Acclaim®

Bonded Silica-Based Columns for HPLC

Pharmaceutical

Environmental

Food & Beverage

Chemical

Bioscience

Specialty Columns

DIONEX

Passion. Power. Productivity.

Pharmaceutical

Acclaim 120
Acclaim 300
Acclaim PA/PA2
Acclaim OA

Environmental

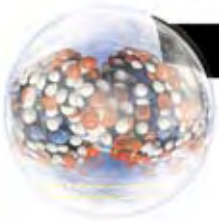
Acclaim 120
Acclaim PA
Acclaim Explosives

Food and Beverage

Acclaim OA
Acclaim PA/PA2
Acclaim 120

Chemical

Acclaim 120
Acclaim PA/PA2
Acclaim OA
Acclaim Surfactant



Bioscience

Acclaim 300
Acclaim 120
Acclaim PA2

Ordering and Warranty Information

In the U.S.

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

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) 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.

Outside the U.S.

Please contact your local Dionex representative.

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.

©2007 by Dionex Corporation
All rights reserved
LPN 1668-03

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

AQA and MSQ are trademarks of Thermo Electron Corporation.
One-A-Day is a registered trademark of Bayer Corporation.
NyQuil is a registered trademark of Proctor and Gamble.
All other trademarks and registered trademarks are property of Dionex Corporation.

CONTENTS

PART ONE

Introduction 4

Acclaim Column Familes at a Glance 4

Selecting an Acclaim Column 6

Understanding More about the Bonded Phase 8

Mobile Phase Considerations 9

Acclaim Quality 10

PART TWO

Chapter 1: Acclaim 120 C18 12

Chapter 2: Acclaim 120 C8 14

Chapter 3: Acclaim 300 C18 16

Chapter 4: Acclaim Polar Advantage (PA) 18

Chapter 5: Acclaim Polar Advantage II (PA2) 20

Chapter 6: Acclaim OA for Organic Acids 22

Chapter 7: Acclaim Surfactant 24

Chapter 8: Acclaim Explosives E1 and E2 26

PART THREE

Applications 28

Applications Contents 28

Pharmaceutical Applications 29

Environmental Applications 34

Food and Beverage Applications 37

Chemical (Surfactant) Applications 42

Bioscience Applications 46

Natural Products Applications 48

Organic Acid 49

PART FOUR

Appendix 50

Lot Qualification Tests 50

Topical Index 52

Part Number Index 53

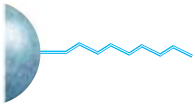
Analyte Index 54

Part I: INTRODUCTION

Acclaim Column Families at a Glance

Dionex Acclaim columns are silica-based columns designed for high efficiency separations and manufactured using ultra-high purity silica. Product families consists of a broad spectrum of stationary phases in the form of the conventional C8 and C18 phases. polar embedded phases. and several specialty phases, each designed for a specific application or group of applications. All Acclaim columns are designed and manufactured at Dionex, to tight specifications, to ensure consistent performance.

General Purpose HPLC Columns



Acclaim 120

High purity silica for small molecule separations

Acclaim 120 reversed-phase C18 and C8 phases are manufactured using high purity silica with a 120-Å pore diameter, very high surface coverage, low silanol activity, and very low metal content. All the phases are LC-MS compatible with good capacity ideal for use with high organic mobile phases.

Characteristics

High purity spherical silica, LC/MS compatible, monomeric bonding, endcapped, 120 Å pore size, 3 µm, 5 µm.

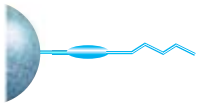
Acclaim 300

Wide pore columns for macromolecule applications

Acclaim 300 columns are ideal for the reversed-phase separation of proteins, peptides, and other biological macromolecules. The unique bonding chemistry results in a high-density, highly uniform phase coverage. The use of a 3-µm silica particle results in fast, high-resolution separations.

Characteristics

High purity spherical silica, LC/MS compatible, monomeric bonding, endcapped, 300 Å pore size, 3 µm, 5 µm.



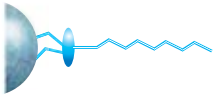
Acclaim PA

Polar-embedded Reversed-phase columns for separating a wide range of analytes

Acclaim PolarAdvantage (PA) columns feature a patented bonding chemistry that incorporates a polar sulfonamide group near the surface of the silica particle. This unique chemistry provides enhanced hydrolytic stability and allows a single column to resolve both polar and nonpolar analytes. The Acclaim PA is compatible with 100% aqueous mobile phases and exhibits high reversed-phase capacity.

Characteristics

High purity spherical silica, LC/MS compatible within guidelines, endcapped, 120 Å pore size, 3 µm, 5 µm.



Acclaim PA2

Polar-embedded columns with enhanced hydrolytic stability

Acclaim PolarAdvantage II (PA2) columns feature a patented bonding chemistry that provides enhanced hydrolytic stability from pH 1.5–10 and allows a single column to resolve both polar and nonpolar analytes. The Acclaim PA2 is compatible with 100% aqueous mobile phases and exhibits high reversed-phase capacity, with selectivity complementary to conventional C18 columns such as the Acclaim 120 C18.

Characteristics

High purity spherical silica, LC/MS compatible, endcapped, 120 Å pore size, 3 µm, 5 µm.

Acclaim General Purpose Column Specifications					
Dionex phase	Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim PA	Acclaim PA2
Bonded phase	C18	C8	C8	Sulfamido C16	Amide C18
USP type	L1	L7	L1	na	na
Endcapped	Yes	Yes	Yes	Yes	Yes
Substrate	Ultrapure silica	Ultrapure silica	Ultrapure silica	Ultrapure silica	Ultrapure silica
Particle shape	Spherical	Spherical	Spherical	Spherical	Spherical
Particle size	3 and 5 µm	3 and 5 µm	3 µm	3 and 5 µm	3 and 5 µm
Metal impurity (ppm) Na, Fe, Al	<10 ppm	<10 ppm	<10 ppm	<10 ppm	<10 ppm
Average pore diameter	120 Å	120 Å	300 Å	120 Å	120 Å
Surface area (m^2/g)	300	300	100	300	300
Total carbon content	18%	11%	7%	17%	17%

Specialty HPLC Columns

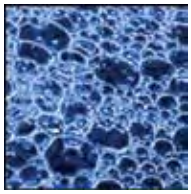
Acclaim OA

Specialty column for fast organic acid analysis

The Acclaim OA column features a patented polar-embedded stationary phase optimized and use tested for hydrophilic organic acid separations. The Acclaim OA stationary phase is compatible with 100% aqueous mobile phases and has excellent hydrolytic stability at low pHs.

Characteristics

High purity spherical silica, LC/MS compatible, monomeric bonding, endcapped, 120 Å pore size3 µm, 5 µm.



Acclaim Surfactant

Specialty column for the separation of surfactants

The Acclaim Surfactant column uses high purity 120-Å silica and a proprietary bonded phase to separate anionic, non-ionic, cationic and amphoteric surfactants in various matrices, including consumer products, pharmaceutical formulations and environmental samples.

Characteristics

High purity spherical silica, monomeric bonding, endcapped, 120 Å pore size.

Acclaim Explosives

Specialty columns for the separation of explosives.

A total solution to EPA Method 8330; two columns each of which provides guaranteed baseline resolution of all 14 explosives targeted by the EPA. With their complementary selectivities, the E1 and E2 provide both primary and confirmatory columns.

Characteristics

High purity spherical silica, LC/MS compatible, endcapped, 120 Å pore size

Acclaim Specialty Column Specifications				
Dionex phase	Acclaim OA	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2
Bonded phase	Proprietary	Proprietary	Proprietary	Proprietary
USP type	na	na	na	na
Endcapped	Yes	Yes	Yes	Yes
Substrate	Ultrapure silica	Ultrapure silica	Ultrapure silica	Ultrapure silica
Particle shape	Spherical	Spherical	Spherical	Spherical
Particle size	5 µm	5 µm	5 µm	5 µm
Metal impurity (ppm) Na, Fe, Al	<10 ppm	<10 ppm	<10 ppm	<10 ppm
Average pore diameter	120 Å	120 Å	120 Å	120 Å
Surface area (m^2/g)	300	300	300	300
Total carbon content	17%	na	na	na

Selecting an Acclaim Column

There are four questions to consider when choosing an HPLC column:

1. What are the goals of the separation?
   - Speed of analysis
   - Critical peaks requiring resolution
   - Solvent consumption/minimal waste
   - Sensitivity requirements
   - Operating costs
   - Other criteria
2. What are the best silica characteristics for this separation? This is explained in the section below.
3. What is the most appropriate column format? see page 8 for a summary of the impact of column dimensions on the separation
4. What is an appropriate stationary phase chemistry? see pages 4–5 for a summary of the Acclaim family of columns and Part 2 for detailed information.

Choosing the Best Column Chemistry

When a chromatographer designs a separation, 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. Determining the column with optimal selectivity is the essential starting point. This determination depends on the nature of the desired separation.

The easiest first step in choosing a column is to identify whether there is a column designed specifically for your separation. This can be readily established if the column is named according to its application, as in the case of some of the Dionex Acclaim columns. Thus, if you are separating surfactants, the Acclaim Surfactant column is the best first choice; likewise Acclaim Explosives column are used for EPA Method 8330, and Acclaim OA is for organic acid analysis.

If there is no specialty column for your application, then you will need some understanding of chemistry of your sample to choose the best column chemistry. For those cases where the nature of the sample is completely unknown, the best first choice is usually C18 because of its excellent peak efficiency, low silanol activity, and ability to separate many organic molecules. The C8 phase is a good choice if you want less retention of the sample on the column. If you need highly aqueous mobile phase conditions, or different selectivity, then the polar embedded phases of the Acclaim Polar Advantage are the best option.

For samples that are well characterized, various tests are useful to characterize the stationary phases and to compare different columns. The tests include studies of effects such as hydrophobic interactions, molecular shape, and ion-exchange interactions between the stationary phase and the sample analytes. A good, purely reversed-phase interaction will exhibit strong retention of strongly hydrophobic molecules and this will be the only retention mechanism. As the density of the bonded phase decreases, other retention mechanisms will come into play, such as ion exchange interactions between the sample and residual silanol groups on the surface of the silica gel, or sieving effects for similar sized molecules of different shapes. Thus, these studies can be used to choose a column with specific characteristics, or to find a new column with characteristics similar to an old favorite.

