Detection



Column: Acclaim PA, 3 µm
Dimensions: 2.1 × 150 mm
Mobile Phase (A) 10 mM acetic acid, 3.3 g/L pentanesulfonic acid, pH 4.0 with NaOH
(B) 10 mM acetic acid, 0.55 g/L pentanesulfonic acid, pH 4.0 with NaOH
(C) Acetonitrile

Gradient:

Time	*% A*	*% B*	*% C*
0.0	70	30	0
10.0	70	30	0
10.1	0	100	0
20.0	0	100	0
23.0	0	75	25
37.0	0	75	25

Flow Rate: 0.30 mL/min
Temperature: 40 °C
Inj. Volume: 10 µL
Detection: Postcolumn addition of 0.5 M NaOH at 0.12 mL/min; pulsed amperometric detection

Peaks:
1. Benzyl alcohol
2. Spectinomycin anomer 1
3. Spectinomycin anomer 2
4. Spectinomycin
5. Lincomycin

20384

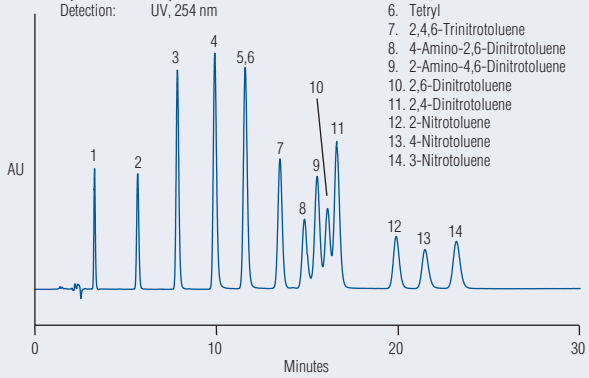
APPLICATIONS

Environmental

Explosives by EPA Method 8330 on Acclaim 120 C18

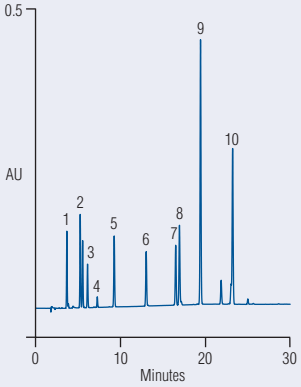
Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 250 mm
Mobile Phase: 51/49 v/v MeOH/H_2O
Temperature: 25 °C
Flow Rate: 1.25 mL/min
Inj. Volume: 10 µL
Detection: UV, 254 nm

Peaks: (10 µg/mL each)
1. HMX
2. RDX
3. 1,3,5-Trinitrotoluene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 4-Amino-2,6-Dinitrotoluene
9. 2-Amino-4,6-Dinitrotoluene
10. 2,6-Dinitrotoluene
11. 2,4-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene



20113a

Carbamate Insecticide Standards in EPA 531.1 on Acclaim PA



Column: Acclaim PA, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) D. I. H_2O
(B) CH_3CN

Gradient:

Time (min)	*% B*
0	10
30	70

Temperature: 25 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks: (100 µg/mL each)
1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxycarbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. Methiocarb

20105

Carbamate Insecticides in EPA 531.1 on Acclaim 120 C8 with MS Detection

Background Information:
LC/MS of 12 insecticides, 1 ng each on column. Overlay of 12 simultaneous single-ion chromatograms.

Column: Acclaim 120 C8, 5 µm, 2.1 × 100 mm
Mobile Phase: (A) MeOH
(B) 20 mM NH_4HCO_2, pH 3.4

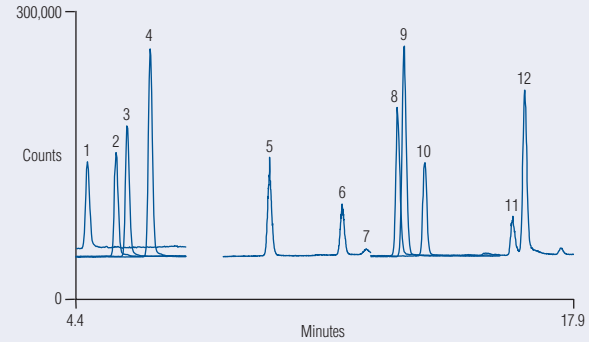
Gradient:

Time	*%A*	*%B*	*Flow*
0	12	88	0.25
1	12	88	
15	70	30	
17	70	30	
18	100	0	

Inj. Volume: 1–50 µL
Sample: 100 ppm stock diluted 1000× in Eluent B

Detection: MSQ™ MS +ESI
MS Settings: Probe 400 °C
Needle 2.0 kV
Cone voltage set per ion

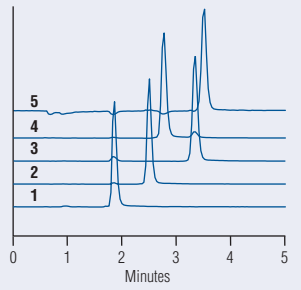
Peaks:	*m/z*
1. Aldicarb sulfoxide	207
2. Aldicarb sulfone	240
3. Oxamyl	237
4. Methomyl	163
5. 3-Hydroxy carbofuran	220
6. Aldicarb	208
7. Unknown	208
8. Propoxur	168
9. Carbofuran	222
10. Carbaryl	145
11. Unknown	169
12. Methiocarb	169



Note: MSQ is a trademark of Thermo Electron Corporation

20399

LC/MS Detection of Herbicides on Acclaim 120 C18



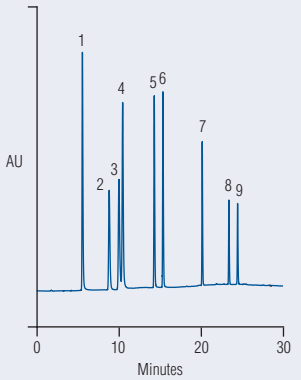
Column: Acclaim 120 C18, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phase: 70/30% methanol/ 0.1% formic acid
Flow Rate: 0.25 mL/min
Detection: AQA™ MS, +ESI, 30 V, 200 °C, SIM as indicated