Hydrophobicity

The strength of the hydrophobic interaction between an analyte and the bonded silica is usually characterized by measuring the selectivity of ethylbenzene relative to toluene. Because of their high-density bonding, Acclaim C18 and C8 columns have near maximum hydrophobicity compared with other C18 and C8 columns, and therefore almost pure reversed-phase behavior.

Ion-Exchange Activity

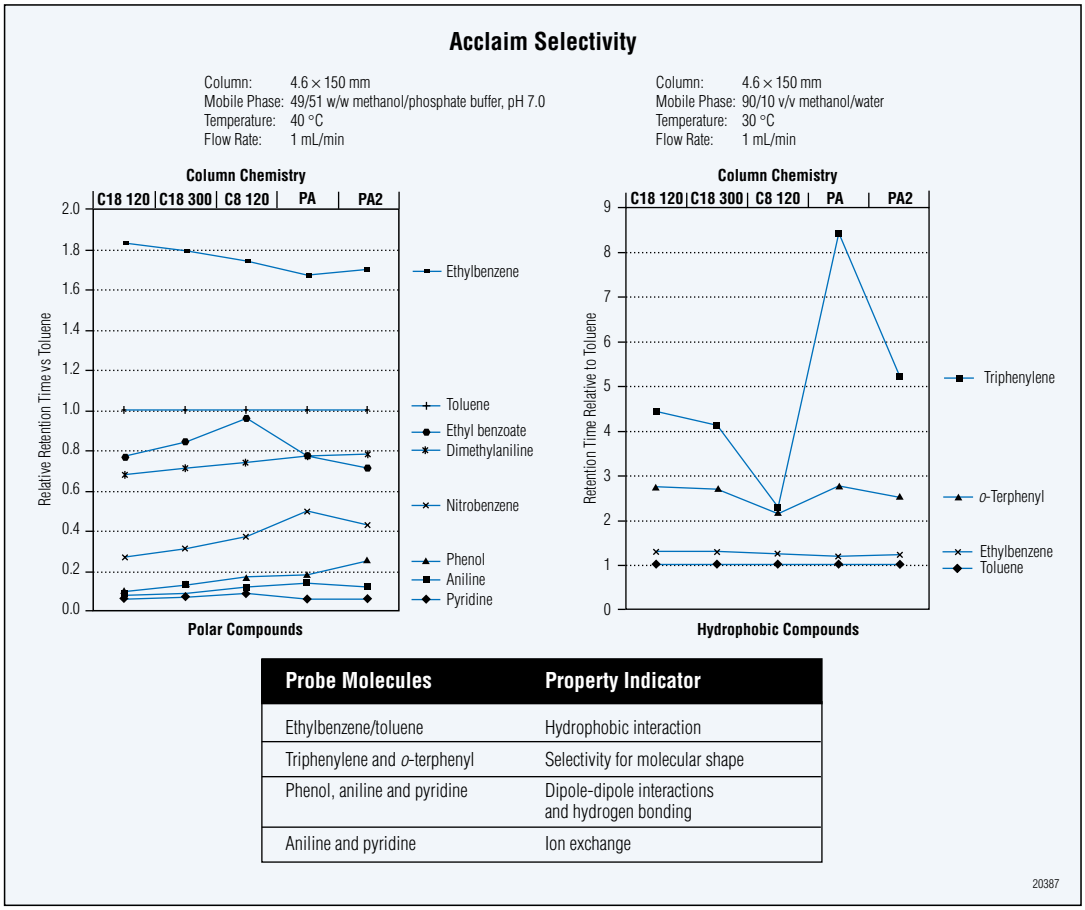
Ion-exchange activity is usually present where low surface coverage of the bonded phase, or insufficient endcapping is present. Since this effect is generally not manufactured into reversed phase stationary phases on purpose, ion-exchange activity of reversed phase columns is not reproducible and separations reliant on this effect are not reliable. Selectivity between phenol, aniline and pyridine is affected by the degree of their ion-exchange activity with residual surface silanol groups at pH 7, because under these conditions the two bases are protonated but phenol is neutral. As illustrated in Acclaim Selectivity figure, overall the Acclaim columns exhibit low ion-exchange activity under neutral conditions and the general purpose C8 and C18 show particularly low ion-exchange activity, emphasizing their high surface coverage of bonded phase.

Molecular Shape

When hydrophobic interaction alone is insufficient to produce the required separation, it is often convenient to have secondary effects that will have an impact. Triphenylene and o-terphenyl are both tricyclic hydrocarbons of similar size but with different shapes. The type of bonding chemistry of the stationary phase and the bonding density will both influence how these compounds interact with a stationary phase. These compounds are unresolved on the C8 phase, similarly resolved on the 120-Å or 300-Å C18 phases but as shown in the figure above, shape differences have a much larger effect with the polar embedded phases than they do with C18 phases.

Hydrogen Bonding and Dipole-dipole Interactions

In addition to hydrophobic retention, and ion exchange if the stationary phase has exposed charged sites, phenol, aniline and pyridine are also retained by dipole-dipole interactions and hydrogen bonding. These secondary effects can be exploited to improve a separation by choosing a stationary phase that has stronger interactions of this type, such as the polar embedded phases.



Probe Molecules	Property Indicator
Ethylbenzene/toluene	Hydrophobic interaction
Triphenylene and *o*-terphenyl	Selectivity for molecular shape
Phenol, aniline and pyridine	Dipole-dipole interactions and hydrogen bonding
Aniline and pyridine	Ion exchange

Choosing the Appropriate Particle Size

Particle size affects peak efficiency, which in turn impacts resolution or the amount of space between peaks. Smaller particles sizes give higher efficiencies and higher resolution than larger ones. However, at the same flow rate, the backpressure will be greater using smaller particle sizes than larger ones. Therefore, smaller particle sizes are recommended for fast analyses, using shorter columns.

Acclaim columns are available packed with either 3-µm or 5-µm bonded silica. A column packed with 3-µm material will give almost twice the separation efficiency as a column of identical proportions, packed with 5-µm material, but the backpressure will be four times as high on the 3-µm column at the same linear velocity.

3 µm *5 µm*

Choosing the Pore Size and Surface Area

Select a pore size appropriate for the molecular weight of your sample analytes. The pore size should be large enough to allow the sample molecules of interest to enter and pass through. If the pore size is too small, size exclusion effects can cause unwanted peak broadening.

MW < 15 kDa: use Acclaim 120, Acclaim PA or Acclaim PA2

MW < 150 kDa: use Acclaim 300

Generally speaking, the smaller the pore size, the greater the number of pores and the higher the surface area. The higher the surface area, the higher the capacity of the packing material. This means that the retention time of a given analyte will be shorter on a wide-pore material than on a narrow-pore one.

Smaller pores = Larger surface area *Larger pores = Smaller surface area*

Understanding More about the Bonded Phase

Dionex offers highest quality silica-based reversed phase packing materials. The type of bonding, carbon load and endcapping are all factors that contribute to the superior bonded phase.

Type of Bonding

Functional groups are attached to the silica substrate either via a single attachment (monomeric bonding), or via multiple attachments (multidentate bonding). Monomeric bonded phases provide higher column efficiencies, than polymeric phases, but polymeric phases are very stable under pH extremes.

Carbon Load and Endcapping

The Percent (%) carbon is a rough guide to the capacity of the column. Given the same type of silica the higher carbon load is an indicator of surface coverage. Phases with higher carbon loads are more strongly hydrophobic, resulting in higher capacity, longer retention times, and often better resolution.

When the functional groups are attached to the silica particle, not all silanol groups on the surface of the silica particle are covered. Free silanol groups will interact with polar analytes, altering the retention times and often causing peak tailing for organic bases. To minimize these secondary interactions, the free silanol groups are endcapped. All Acclaim column packings are endcapped.

Choosing the Optimum Column Format

Column efficiency, which is measured in theoretical plates (N), is mostly a function of the column length and particle size. Acclaim columns are available in many formats, therefore it is important to carefully select the best one for a particular application.

Column Length

Shorter columns provide faster run times and longer columns provide better resolution. If all else is constant, peaks are better separated on longer columns. However, the pressure, mobile phase consumption and time of analysis all increase in proportion to column length, thereby raising the cost per analysis. Detection limits are better on shorter columns.

Column Length Selection

Column Length	Application
10–75 mm	Fast analysis. Works best with 3-µm particles size
100–150 mm	Standard separations. Works well with 3- or 5-µm particle sizes
>250 mm	High resolution applications. Works best with 5-µm particle size

Column Diameter

Select a column diameter according to your requirements for sample size, solvent consumption and sensitivity. Column diameter impacts sensitivity (narrow columns provide better sensitivity), loading capacity (wider is better for larger sample volumes and for purifying samples), solvent consumption (narrow columns use less solvent) and backpressure (narrow columns exhibit higher backpressure so you will need to decrease the flowrate). Scale the flow rate by the square of the column diameter to preserve backpressure and retention time. To gain the full benefits of smaller column diameters, your HPLC instrumentation needs to be optimized for the flow rate.

Column Diameter Selection

Column Diameter	Application
0.075 to 1.0 mm I.D	High sensitivity or limited volume samples. Use with LC/MS instrumentation
2.1 mm I.D	High sensitivity or limited volume samples. Use with low dead-volume instrumentation
3.0 mm I.D	Lower solvent consumption. Use with standard instrumentation
4.0–4.6 mm I.D	Standard formats. Use with standard instrumentation

Quick Tips when Choosing Column Format

Optimize the analysis time and chromatographic efficiency by evaluating the trade-offs between the particle diameter and column lengths.

- 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.
- A 3-μm, 4.6 × 150-mm column delivers similar peak widths as a 5-μm, 4.6 × 250-mm column in 60% of the time.
- 50-mm columns sometimes provide adequate resolution while saving time and solvent.
- LC-MS applications can increase throughput using shorter column or larger particle size than required 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.
- By default, a 5-μm, 150-mm-long column is always a good starting point.

Mobile Phase Considerations

The effect of mobile phase composition on column lifetime should be part of the consideration for designing a method.

Use the highest practical quality of water, solvent, and buffer components; HPLC-grade material has low UV absorbance and is 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.

Both alkaline conditions and strong acid conditions will 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 co-solvents. When organic solvents are added to buffers of inorganic anions such as phosphate, the buffer becomes more alkaline; when organic solvents are added to buffers of organic bases, such as TRIS, the buffer becomes less alkaline

Acid conditions hydrolyze the bonded layer, rather than the silica itself, and the rate of this effect is proportional to the hydrogen ion concentration. The rate of hydrolysis is slower toward the middle of the pH range. Fortunately, organic modifiers usually provide protection against degradation.

Acclaim Columns Dedicated to High Quality

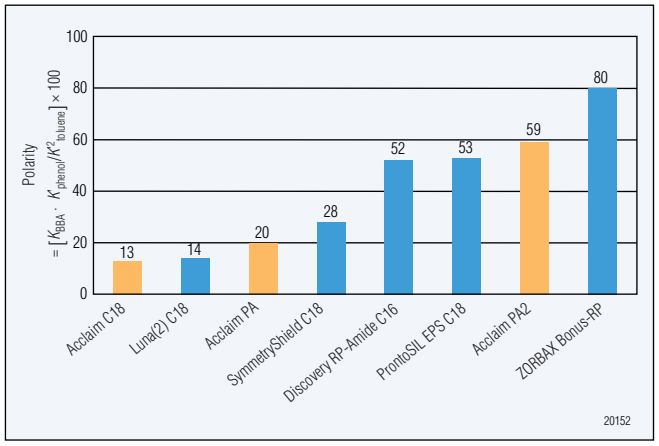
Reliability and Durability

At Dionex we know that quality and reliability are essential to a successful analysis. Our columns are thoroughly tested individually, so that chromatographers can have full confidence in them. Manufacturing starts with an ultrapure silica substrate, using only carefully selected lots with narrow ranges of physical parameters. By design, the bonding processes are clean and repeatable with no unexpected changes in performance. 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 processes. Each packed column is tested to ensure the same great performance every time. The quality assurance reports for silica lot validation and column performance explain the test protocols, list the specifications, and show the actual chromatograms.

Performance Indicators

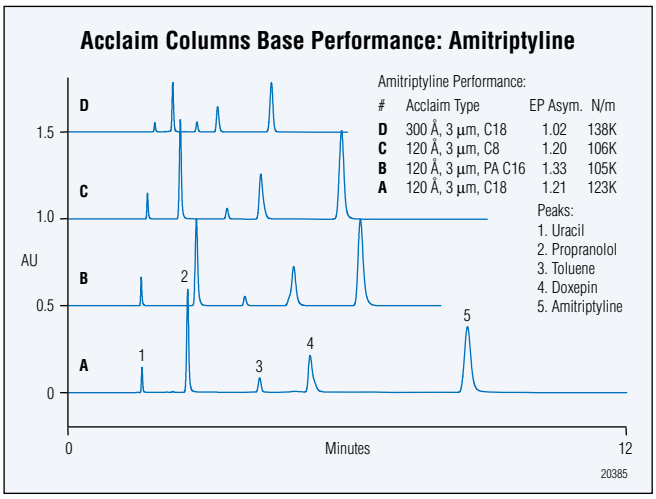
Acclaim bonded-silica columns have been designed to meet the high quality standard needed in laboratories today. The innovative surface chemistries deliver exceptional peak efficiencies for a broad range of analytes. To ensure optimal performance, all Dionex Acclaim products are thoroughly characterized using a number of performance indicators, including surface coverage of the bonded phase, metal contamination, steric selectivity, column polarity, column hydrophobicity, and low silanol activity for bases. The specialty columns are also application-tested for their specific analysis, to ensure that each lot of bonded silica provides high-performance separations. The following charts and chromatograms explain how to interpret each of these tests.

Polarity Index



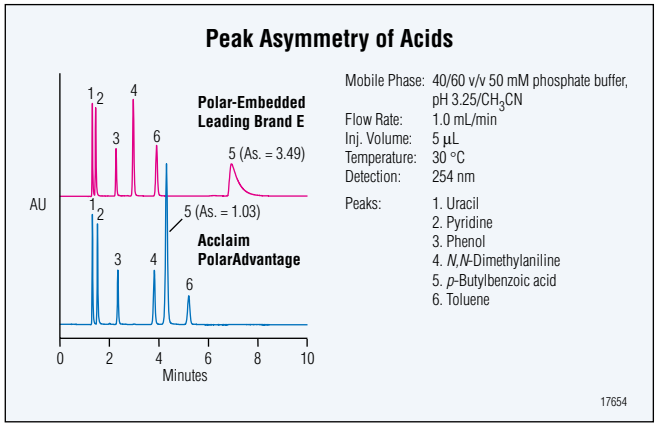
The polarity index chart above ranks commercially available stationary phases according to their polarity relative to one another, under the same conditions. The more similar the polarity index of two stationary phases, the more similar their separation of polar compounds should be. If one column does not provide adequate resolution, choosing a column with a different polarity index may deliver a better separation.

Base Asymmetry



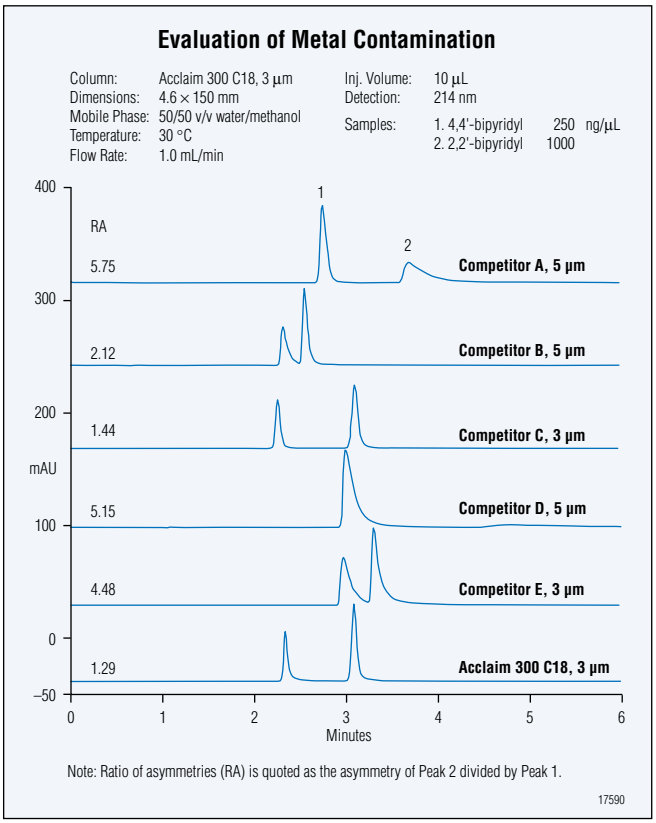
Good surface coverage of the silica gel with the bonded phase, and exhaustive endcapping ensure that Acclaim columns provide symmetrical peaks and superior performance for the separation of basic drugs. Amitriptyline is a well-known example of a basic pharmaceutical that interacts strongly with residual silanol groups on the silica gel, to produce asymmetrical peaks.

Acid Asymmetry



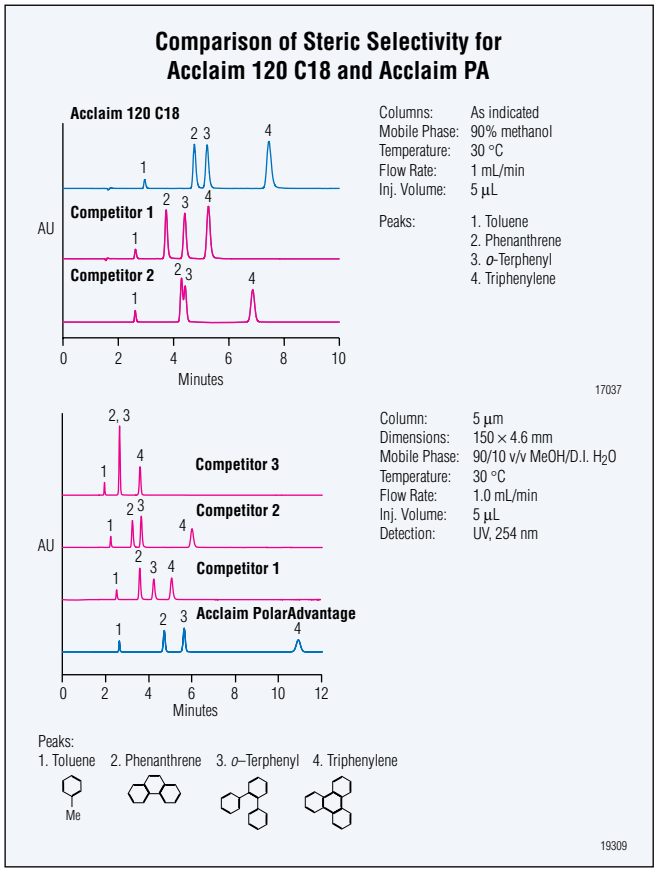
Many polar-embedded columns perform well for a limited group of compounds. Acclaim PolarAdvantage columns use state of the art methods of synthesis to eliminate unwanted secondary interactions. The result is outstanding performance for hydrophilic and hydrophobic acidic, basic, and neutral analytes.

Metal Contamination



Metal contamination can interfere with the separation of many different substances, resulting in peak tailing and concomitant poor peak resolution. 2,2′-bipyridyl is a strong chelating agent, and will reveal any contamination by metal ions. The figure above shows that Acclaim outperforms five competitor columns in this test.

Steric Selectivity

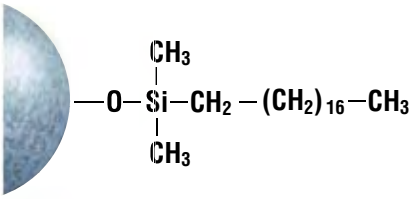


Depending on the bonding chemistry, various C18 columns can have different selectivity to molecular shape. Acclaim may be just what you need for your unique separation challenge.