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

Note: AQA is a trademark of Thermo Electron Corporation

17922

LC Separation of Nitrosamines in EPA 8270 Using Acclaim PA



Column: Acclaim PA, 5 µm
Dimensions: 4.6 × 250 mm
Mobile Phase: (A) D.I. H_2O
(B) Acetonitrile

Gradient:

Time (min)	*% B*
0	1
5	1
25	90
30	90

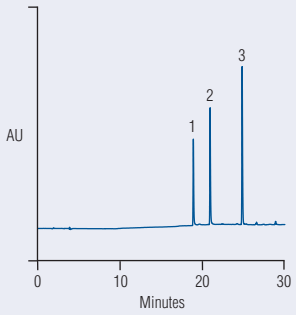
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 15 µL
Detection: UV, 230 nm

Peaks: (20 µg/mL)
1. *N*-Nitroso-dimethylamine
2. *N*-Nitroso-morpholine
3. *N*-Nitroso-methylethylamine
4. *N*-Nitroso-pyrrolidine
5. *N*-Nitroso-diethylamine
6. *N*-Nitroso-piperidine
7. *N*-Nitroso-dipropylamine
8. *N*-Nitroso-dibutylamine
9. *N*-Nitroso-diphenylamine

Note: This gradient will also resolve benzidines.

20109a

LC Separation of Benzidines in EPA 8270 Using Acclaim PA



Column: Acclaim PA, 5 µm
Dimensions: 4.6 × 250 mm
Mobile Phase: (A) D.I. H_2O
(B) Acetonitrile

Gradient:

Time (min)	*% B*
0	1
5	1
25	90
30	90

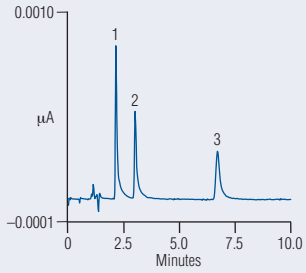
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 15 µL
Detection: UV, 230 nm

Peaks: (20 µg/mL)
1. Benzidine
2. 3.3'-Dimethylbenzidine
3. 3.3'-Dichlorobenzidine

Note: This gradient will also resolve *N*-nitrosamines

20109b

Benzidines by EPA Method 605 on Acclaim 120 C18 with Electrochemical Detection



Column: Acclaim 120 C18, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phase: 50/50 acetonitrile/
100 mM NaOAc, pH 4.7
Flow Rate: 0.17 mL/min
Inj. Volume: 1 µL
Detection: Glassy carbon electrode, 800 mV
(1 µg/mL)

Peaks
1. Benzidine
2. 3.3'-Dimethylbenzidine
3. 3.3'-Dichlorobenzidine

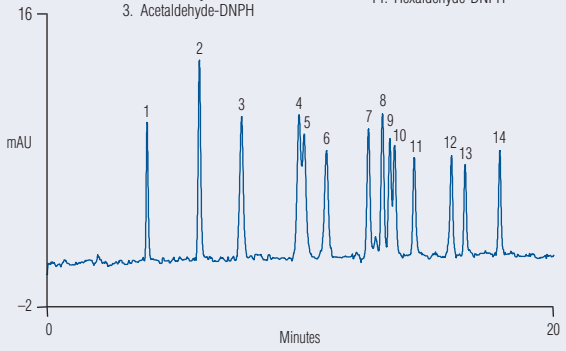
Note: Scaling down the column size and flow rate produces a superproportional increase in sensitivity in the electrochemical detector.

20400

Thirteen Carbonyl-DNPH Derivatives by CARB Method 1004 on Acclaim 120 C18

Column: Acclaim 120 C18, 5 µm
Dimensions: 2.1 × 250 mm
Mobile Phase: 40% acetonitrile at 0–7 min,
gradient to 100% acetonitrile at 20 min
Flow Rate: 0.25 mL/min
Inj. Volume: 10 µL
Detection: UV, 360 nm

Peaks (DNPH and 13 carbonyl derivatives) 0.3 µg/mL:
1. 2,4-DNPH
2. Formaldehyde-DNPH
3. Acetaldehyde-DNPH
4. Acrolein-DNPH
5. Acetone-DNPH
6. Propionaldehyde-DNPH
7. Crotonaldehyde-DNPH
8. Methacrolein-DNPH
9. 2-Butanone-DNPH
10. Butyraldehyde-DNPH
11. Benzaldehyde-DNPH
12. Valeraldehyde-DNPH
13. *m*-Tolualdehyde-DNPH
14. Hexaldehyde-DNPH



Note: CARB = California Air Resource Board

20111

Improved Separation of 13 Carbonyl-DNPH Derivatives by CARB Method 1004 Using Coupled Acclaim PA and Acclaim 120 C18 Columns

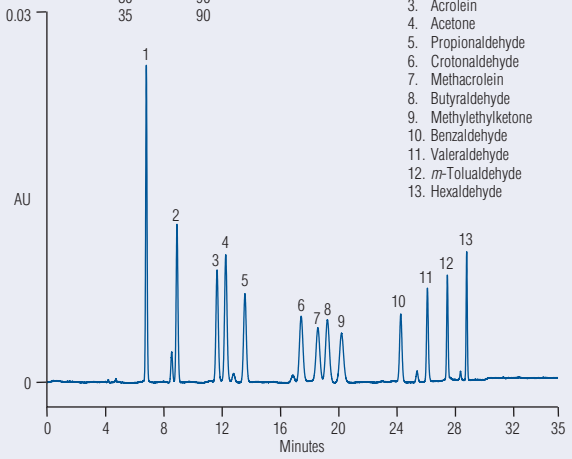
Columns: Acclaim PA, 5 µm, 4.6 × 150 mm, coupled with Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Mobile Phase: (A) H_2O
(B) CH_3CN

Gradient:

Time (min)	*% B*
0	54
20	54
30	90
35	90

Temperature: 30 °C
Flow Rate: 1.5 mL/min
Inj. Volume: 10 µL
Detection: UV, 360 nm