PART TWO: COLUMNS

Chapter 1: Acclaim 120 C18

High performance reversed-phase columns for the separation of small molecules



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:

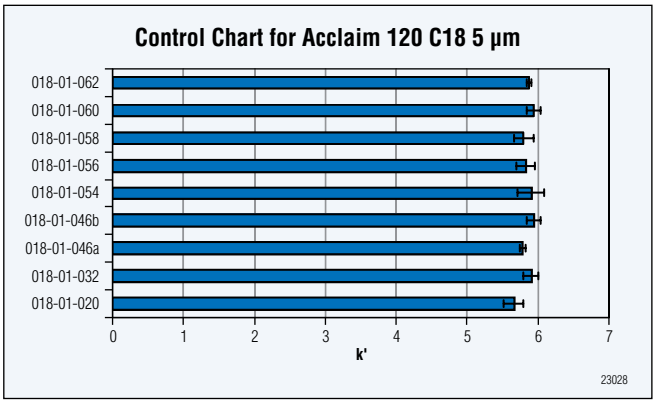
- Highly efficient, symmetrical peaks for difficult basic and chelating analytes
- Ultrapure silica substrate
- Optimized surface pretreatment, proprietary high-density bonding process, and double endcapping
- Reliability designed into the manufacturing process and assured by thorough and appropriate 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

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

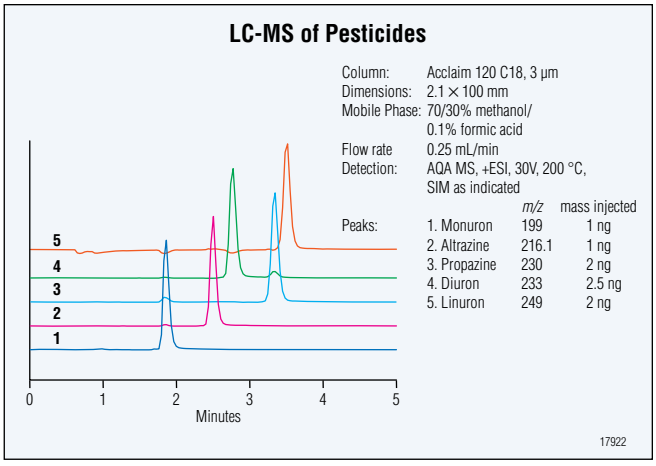
Recommended Ranges of Operation	
To maintain wetting of the hydrophobic surface, the recommended range for organic composition is 20–100%. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, high concentrations of halide ions at pH <2.5 should be avoided.	
pH Range	2.0–8.0
Pressure limit	4500 psi
Temperature	< 80 °C

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)	90,000 (5 µm) 120,000 (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. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.

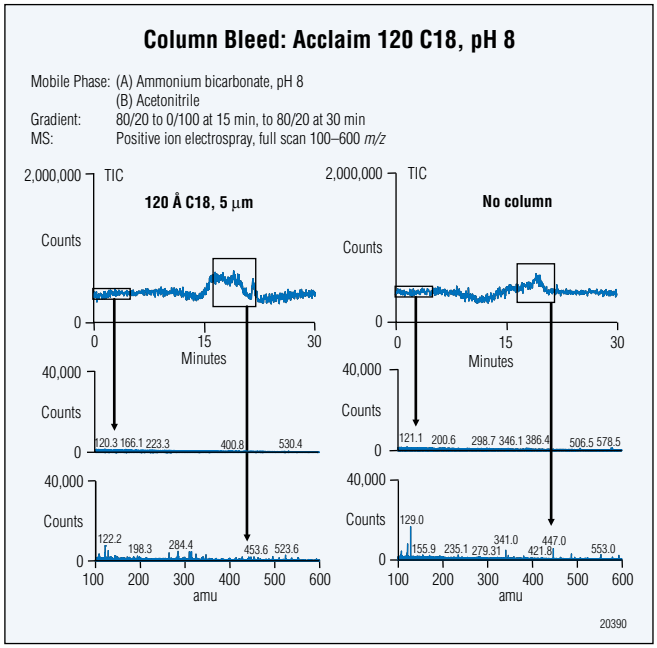


Control chart showing k' (phenanthrene) for nine lots of Acclaim 120 5-µm C18. The data show the average and standard deviation for all columns shipped from that lot.



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.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim 120 C18 Analytical Columns		
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	2.1 × 50 mm	059142
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

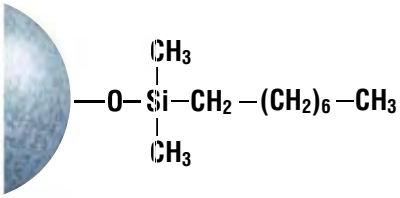
Acclaim 120 C18 Micro and Nano Columns		
3 µm	75 µm × 50 mm (NAN75)	162238
3 µm	75 µm × 150 mm (NAN75)	162239
3 µm	300 µm × 50 mm (FUS)	162236
3 µm	300 µm × 150 mm (FUS)	162237
3 µm	1 × 50 mm (MIC)	162234
3 µm	1 × 150 mm (MIC)	162235
5 µm	75 µm × 50 mm (NAN75)	162456
5 µm	75 µm × 150 mm (NAN75)	162457
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

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

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

Chapter 2: Acclaim 120 C8

High performance reversed-phase columns for the separation of small molecules



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:

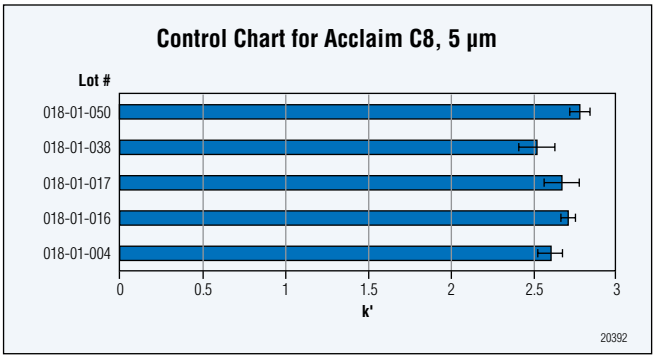
- Highly efficient, symmetrical peaks with difficult basic and chelating analytes
- Ultrapure silica substrate
- Optimized surface pretreatment, proprietary high-density bonding process, and vigorous endcapping
- Reliability designed into the manufacturing process and assured by thorough and appropriate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

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

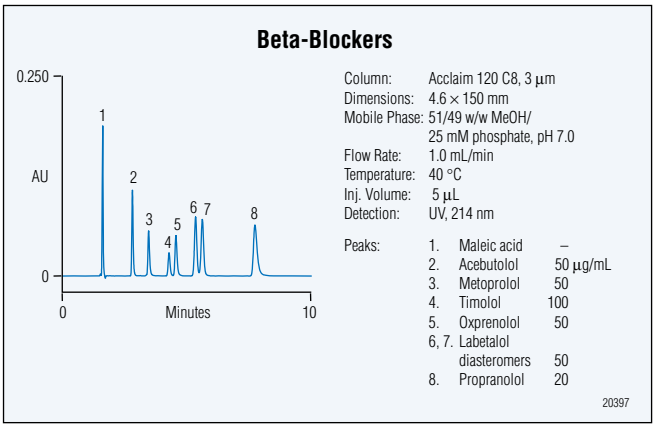
Recommended Ranges of Operation	
To maintain wetting of the hydrophobic surface, the recommended range for organic composition is 20–100%. The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, high concentrations of halide ions at pH <2.5 should be avoided.	
pH Range	2.0–8.0
Pressure limit	4500 psi
Temperature	< 80 °C

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)	90,000 (5 µm) 120,000 (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. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.



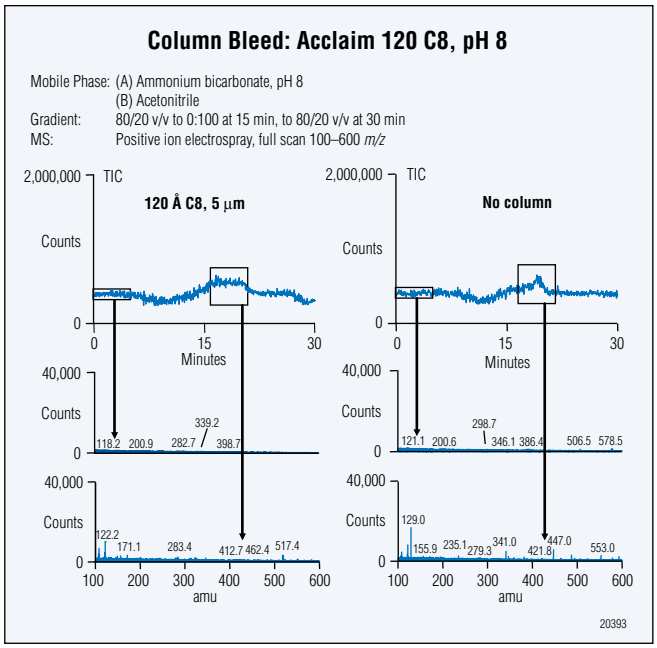
Control chart showing k′ (phenanthrene) for five lots of 5-µm C8. The data show the average and standard deviation for all columns shipped from that lot.



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.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim 120 C8 Analytical Columns		
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	2.1 × 50 mm	059134
5 µm	2.1 × 100 mm	059135
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

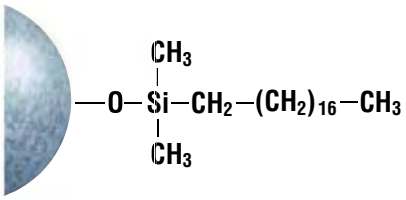
Acclaim 120 C8 Micro and Nano Columns		
3 µm	75 µm × 50 mm (NAN75)	162208
3 µm	75 µm × 150 mm (NAN75).	162209
3 µm	300 µm × 50 mm (FUS).	162206
3 µm	300 µm × 150 mm (FUS).	162207
3 µm	1 × 50 mm (MIC).	162204
3 µm	1 × 150 mm (MIC).	162205
5 µm	75 µm × 50 mm (NAN75)	162216
5 µm	75 µm × 150 mm (NAN75).	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

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

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

Chapter 3: Acclaim 300 C18

Reversed-phase columns for the separation of proteins, peptides, and other biological macromolecules



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
- Minimal secondary interactions for repeatable results day-to-day and column-to-column

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.