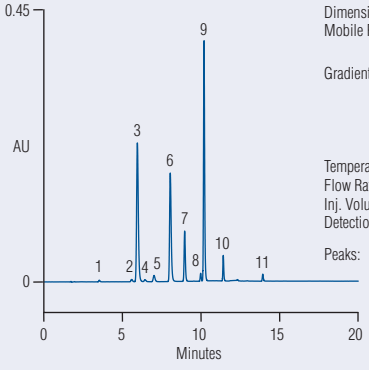
Peaks: (0.3 µg/mL each)
DNPH derivatives of
1. Formaldehyde
2. Acetaldehyde
3. Acrolein
4. Acetone
5. Propionaldehyde
6. Crotonaldehyde
7. Methacrolein
8. Butyraldehyde
9. Methylethylketone
10. Benzaldehyde
11. Valeraldehyde
12. *m*-Tolualdehyde
13. Hexaldehyde



Notes: (1) No conventional C18 column can produce equivalent resolution.
(2) CARB = California Air Resource Board

20112

Phenol Standards by EPA Method 604 on Acclaim PA



Column: Acclaim PA, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 2.5 mM MSA in H_2O
(B) Methanol

Gradient:

Time (min)	*% B*
0	60
4.5	60
10.5	95
20	95

Temperature: 25 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 285 nm

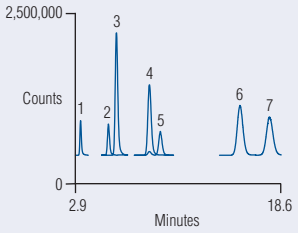
Peaks: (50– 250 µg/mL each)
1. Phenol
2. 2-Chlorophenol
3. 4-Nitrophenol
4. 2-Nitrophenol
5. 2,4-Dimethylphenol
6. 4-Chloro-3-methylphenol
7. 2,4-Dichlorophenol
8. 2,4-Dinitrophenol
9. 2-Methyl-4,6-dichlorophenol
10. 2,4,6-Trichlorophenol
11. Pentachlorophenol

Note: The lower pH provided by methanesulfonic acid (compared to acetic acid in the standard method) gives optimum separation for the Acclaim PA column.

20106

APPLICATIONS

Sensitive Detection of Triazine Herbicides on Acclaim 120 C18 with MS Detection



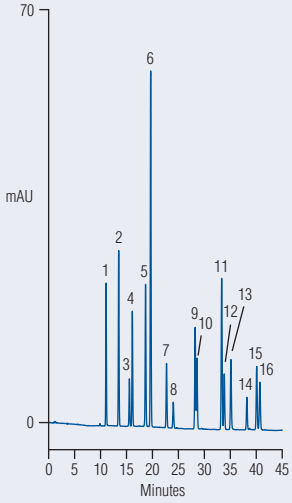
Background Information: Seven herbicides at 1 ng on column. Seven simultaneous single-ion chromatograms optimized for positive identification.

Column:	Acclaim 120 C18, 5 µm
Dimensions:	2.1 × 100 mm
Mobile Phase:	35% Acetonitrile 65% Ammonium acetate, pH 5
Temperature:	30 °C
Flow Rate:	0.25 mL/min
Inj. Volume:	10 µL
Detection:	MSQ MS
ESI:	Positive
Probe:	400 °C
Needle:	2.5 kV
Cone Voltage:	Set per ion

Peaks	*m/z*
1. Simazine	202
2. Atrazine	216
3. Prometon	226
4. Ametryn	228
5. Propazine	230
6. Prometryn	242
7. Terbutryn	242

20401

Polynuclear Aromatic Hydrocarbons by EPA Method 8310 on Acclaim PA

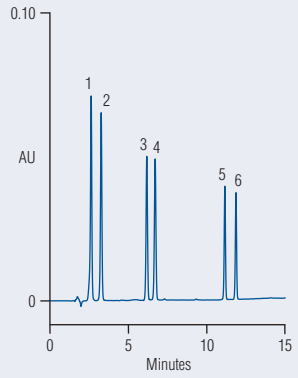


Column:	Acclaim PA, 5 µm
Dimensions:	4.6 × 250 mm
Mobile phase:	(A) CH_3CN (B) D.I. H_2O
Gradient:	Hold A/B (50/50) for 5 min, A/B (50/50) to A/B (85/15) in 35 min, Hold A/B (85/15) for 5 min
Temperature:	25 °C
Flow Rate:	2 mL/min
Inj. Volume:	10 µL
Detection:	UV, 254 nm

Peaks:
1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a] anthracene
10. Chysene
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

19305

Separation of Phthalate Esters in EPA Method 606 Using Acclaim PA



Column:	Acclaim PA, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) D.I. H_2O (B) MeOH

Gradient:	*Time (min)*	*% B*
	0	25
	10	100
	15	100

Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	UV, 254 nm

Peaks: (100 µg/mL each)
1. Dimethyl phthalate
2. Diethyl phthalate
3. Bis(2-ethylhexyl) phthalate
4. Di-n-butyl phthalate
5. Di-n-octyl phthalate
6. Butyl benzyl phthalate

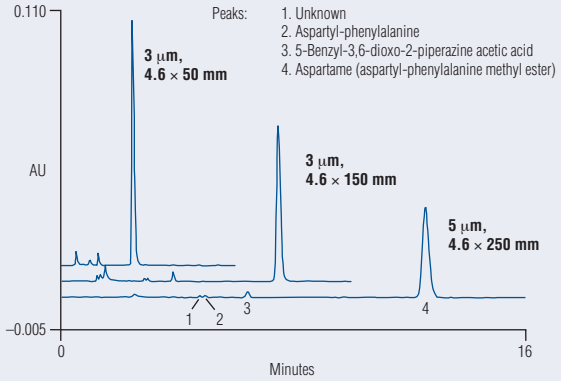
20107

Food and Beverage

Assay of Aspartame and Its Impurities on Three Formats of Acclaim 120 C18 Columns

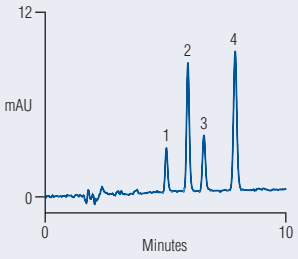
Columns: Acclaim 120 C18
Mobile Phase: 67/33 NH_4HCO_2, pH 3.7/ MeOH
Flow Rate: 1.0 mL/min
Inj. Volume: 25 µL
Detection: UV, 254 nm
Sample: 1000 ppm commercial-grade aspartame

Peaks:
1. Unknown
2. Aspartyl-phenylalanine
3. 5-Benzyl-3,6-dioxo-2-piperazine acetic acid
4. Aspartame (aspartyl-phenylalanine methyl ester)



20402

Four Aflatoxin Standards on Acclaim 120 C18

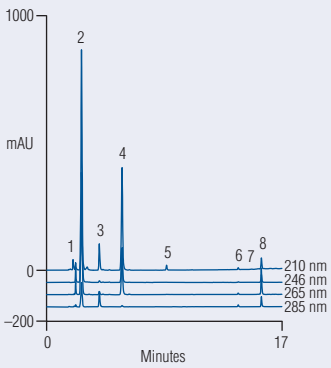


Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: Isocratic, 22.5/22.5/55 methanol/acetonitrile/water
Flow Rate: 1.0 mL/min
Temperature: 30 °C
Inj. Volume: 50 µL
Detection: UV, 360 nm

Peaks:		
1. G2	7.5 ng
2. G1	25
3. B2	7.5
4. B1	25

20403

Assay for Water-Soluble Vitamins in Vitamin Tablets Using Acclaim PA



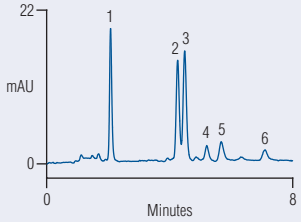
Column: Acclaim PA, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: Phosphate pH 3.4, gradient 0–20% CH_3CN
Detection: UVD 340 at listed wavelengths
Sample: One-A-Day® brand "Active Formula"
Sample Prep.: 1. Grind a 1500 mg tablet
2. Extract 150 mg with 100 mL of pH 3.4 buffer
3. Sonicate 10 min
4. Filter
5. Inject 20 µL

Peaks:
1. Thiamine
2. Ascorbic acid
3. Pyridoxine
4. Niacinamide
5. Pantothenic acid
6. Folic acid
7. Vitamin B_{12}
8. Riboflavin

Note: One-A-Day is a registered trademark of Bayer Corp.

19350

Fat-Soluble Vitamin Standards Using Acclaim PA

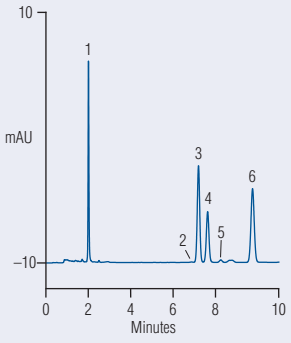


Column: Acclaim PA, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phase: 97/3 acetonitrile/NH_4HCO_2, pH 3.4
Flow Rate 0.25 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: UV, 385 nm

Peaks:
1. Vitamin A acetate
2. Vitamin D_2
3. Vitamin D_3
4. Vitamin E acetate
5. Vitamin E
6. Vitamin K_1

19302

Fat-Soluble Vitamin Standards on Acclaim 120 C18



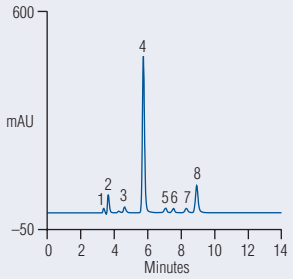
Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 95/5 v/v acetonitrile/methanol
Flow Rate: 2.0 mL/min
Temp.: 30 °C
Inj. Volume: 5 µL
Detection: UV, 280 nm

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

APPLICATIONS

Amino Acids in Vitamin Premix on Acclaim OA

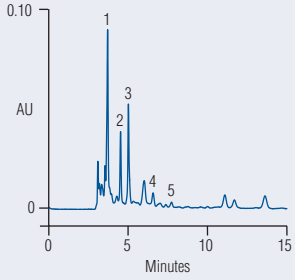


Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 40 mM Na_2SO_4 adjusted to pH 3.05 with methanesulfonic acid
Flow Rate: 0.60 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks:
1. Lysine
2. Glutamine
3. Valine
4. Ascorbic acid
5. Isoleucine
6. Leucine
7. Unknown
8. Unknown

20412

Organic Acids in White Wine on Acclaim OA



Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample prep: OnGuard II P

Peaks:
1. Tartaric acid
2. Malic acid
3. Lactic acid
4. Citric acid
5. Succinic acid

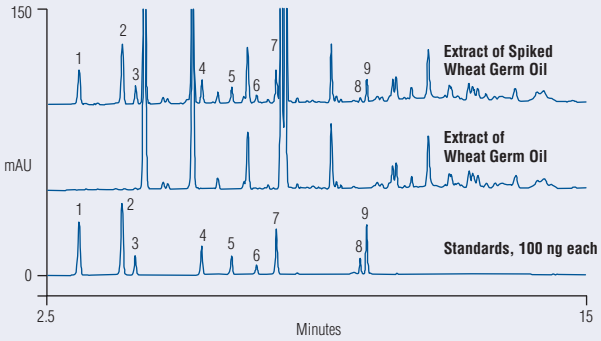
20024

Antioxidants in Edible Oils by AOAC 983.15 on Acclaim 120 C18

Background Information: Nine antioxidants for AOAC 983.15 spiked into wheat germ oil.
Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 0.75% HOAc in H_2O
(B) 50/50/0.5 MeOH/acetonitrile/HOAc
Inj. Volume: 10 µL
Detection: UV, 280 nm; diode array confirmation
Gradient:

Time	*% A*	*% B*	*Flow*
0.0	30	70	2.0
9.6	0	100	2.0
11.4	0	100	2.0
12.0	0	100	4.0
17.0	0	100	4.0

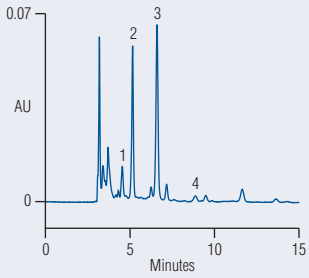
Peaks:
1. Propyl gallate
2. THBP
3. TBHQ
4. NDGA
5. BHA
6. Ionox-100
7. Octyl gallate
8. BHT
9. Dodecyl gallate



Note: Ethoxyquin (not shown) has been resolved under the same conditions.