Physical Specifications

Bonding	C18 monomeric
USP code	L1
Endcapping	Double
% C	6.7–7.5
Pore size	260–300 Å
Surface area	90–120 m^2/g
Particle diameters	2.9–3.2 µm

Performance Specifications

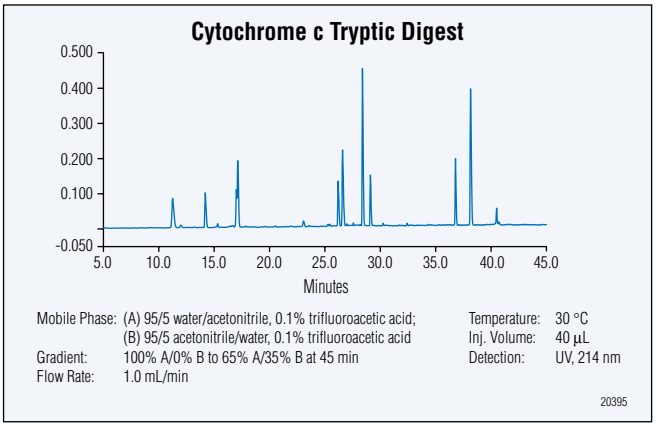
Lot	Base asymmetry	0.98–1.30
	Metal activity ratio	0.80–1.30
	Cytochrome c tryptic digest peptide map	P2-P1: 2.8–3.1 min P7-P6: 0.6–1.0 P9-P8: 1.1–1.5
Column	Efficiency (N/m)	115,000
	Asymmetry (EP)	0.98–1.20
	Retention time	4.30–4.80

Example chromatographic specifications for Acclaim 300 C18 4.6 × 150 mm columns. Please refer to Part 4 for a detailed description of the tests and specifications.

Recommended Ranges of Operation

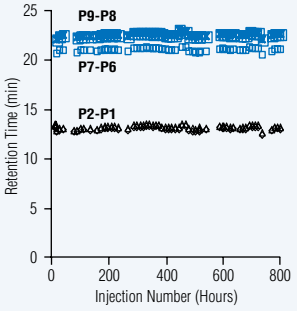
The type 316 stainless steel column hardware is compatible with common HPLC mobile phases. To prevent corrosion, high concentrations of halide ions at pH <2.5 should be avoided.

pH Range	2.0–8.0
Pressure limit	4500 psi
Organic solvents	0–100%
Temperature	< 80 °C



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.

Stability Study for 3-µm C18, 300 Å at 50 °C



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

Gradient:	*Time (min)*	*% A*	*% B*
	–10.0	95	5
	0.0	95	5
	45.0	50	50
	50.0	50	50

Temperature: 50 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 40 µL
Inj. Amount: 0.5 µg/µL
Detection: UV, 214 nm
Samples: Tryptic digest of cytrochrome c (retention data plotted for the two least separated pairs of peptides, P2-P1, P7-P6, and P9-P8)

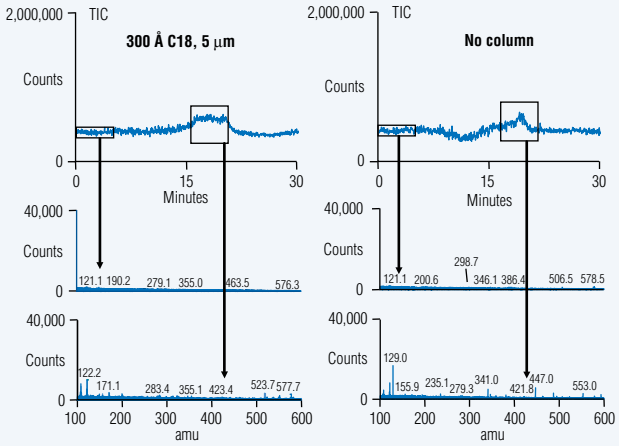
17592

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.

Column Bleed: Acclaim 300 C18, pH 8

Mobile Phase: (A) Ammonium bicarbonate, pH 8
(B) Acetonitrile
Gradient: 80/20 v/v to 0/100 at 15 min, to 80/20 v/v at 30 min
MS: Positive ion electrospray, full scan 100–600 *m/z*



20394

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.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim 300 C18 Analytical Columns		
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

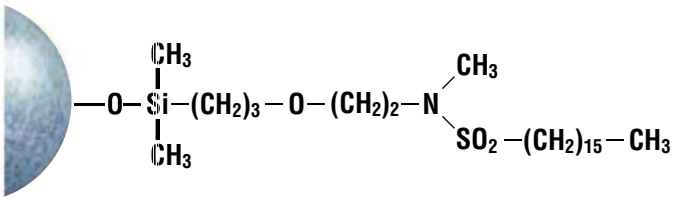
Acclaim 300 C18 Guard Columns		
3 µm	2.0 × 10 mm.	060395
3 µm	4.3 × 10 mm	060393
Biocompatible cartridge holder for 2.0 × 10 and 4.3 × 10 mm guards		059456
Guard to analytical column coupler		059457
Holder and coupler kit		059526

Acclaim 300 C18 Micro and Nano 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

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

Chapter 4: Acclaim PolarAdvantage (PA)

Polar Embedded Reversed-Phase Columns for separating polar compounds



The Acclaim PA column has a patented surface chemistry that renders it 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 synthesis procedure 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

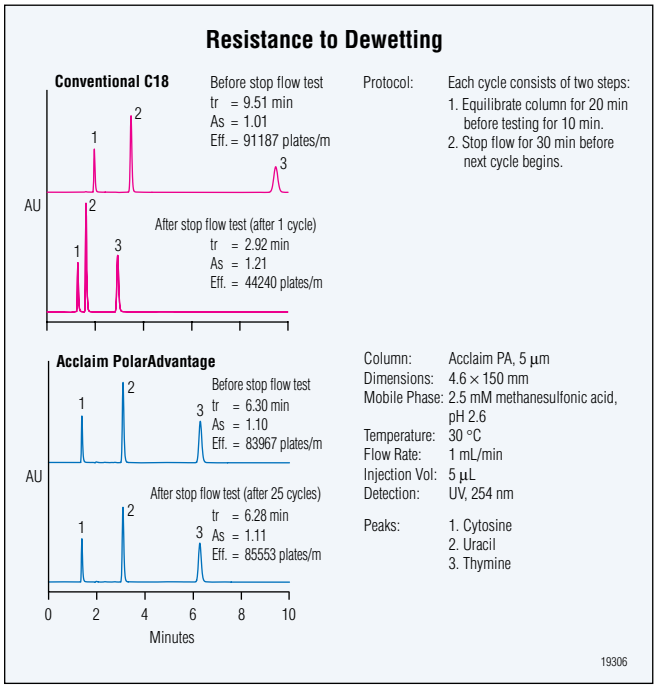
Physical Specifications	
Bonding	C16 monomeric, polar-embedded
USP code	na
Endcapping	Double
% C	16.0–18.0
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameters	2.9–3.2 µm or 4.2–4.5 µm

Recommended Ranges of Operation	
The 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.	
pH Range	2.0–8.0
Pressure limit	4500 psi
Organic solvents	0–100%

Resistance to Dewetting

The surface of a conventional C18 phase is very hydrophobic. When hydrophobic surfaces are in contact with highly aqueous mobile phases, the partial pressure of dissolved gases can expel the mobile phase from the pores of the stationary phase. This process is called dewetting and it adversely affects chromatographic performance. By design, the mildly hydrophilic surface of the Acclaim PA remains in contact with aqueous-only mobile phases, negating the problem of dewetting.

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

In order to reduce tailing and improve the peak shape of amine-containing compounds, HPLC separations of polar analytes are often run under acidic conditions. Separations under these conditions can shorten column life due to cleavage of the bonded phase. This cleavage results in frequent column replacement and instrument downtime. The proprietary bonding of the Acclaim PA column resists hydrolytic attack by protecting the bonded phase at low pH values.

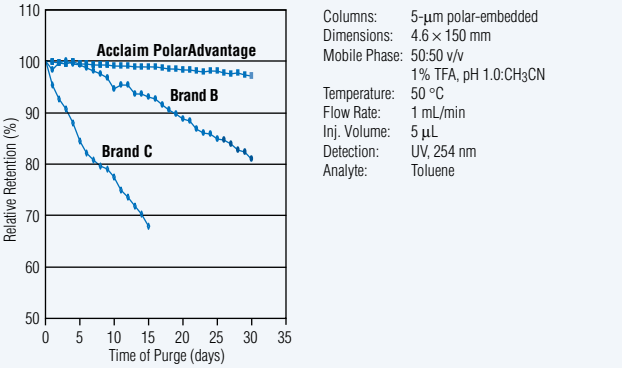


Chart of retention vs time at 50% acetonitrile, 0.5% TFA for polar-embedded columns.

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)

Example chromatographic specifications for Acclaim PA, C16, 4.6 × 150 mm. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.

LC/MS Compatibility

Acclaim PA may be used for LC/MS applications. 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.