20404

Organic Acids in Orange Juice on Acclaim OA



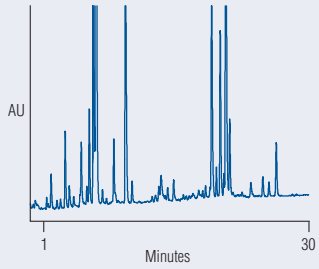
Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample Prep.: OnGuard II P, diluted 2× with D.I. H_2O

Peaks:
1. Malic acid
2. Ascorbic acid (Vitamin C)
3. Citric acid
4. Fumaric acid

20023

Proteins and Peptides

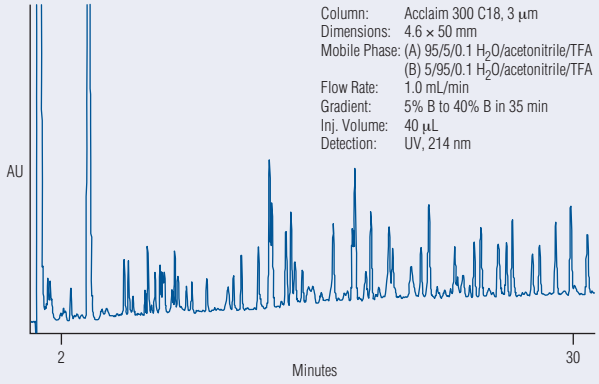
Tryptic Peptide Map of Cytochrome c on Acclaim 300 C18



Column: Acclaim 300 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 95/5/0.1 H_2O/acetonitrile/TFA
(B) 5/95/0.1 H_2O/acetonitrile/TFA
Flow Rate: 1.0 mL/min
Gradient: 15% B to 50% B in 35 min
Inj. Volume: 40 µL
Detection: UV, 214 nm

20405

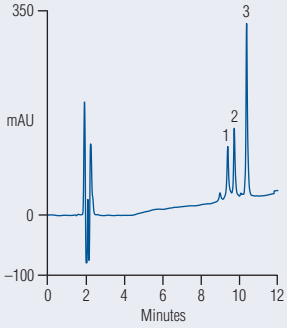
Tryptic Peptide Map of Bovine Serum Albumin on Acclaim 300 C18



Column: Acclaim 300 C18, 3 µm
Dimensions: 4.6 × 50 mm
Mobile Phase: (A) 95/5/0.1 H_2O/acetonitrile/TFA
(B) 5/95/0.1 H_2O/acetonitrile/TFA
Flow Rate: 1.0 mL/min
Gradient: 5% B to 40% B in 35 min
Inj. Volume: 40 µL
Detection: UV, 214 nm

20406

Cytochrome c from Three Species on Acclaim 300 C18



Column: Acclaim 300 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 95/5/0.1 v/v water/acetonitrile/TFA
(B) 95/5/0.1 v/v acetonitrile/water/TFA

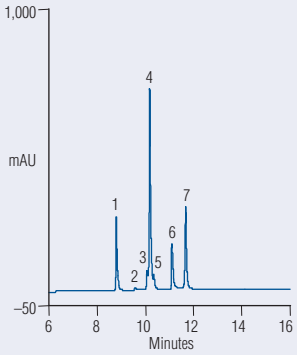
Gradient:	*Time (min)*	*% A*	*% B*
	–5	80	20
	0	80	20
	15	45	55
	20	45	55

Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL (1 µg/µL)
Detection: UV, 214 nm

Peaks:
1. Yeast cytochrome c
2. Equine cytochrome c
3. Bovine cytochrome c

19104

Protein Mixture by Reversed-Phase HPLC on Acclaim 300 C18



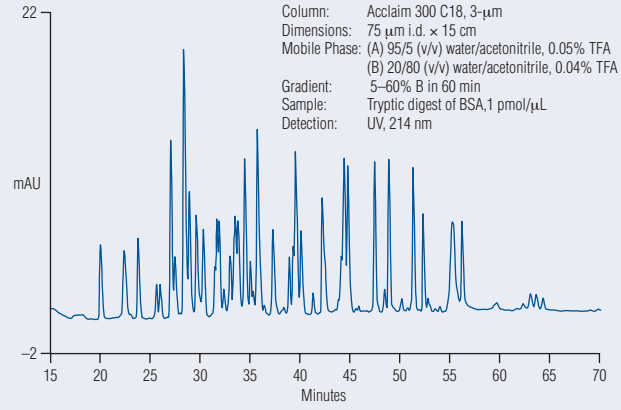
Column: Acclaim 300 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 5 mM MSA in 95/5 H_2O/acetonitrile
(B) 5 mM MSA in 5/95 H_2O/acetonitrile
Inj. Volume: 25 µL
Detection: UV, 214 nm

Gradient:	*Time*	*% A*	*% B*	*Flow*
	0	100	0	1.0
	3	100	0	
	18	0	100	
	23	0	100	

Peaks:
1. RNAse A
2. Impurity in carbonic anyhdrase
3. Impurity in lysozyme
4. Lysozyme
5. Impurity in carbonic anhydrase
6. Myoglobin
7. Carbonic anhydrase

20407

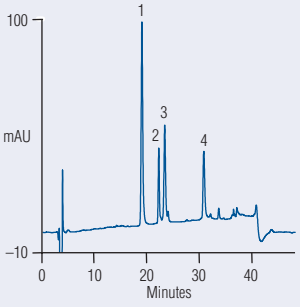
Capillary LC for Peptide Mapping Using Acclaim 300 C18



Column: Acclaim 300 C18, 3-µm
Dimensions: 75 µm i.d. × 15 cm
Mobile Phase: (A) 95/5 (v/v) water/acetonitrile, 0.05% TFA
(B) 20/80 (v/v) water/acetonitrile, 0.04% TFA
Gradient: 5–60% B in 60 min
Sample: Tryptic digest of BSA,1 pmol/µL
Detection: UV, 214 nm

20408

Separation of Protein Standards by Micro LC on Acclaim 300 C18



Column: Acclaim 300 C18, 3 µm
Dimensions: 300-µm i.d. × 15 cm
Mobile Phase: (A) Water. 0.05% TFA
(B) 8/2 CH_3CN/ water, 0.04 % TFA
Gradient: 15–60% B in 30 min