PA

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.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim PA Analytical Columns		
3 µm	2.1 × 50 mm	061374
3 µm	2.1 × 100 mm	061316
3 µm	2.1 × 150 mm	061317
3 µm	4.6 × 150 mm	061318
5 µm	4.6 × 50 mm	061319
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

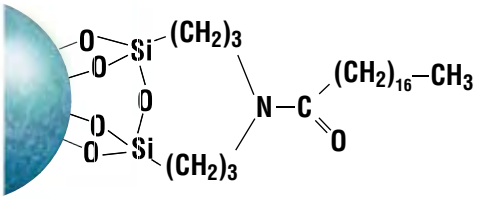
Acclaim PA Micro and Nano Precolumns		
5 µm	300 µm × 1 mm (pack of 5)	162310
5 µm	300 µm × 5 mm (pack of 5)	162302
5 µm	500 µm × 5 mm (pack of 5)	162336
5 µm	500 µm × 15 mm (pack of 5)	162337
5 µm	800 µm × 5 mm (pack of 5)	162335
5 µm	1 × 5 mm (pack of 5)	162333
5 µm	1 × 15 mm (pack of 5)	162334

Acclaim PA Micro and Nano 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
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

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

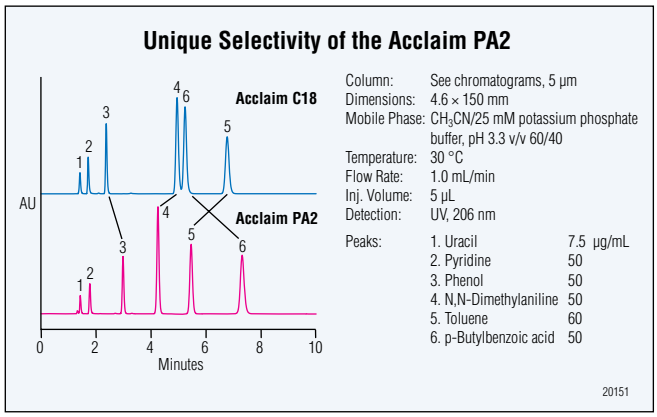
Chapter 5: Acclaim PolarAdvantage II (PA2)

Polar-embedded reversed-phase columns with enhanced hydrolytic stability



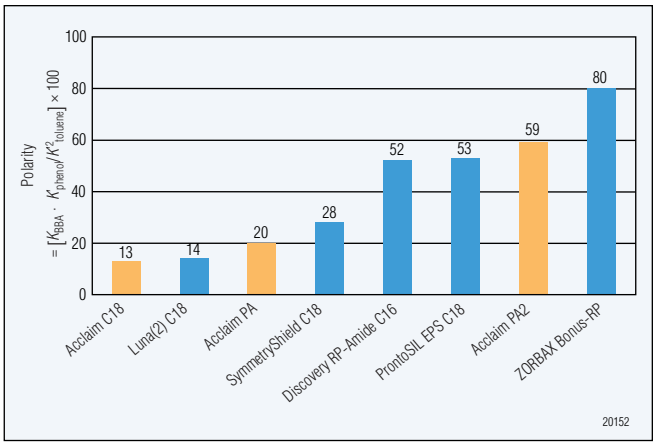
Acclaim PA2

The Acclaim PA2, like the Acclaim PA, is a high-efficiency, silica-based, reversed phase column with a polar enhanced stationary phase for operation over a wider range of chromatographic conditions than is possible with conventional reversed phase stationary phases. The Acclaim PA2 is an amide polar-embedded phase, with all the advantages of conventional polar-embedded phases, but with enhanced hydrolytic stability at both low and high pH (pH 1.5-10). The Acclaim PA2 provides selectivity that is complementary to conventional C18 columns, and to our Acclaim PA, providing a well-rounded column portfolio for methods development. This column is fully compatible with 100% aqueous mobile phases and provides symmetrical peaks for both polar and non-polar analytes.



The Acclaim PA2 benefits include:

- Ability to separate polar and non-polar compounds
- Exceptional hydrolytic stability (pH 1.5–10)
- High polarity for complementary selectivity to C18 columns
- Compatibility with 0–100% aqueous or 0–100% organic solvent mobile phases
- Good peak shapes for both acidic and basic compounds
- High column efficiency
- Broad range of applications in pharmaceutical, environmental, food testing and product-quality testing.



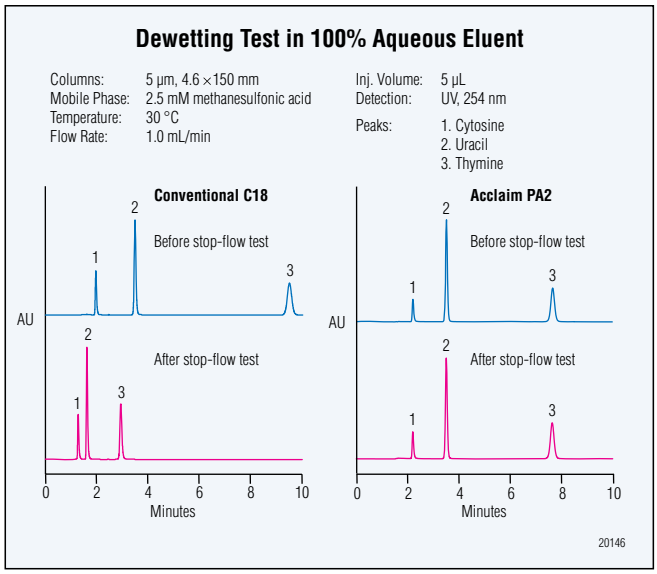
The polarity index chart above ranks commercially available stationary phases according to their polarity relative to one another, under the same conditions. The more similar the polarity index of two stationary phases, the more similar their separation of polar compounds should be. If one column does not provide adequate resolution, choosing a column with a different polarity index may deliver a better separation.

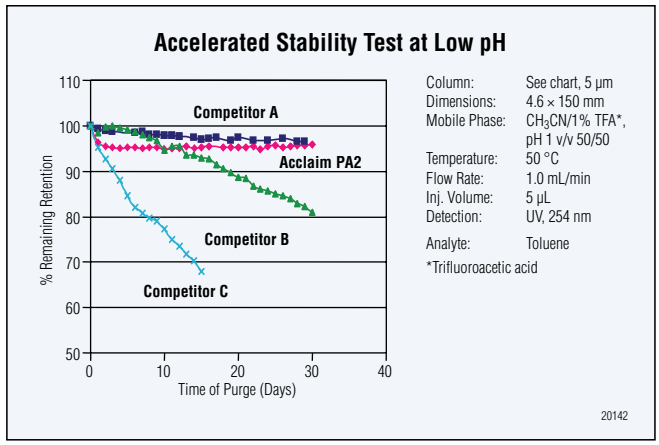
Physical Specifications	
Surface chemistry	C18 polar-embedded
USP code	na
Endcapping	Yes
%C	15.5–17.5%
Pore size	120–140 Å
Surface area	260–320 m^2/g
Particle diameters	2.9–3.2 µm or 4.2–4.5 µm

Recommended Ranges of Operation	
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.	
pH Range	1.5–10
Pressure limit	4500 psi
Organic solvents	0–100%

Resistance to Dewetting

The carefully-designed bonding technology of the Acclaim PA2 incorporates hydrophilic functional groups between the hydrophobic alkyl chain and the silica surface that allows the surface to remain wetted, even in 100% aqueous mobile phase conditions. The accompanying figure shows the effect of repeatedly stopping the flow through a C18 and PA2 column. The C18 column is dewetted in a single cycle, but the PA2 provides reliable retention times for even very hydrophilic nucleic acid bases such as cytosine, uracil, and thymine, through many cycles.





Hydrolytic Stability

Despite their many advantages, the hydrolytic stability of polar embedded phases is often inferior to conventional C18 phases as a result of lower ligand coverage and the hydrophilic nature of the embedded group. The Acclaim PA2 has been designed to provide all the advantages of polar embedded phases, but with enhanced stability at both low and high pH.

HPLC separations of polar analytes are often run at low pH (PH <3.0) to reduce tailing of amine-containing compounds. The accompanying figure shows that even at pH 1.0, the Acclaim PA2 is stable to hydrolysis unlike several other polar-embedded phases. Similarly, at pH 11.5 the Acclaim PA2 retains 90% of its retention capacity after 60 hours, while other columns with extended pH compatibility drop to 50-80%. Thus, the proprietary bonding of the Acclaim PA2 provides protection against hydrolytic attack at both low and high pH.

Performance Specifications

Lot	Polar selectivity ratio	0.28–0.32
	Base asymmetry	0.98–1.6
	Metal activity ratio	0.90–1.70
	Acid asymmetry	0.98–1.30
Column	Efficiency (N/m)	12,150 (5 µm) 16,200 (3 µm)
	Asymmetry (EP)	0.98–1.32
	Retention time	7.05–8.26 (5 µm) 7.05–8.15 (3 µm)

Example chromatographic specifications for Acclaim PA, C16, 4.6 × 150 mm. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.

Ordering Information: Acclaim PolarAdvantage II (PA2)

The standard particle sizes for the Acclaim PA2 columns are nominally 3- and 5-µm. Analytical columns are stocked in 2.1 and 4.6–µm 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-mm analytical columns. Other geometries can be made to order. Inquire with your Dionex sales representative.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim PA2 Analytical Columns

3-µm	4.6 × 150 mm	063191
3-µm	4.6 × 50 mm	063189
3-µm	2.1 × 150 mm	063187
5-µm	4.6 × 250 mm	063199
5-µm	4.6 × 150 mm	063197

Acclaim PA Guard Columns

5 µm	2.0 × 10 mm (pack of 2)	063193
5 µm	4.3 × 100 mm (pack of 2)	063195
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

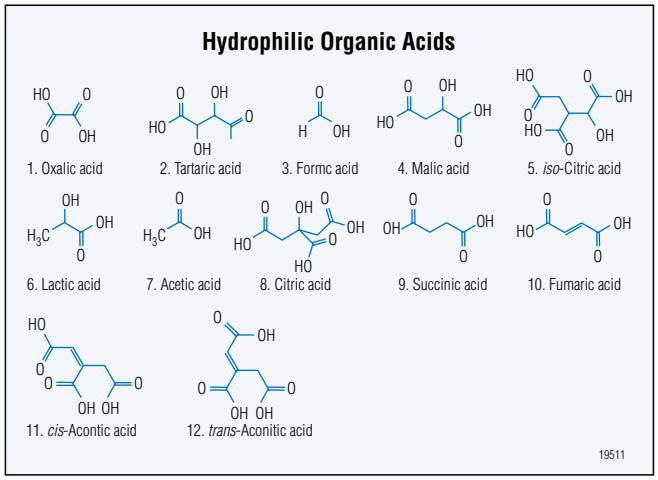
These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

Chapter 6: Acclaim OA for Organic Acids

Optimized and application-tested for the analysis of hydrophilic organic acids

Acclaim OA organic acid columns are special 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 is the recommended column for determining small hydrophilic organic acids, C1 to C7 aliphatic acids, and hydrophilic aromatic acid and is also valuable for the analysis and quality assurance of food and beverage products, pharmaceutical preparations, plating baths for semiconductor manufacturing, manufacturing chemicals, and chemical intermediates.



Recommended Ranges of Operation	
pH Range	2.0–8.0
Pressure limit	4500 psi (2000 psi above 50% organic solvent content)
Organic solvents	0–100% acetonitrile or methanol

The Acclaim OA columns are packed in metal-free PEEK column bodies, to eliminate unwanted interactions between the analytes and the column body. Benefits of the Acclaim OA columns include:

- Use-tested to guarantee consistent organic acid separations
- Compatible with 100% aqueous mobile phases, optimum for reversed-phase retention of hydrophilic aliphatic organic acids
- Hydrolytic stability at low-pH conditions, optimum for reversed-phase retention of organic acids
- Ideal selectivity for separating a wide spectrum of organic acids, including hydrophilic aliphatic acids, C1 to C7 aliphatic acids, aromatic acids, and selected amino acids
- Excellent peak shapes for organic acids
- High reversed-phase capacity with retention similar to standard C18 phases
- Suitable for analysis of foods, beverages, pharmaceuticals, chemical intermediates, and environmental samples
- Ideal for quality assurance offering fast run times and minimal sample preparation

Physical Specifications	
Surface chemistry	Proprietary
USP code	na
Endcapping	Yes
Pore size	120–140 Å
Surface area	290–320 m^2/g
Particle diameter	4.2–4.5 µm

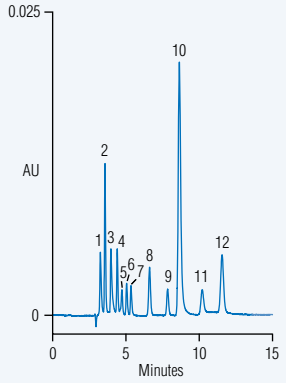
Guaranteed Performance for Organic Acids

The performance of the Acclaim OA columns is guaranteed for the separation of aliphatic and aromatic organic acids at low pH. These 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.

Performance Specifications		
Lot	Metal activity ratio	0.80–1.50
	Gradient organic acids chromatogram	Baseline drift <12 mAU Resolution (Caproic-isocaproic) ≥2.80
Column	Efficiency (N/m)	72,000
	Asymmetry	0.95–1.27
	Retention time	7.52–8.62
	Resolution (Lactic-Acetic)	≥1.58

Example chromatographic specifications for Acclaim OA 4.0 × 250 mm. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.

Isocratic Separation of Hydrophilic Organic Acids



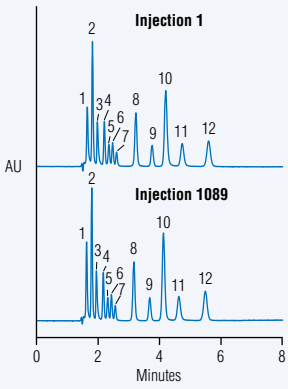
Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 100 mM Na_2SO_4, pH 2.65 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks:		15 mg/L (ppm)
1.	Oxalic acid	
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

Ruggedness Test at pH 2.68



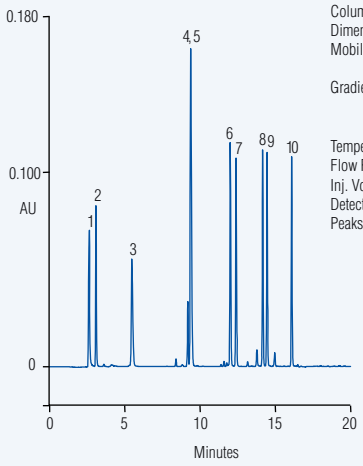
Column: Acclaim OA, 5 µm
Dimensions: 4 × 150 mm
Mobile Phase: 40 mM Na_2SO_4, pH (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks:		15 mg/L (ppm)
1.	Oxalic acid	
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

Gradient Separation of C1–C7 Aliphatic Organic Acids



Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phases: A: CH_3CN
B: 2.5 mM methanesulfonic acid

Gradient:	-10	0	1	15	20 min.
	2	2	2	60	60 %A
	98	98	98	40	40 %B

Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 30 µL
Detection: UV, 210 nm with blank subtraction

Peaks:		10 mmol/L
1.	Formic acid	
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

23611

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.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

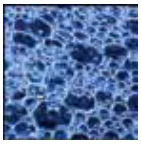
Acclaim OA Analytical Columns	
5 µm 4.0 × 150 mm	062903
5 µm 4.0 × 250 mm	062902

Acclaim OA 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

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

Chapter 7: Acclaim Surfactant

Optimized and application-tested for the analysis of anionic, nonionic and cationic surfactants



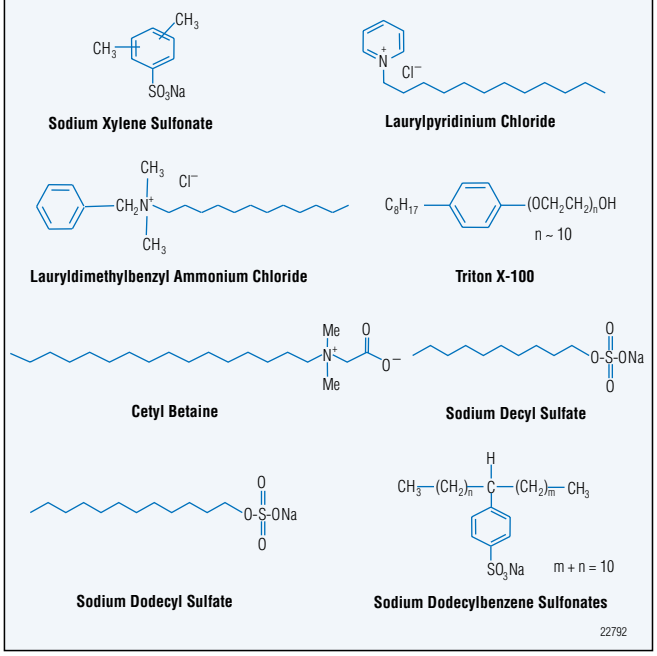
The Acclaim Surfactant Column

The Acclaim Surfactant is a silica-based, reversed phase column that features a proprietary bonding chemistry optimized and application-tested for separating a variety of surfactants. This specialty column is ideal for separating cationic, nonionic, anionic and amphoteric surfactants in a single chromatographic run using simple and volatile mobile phases. The Acclaim Surfactant column is the ideal tool for the analysis of surfactants including linear alkylsulfonates, alkyl sulfates, alkyl quaternary ammonium salts, ethoxylated quats, Triton-X, PEGs and many more. It allows analysis of surfactants in various matrices including consumer products, formulations for pharmaceutical products, and environmental samples.

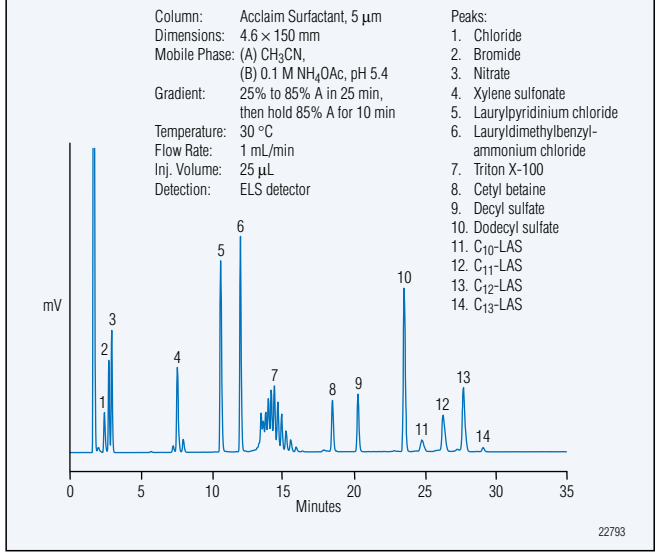
Benefits of the Acclaim Surfactant column include:

- Ideal selectivity for separating different types of surfactants
- Excellent peak shapes, especially for cationic surfactants
- Improved resolution for ethoxylated surfactants compared with other columns
- Ability to analyze hydrophilic hydrotropes
- Methods compatible with various detection methods including UV, ELSD, suppressed conductivity, and more
- Broad range of applications

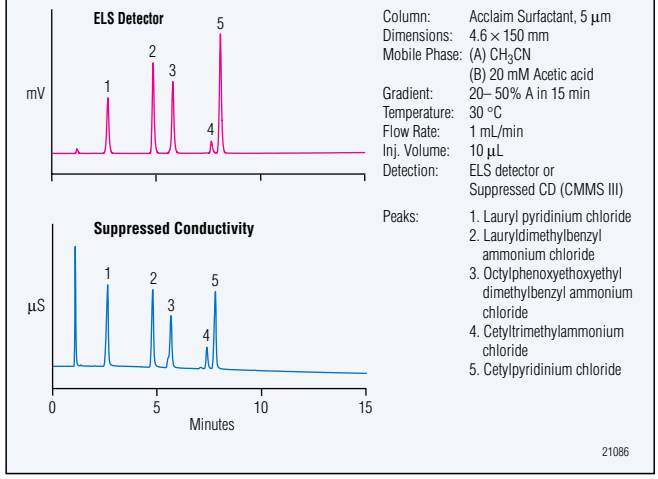
Hydrotrope, Cationic, Nonionic, Amphoteric and Anionic Surfactants



Inorganic Anions, Hydrotropes, Cationic, Nonionic, Amphoteric, and Anionic Surfactants



Separation of Commonly Used Cationic Surfactants



Physical Specifications

Bonding	Proprietary
USP code	na
Pore size	120–140 Å
Surface area	300 m^2/g
Particle diameters	4.2–4.5 µm

Recommended Ranges of Operation

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.