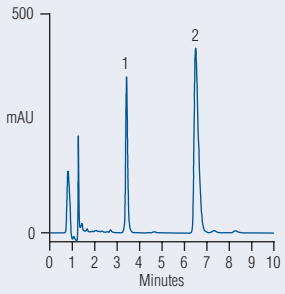
Flow Rate: 4 µL/min
Detection: UV, 214 nm

Peaks:		
1. Ribonuclease	25 ng
2. Insulin	25
3. Cytochrome c	25
4. Myoglobin	25

20409

Natural Products

Alkaloids in Goldenseal Root on Acclaim 120 C18 with ASE Extraction

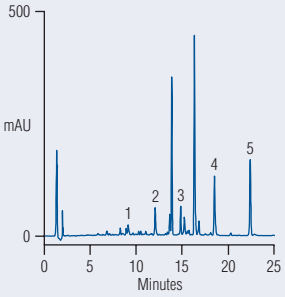


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 73/27 0.1% H_3PO_4 in D.I. H_2O/acetonitrile
Flow Rate: 1.8 mL/min
Inj. Volume: 10 µL
Detection: UV, 235 nm

Peaks:
1. Hydrastine
2. Berberine

17630

Isoflavones in Red Clover on Acclaim 120 C18 with ASE Extraction

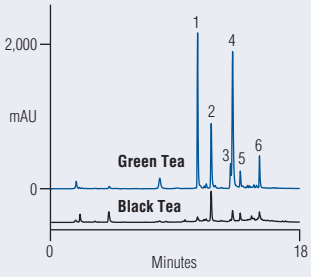


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 0.1% H_3PO_4 in D.I. H_2O
(B) Acetonitrile
Gradient: 10% B to 76% B over 35 min
Flow rate: 1.2 mL/min
Inj. Volume: 10 µL
Detection: UV, 260 nm

Peaks:
1. Genistin
2. Ononin
3. Sissotrin
4. Formononotin
5. Biochanin A

17629

Flavonoids in Green and Black Tea Using Acclaim 120 C18



Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 10 mM H_3PO_4
Temperature: 40 °C
Gradient:

Time	*% A*	*% B*	*Flow*
0	5	95	1.00
5	5	95	
8	15	85	
10	15	85	
15	40	60	
25	60	40	

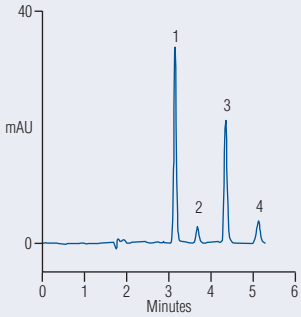
Inj. Volume: 5 µL
Detection: UV 210, with PDA spectra

Peaks:
1. Epigallocatechin
2. Caffeine
3. Epicatechin
4. Epigallocatechin gallate
5. Unidentified flavonoid
6. Epicatechin gallate

20410

Dyes

Sudan Dyes on Acclaim 120 C18



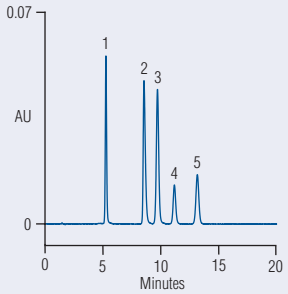
Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 50/50/0.5 MeOH/acetonitrile/HOAc
Flow Rate: 1.0 mL/min
Inj. Volume: 20 µL
Detection: UV, 280 nm

Peaks:
1. Sudan I
2. Sudan I, impurity
3. Sudan II
4. Sudan III

20411

Organic Acids

Resolution of Benzene Polycarboxylic Acids on Acclaim OA

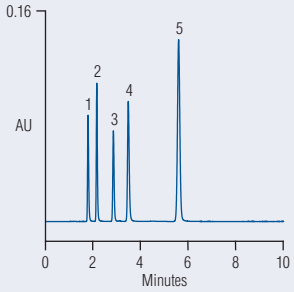


Column: Acclaim OA, 5 µm,
Dimensions: 4 × 150 mm
Mobile Phase: 12.5/87.5 v/v CH_3CN/ 5 mM methanesulfonic acid
Temperature: 30 °C
Flow Rate: 0.75 mL/min
Inj. Volume: 5 µL
Detection: UV, 230 nm

Peaks: (50 ppm each)
1. 1,2,3-Benzenetricarboxylic acid
2. 1,2-Benzenedicarboxylic acid
3. 1,3,5-Benzenetricarboxylic acid
4. 1,4-Benzenedicarboxylic acid
5. 1,3-Benzenedicarboxylic acid

20021

Separation of Hydroxybenzoic Acids on Acclaim OA

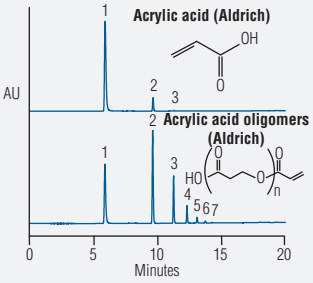


Column: Acclaim OA, 5 µm
Dimensions: 4 × 150 mm
Mobile Phase: 30/70 v/v CH_3CN/5 mM methanesulfonic acid
Temperature: 30 °C
Flow Rate: 0.75 mL/min
Inj. Volume: 5 µL
Detection: UV, 230 nm

Peaks: (80 ppm each)
1. 3,4,5-Trihydroxybenzoic acid
2. 3,5-Dihydroxybenzoic acid
3. 4-Hydroxybenzoic acid
4. 2,5-Dihydroxybenzoic acid
5. Benzoic acid

20018

Acrylic Acid Oligomers on Acclaim OA



Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: (A) CH_3CN
(B) 2.5 mM methanesulfonic acid
Gradient: Hold 5% A for 1 min, then increase to 90% A in 19 min
Temperature: 30 °C
Flow Rate: 0.75 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample prep.: Diluted 1000× with D.I. H_2O

Peaks: 1. Monomer
2. Dimer
3. Trimer
4. Tetramer
5. Pentamer
6. Hexamer
7. Heptamer

20029

APPLICATIONS

PART FOUR

Appendix

Analyte Index

APPENDIX

Topical Index

APPENDIX

Part Number Index

Part No.	Acclaim Type	Size	Main Page No.	Applications Page No.
059122	120, 3 µm, C8	2.1 × 50 mm	9	
059123	120, 3 µm, C8	2.1 × 100 mm	9	
059124	120, 3 µm, C8	2.1 × 150 mm	9	
059125	120, 3 µm, C8	4.6 × 50 mm	9	
029126	120, 3 µm, C8	4.6 × 100 mm	9	
059127	120, 3 µm, C8	4.6 × 150 mm	9	19
059128	120, 3 µm, C18	2.1 × 50 mm	7	
059129	120, 3 µm, C18	2.1 × 100 mm	7	21,22
059130	120, 3 µm, C18	2.1 × 150 mm	7	
059131	120, 3 µm, C18	4.6 × 50 mm	7	19,24
059132	120, 3 µm, C18	4.6 × 100 mm	7	
059133	120, 3 µm, C18	4.6 × 150 mm	7	24,27
059134	120, 5 µm, C8	2.1 × 50 mm	9	
059135	120, 5 µm, C8	2.1 × 100 mm	9	21
059136	120, 5 µm, C8	2.1 × 150 mm	9	
059137	120, 5 µm, C8	2.1 × 250 mm	9	
059138	120, 5 µm, C8	4.6 × 50 mm	9	
059139	120, 5 µm, C8	4.6 × 100 mm	9	
059140	120, 5 µm, C8	4.6 × 150 mm	9	
059141	120, 5 µm, C8	4.6 × 250 mm	9	
059142	120, 5 µm, C18	2.1 × 50 mm	7	
059143	120, 5 µm, C18	2.1 × 100 mm	7	23
059144	120, 5 µm, C18	2.1 × 150 mm	7	
059145	120, 5 µm, C18	2.1 × 250 mm	7	
059146	120, 5 µm, C18	4.6 × 50 mm	7	
059147	120, 5 µm, C18	4.6 × 100 mm	7	
059148	120, 5 µm, C18	4.6 × 150 mm	7	19,20,25,27
059149	120, 5 µm, C18	4.6 × 250 mm	7	21,22,24
059446	120, 5 µm, C18	4.3 × 10 mm	7	
059447	120, 5 µm, C18	2 × 10 mm	7	
059448	120, 5 µm, C8	4.3 × 10 mm	9	
059449	120, 5 µm, C8	2 × 10 mm	9	
059456	Guard Cartridge holder		7,9,11,15,17	
059457	Guard to analyical coupler		7,9,11,15,17	
059526	Guard holder & coupler kit		7,9,11,15,17	
060263	300, 3 µm, C18	2.1 × 50 mm	11	
060264	300, 3 µm, C18	2.1 × 150 mm	11	
060265	300, 3 µm, C18	4.6 × 50 mm	11	26
060266	300, 3 µm, C18	4.6 × 150 mm	11	26
060393	300, 3 µm, C18	4.3 × 10 mm	11	
060395	300, 3 µm, C18	2 × 10 mm	11	
061316	PA, 3 µm, C16	2.1 × 100 mm	15	24
061317	PA, 3 µm, C16	2.1 × 150 mm	15	20
061318	PA, 3 µm, C16	4.6 × 150 mm	15	19,20,22,24
061319	PA, 5 µm, C16	4.6 × 50 mm	15	
061320	PA, 5 µm, C16	4.6 × 150 mm	15	20,21,22,23
061321	PA, 5 µm, C16	4.6 × 250 mm	15	21,23
061331	PA, 5 µm, C16	2 × 10 mm	15	
061332	PA, 5 µm, C16	4.3 × 10 mm	15	
062902	OA, 5 µm	4.0 × 250 mm	17	19,25,28
062903	OA, 5 µm	4.0 × 150 mm	17	28
092925	OA, 5 µm	4.3 × 10 mm	17	
161450	120, 5 µm, C18	1.0 × 50 mm	7	
161451	120, 5 µm, C18	1.0 × 150 mm	7	
161452	120, 5 µm, C18	1.0 × 250 mm	7	
161453	120, 5 µm, C18	300 µm × 50 mm	7	
161454	120, 5 µm, C18	300 µm × 50 mm	7	
161455	120, 5 µm, C18	300 µm × 250 mm	7	
161456	120, 5 µm, C18	75 µm × 50 mm	7	
161457	120, 5 µm, C18	75 µm × 150 mm	7	
161458	120, 5 µm, C18	75 µm × 250 mm	7	
162204	120, 3 µm, C8	1.0 × 50 mm	9	
162205	120, 3 µm, C8	1.0 × 150 mm	9	
162206	120, 3 µm, C8	300 µm × 50 mm	9	

Part Number Index

Part No.	Acclaim Type	Size	Main Page No.	Applications Page No.
162207	120, 3 µm, C8	300 µm × 150 mm	9	
162208	120, 3 µm, C8	75 µm × 50 mm	9	
162209	120, 3 µm, C8	75 µm × 150 mm	9	
162210	120, 5 µm, C8	1.0 × 50 mm	9	
162211	120, 5 µm, C8	1.0 × 150 mm	9	
162212	120, 5 µm, C8	1.0 × 250 mm	9	
162213	120, 5 µm, C8	300 µm × 50 mm	9	
162214	120, 5 µm, C8	300 µm × 150 mm	9	
162215	120, 5 µm, C8	300 µm × 250 mm	9	
162216	120, 5 µm, C8	75 µm × 50 mm	9	
162217	120, 5 µm, C8	75 µm × 150 mm	9	
162218	120, 5 µm, C8	75 µm × 250 mm	9	
162219	300, 3 µm, C18	1.0 × 50 mm	11	
162220	300, 3 µm, C18	1.0 × 150 mm	11	
162221	300, 3 µm, C18	300 µm × 50 mm	11	
162222	300, 3 µm, C18	300 µm × 150 mm	11	26
162223	300, 3 µm, C18	75 µm × 50 mm	11	
162224	300, 3 µm, C18	75 µm × 150 mm	11	26
162234	120, 3 µm, C18	1.0 × 50 mm	7	
162235	120, 3 µm, C18	1.0 × 150 mm	7	
162236	120, 3 µm, C18	300 µm × 50 mm	7	
162237	120, 3 µm, C18	300 µm × 150 mm	7	
162238	120, 3 µm, C18	75 µm × 50 mm	7	
162239	120, 3 µm, C18	75 µm × 150 mm	7	
162240	PA, 3 µm, C16	1.0 × 50 mm	15	
162241	PA, 3 µm, C16	1.0 × 150 mm	15	
162242	PA, 3 µm, C16	300 µm × 50 mm	15	
162243	PA, 3 µm, C16	300 µm × 150 mm	15	
162244	PA, 3 µm, C16	75 µm × 50 mm	15	
162245	PA, 3 µm, C16	75 µm × 150 mm	15	
162246	PA, 5 µm, C16	1.0 × 50 mm	15	
162247	PA, 5 µm, C16	1.0 × 150 mm	15	
162248	PA, 5 µm, C16	1.0 × 250 mm	15	
162249	PA, 5 µm, C16	300 µm × 50 mm	15	
162250	PA, 5 µm, C16	300 µm × 150 mm	15	
162251	PA, 5 µm, C16	300 µm × 250 mm	15	
162252	PA, 5 µm, C16	75 µm × 50 mm	15	
162253	PA, 5 µm, C16	75 µm × 150 mm	15	
162254	PA, 5 µm, C16	75 µm × 50 mm	15	
162261	120, 5 µm, C8	1.0 × 5 mm	9	
162262	120, 5 µm, C8	1.0 × 15 mm	9	
162263	120, 5 µm, C8	800 µm × 5 mm	9	
162264	120, 5 µm, C8	500 µm × 5 mm	9	
162265	120, 5 µm, C8	500 µm × 15 mm	9	
162266	120, 5 µm, C8	300 µm × 5 mm	9	
162302	PA, 5 µm, C16	300 µm × 5 mm	15	
162304	120, 5 µm, C8	300 µm × 1.0 mm	9	
162306	120, 5 µm, C18	300 µm × 1.0 mm	7	
162310	PA, 5 µm, C16	300 µm × 1.0 mm	15	
162321	120, 5 µm, C18	1.0 × 5 mm	7	
162322	120, 5 µm, C18	1.0 × 15 mm	7	
162323	120, 5 µm, C18	800 µm × 5 mm	7	
162324	120, 5 µm, C18	500 µm × 5 mm	7	
162325	120, 5 µm, C18	500 µm × 15 mm	7	
162326	120, 5 µm, C18	300 µm × 5 mm	7	
162333	PA, 5 µm, C16	1.0 × 5 mm	15	
162334	PA, 5 µm, C16	1.0 × 15 mm	15	
162335	PA, 5 µm, C16	800 µm × 5 mm	15	
162336	PA, 5 µm, C16	500 µm × 5 mm	15	
132337	PA, 5 µm, C16	500 µm × 15 mm	15	

Lot Qualification Tests for Acclaim Bonded Silica

Polar Selectivity

This parameter is used to monitor batch-to-batch reproducibility as measured by the selectivity of a polar analyte (dimethylphthalate) relative to a nonpolar analyte (phenanthrene). This test protocol is part of the column performance test; column pressure, retention time, efficiency, and asymmetry for phenanthrene are specified for each column type.

Mobile phase: 70/30 v/v acetonitrile/water
Flow rate: 1.0 mL/min
Temperature: 30 °C
Inj. volume: 5 µL
Detection: UV, 254 nm, 6-mm path length
Peaks: 1. Uracil, 0.015 mg/mL
2. Dimethylphthalate, 0.075 µL/mL
3. Phenanthrene, 0.015 mg/mL

Metal Activity

2,2′-Bipyridyl chelates with metals whereas 4,4′-bipyridyl does not form chelation complexes. If metals are present, the 2,2′ isomer elutes with a high asymmetry factor (tailing). The peak asymmetry ratio for the two analytes should be approximately 1.0 if no metal contamination is present.

Mobile phase: 50/50 v/v methanol/water
Flow rate: 1.0 mL/min
Temperature: 30 °C
Inj. volume: 5 µL
Detection: UV, 254 nm
Peaks: 1. 4,4′-Bipyridyl, 50 ng/µL
2. 2,2′-Bipyridyl, 200 ng/µL

Base Asymmetry

Basic compounds exhibit poor peak shape when the silica surface is incompletely covered during the bonding process, or when the silica substrate is inadequately prepared. This test monitors surface coverage with an application-based analysis.

Mobile phase: 80/20 v/v methanol/30 mM phosphate, pH 6.0
Flow rate: 1.0 mL/min
Temperature: 30 °C
Inj. volume: 5 µL
Detection: UV, 220 nm
Peaks: 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

Hydrophobic Steric Selectivity

This parameter is used to monitor batch-to-batch bonding reproducibility as measured by the relative selectivity of two differently shaped aromatic analytes: *o*-terphenyl and triphenylene. This test is used to characterize all the C18 and polar-embedded phases.

Mobile phase: 90/10 v/v methanol/water
Flow rate: 1.0 mL/min
Temperature: 30 °C
Inj. volume: 5 µL
Detection: UV, 254 nm
Peaks: 1. Toluene, 2 µl/mL
2. Phenanthrene, 0.03 mg/mL
3. *o*-terphenyl, 0.14 mg/mL
4. Triphenylene, 0.03 mg/mL

Acid Asymmetry

This test is used to characterize polar-embedded phases. Acidic compounds exhibit poor peak shape when the bonded phase has basic sites due to degradation or contamination.

Mobile phase: 35/65 v/v acetonitrile/0.1% TFA in water
Flow rate: 1.0 mL/min
Temperature: 30 °C
Inj. volume 5 µL
Detection: UV, 240 nm
Peaks: 1. 4-hydroxybenzoic acid, 50 ng/µL
2. Benzoic acid, 70 ng/µL
3. Ethyl 4-hydroxybenzoate, 50 ng/µL

Some application-specific tests are used for lot validation. Read about these tests in the chapters for Acclaim 300 and Acclaim OA.

Peak asymmetries are calculated by the EP method at 5% of peak height (same as USP method). Theoretical plates are calculated from the peak width at half-height.

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