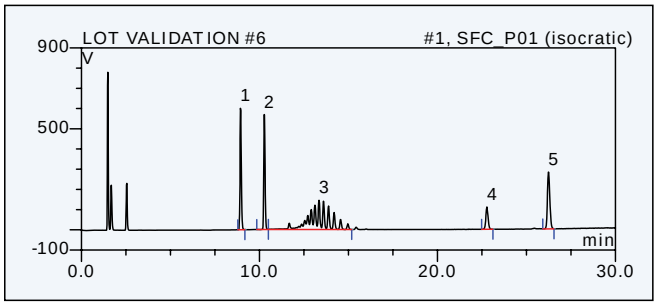
pH Range	2.5–7.5
Pressure limit	4500 psi
Organic solvents	0–100%

Guaranteed Performance for Surfactants

The exceptional performance of Acclaim Surfactant columns for the separation of anionic, cationic and non-ionic surfactants is confirmed with every new lot of bonded silica that is manufactured. In addition each column is individually tested with a simple mixture of anionic, cationic and non-ionic probes, to ensure that every column is packed consistently and well.

Performance Specifications		
Column	Efficiency (N/m)	76,500
	Asymmetry (EP)	0.95–1.32
	Retention time	7.50–8.60

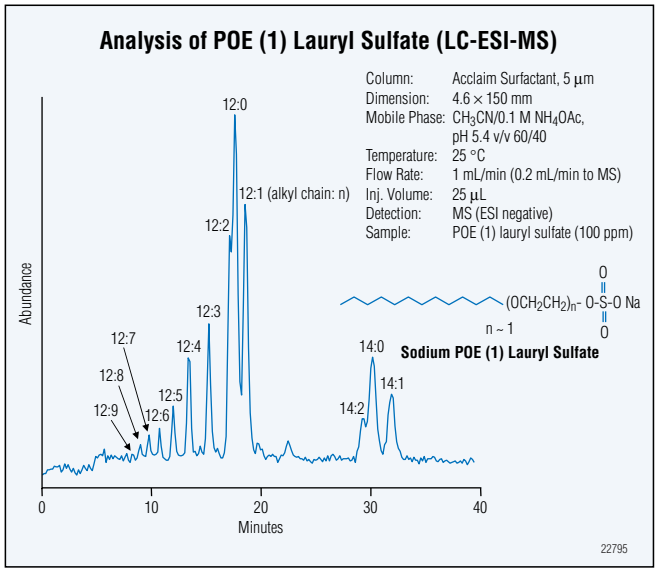
Example chromatographic specifications for Acclaim Surfactant 4.6 × 150 mm. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex.

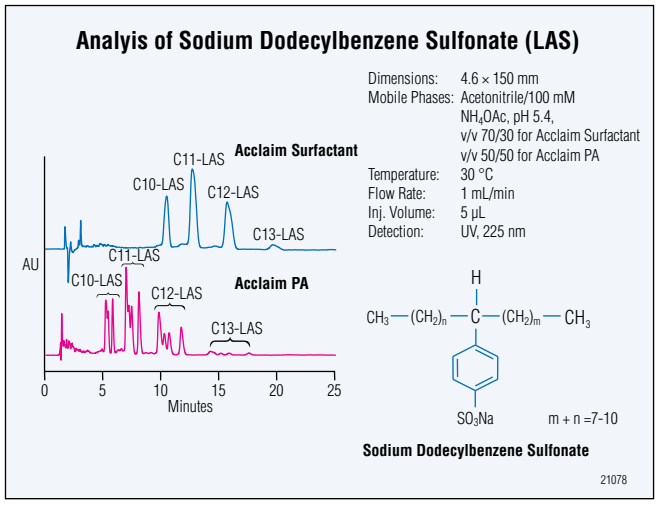


Chromatogram of a group of surfactants, taken from the certificate for lot #013-05-002. Dionex monitors and controls the selectivity of cationic, nonionic and anionic surfactants using the recommended standard conditions for this column.

Detector Compatibility

Although UV detection is the most commonly used of HPLC detectors, not all surfactants possess chromophores. This problem can be overcome by the use of a universal detector, such as evaporative light-scattering detection (ELSD) or refractive index (RI) detection. ELSD is compatible with gradient methods and is far more sensitive than RI. In addition, methods developed with ELSD can be easily transferred to LC-ESI-MS applications with little or no modifications, because both detectors share the same mobile phase requirements. Conductivity detection of cationic surfactants can also be used. Because of the versatility of this column, methods using all these detectors have been developed and can be found in this catalog, in the Acclaim product information bulletin, or on the Dionex website.





Ordering Information: Acclaim Surfactant

Standard particle size is nominally 5-mm. Analytical columns are stocked in 4.6–mm-diameter widths and a couple of lengths. Guard cartridges in 4.3-mm sizes, packed with 5-mm particles are recommended to protect the analytical columns. Other geometries can be made to order. Enquire with your Dionex sales representative.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim Surfactant Analytical Columns		
5 µm	4.6 × 150 mm	063201
5 µm	4.6 × 250 mm	063203

Acclaim Surfactant Guard Columns		
5 µm	4.3 × 10 mm (pack of 2)	063215
Biocompatible guard cartridge holder		059456
Guard for analytical column coupler		059457
Acclaim holder and coupler kit		059526

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

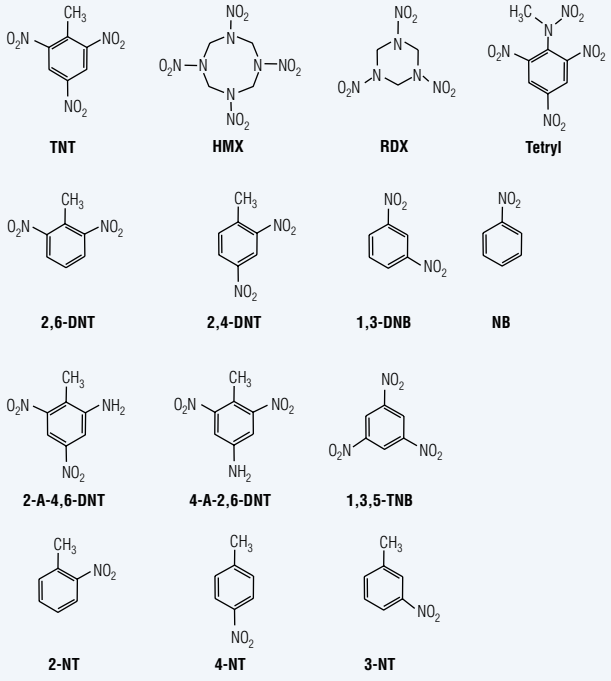
Chapter 8: Acclaim Explosives E1 and E2 Columns

For the separation of the 14 explosives targeted by EPA SW-846 Method 8330

Acclaim Explosives Columns

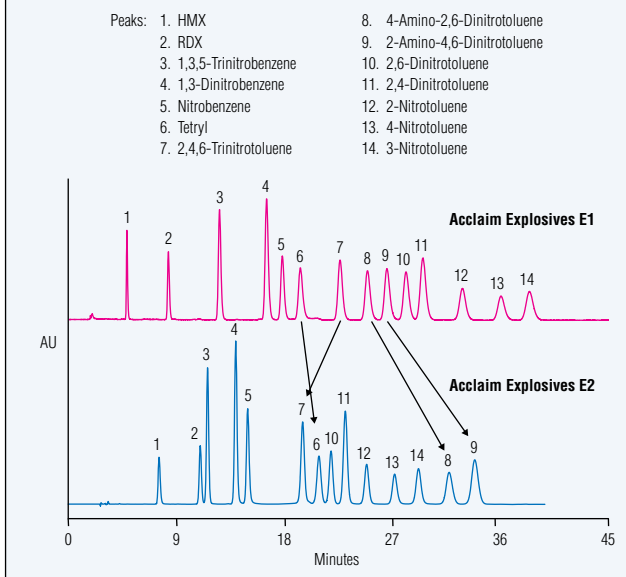
The Acclaim Explosives E1 and E2 are specialty reversed phase columns that have been optimized and application tested for the separation of the 14 explosives targeted by EPA SW-846 Method 8330, "Nitroaromatics and Ntramines by HPLC."

Fourteen Target Explosives Listed in EPA Method 8330



The novel and unique chemistries of these columns provide superior resolution to other commercially available columns. The Acclaim Explosives columns provide a "Total Solution" to EPA Method 8330; two columns each of which provides baseline resolution of all 14 explosives. The Acclaim Explosives E1 provides selectivity similar to conventional C18 columns. The Acclaim E2 column provides selectivity that is complementary to that obtained using the Acclaim E1 and is recommended for the confirmatory analysis. With their complementary selectivities, the E1 and E2 provide both primary and confirmatory columns.

Baseline Separation of 14 Target Explosive Compounds Listed in EPA SW-846 Method 8330

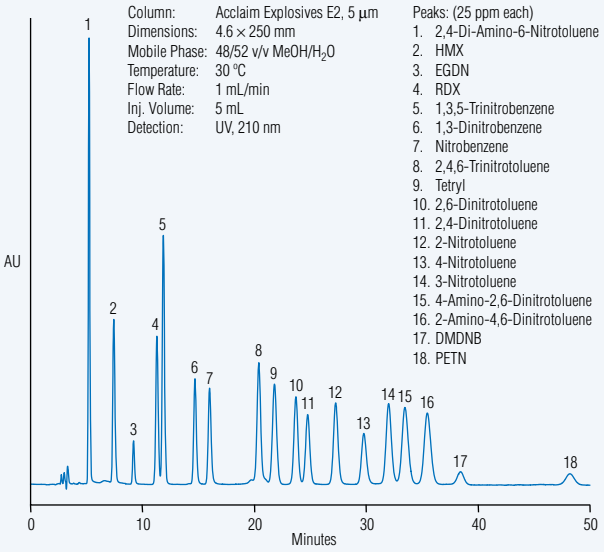


Benefits of the Acclaim Explosives E1 and E2 columns

- Superior resolution for all 14 explosives listed in EPA Method 8330
- Complementary selectivity between Acclaim E1 and E2, for a complete solution for explosives analysis
- 5-µm, 120 Å high purity silica gel for high-resolution separations, with good mechanical stability
- Unique selectivity of the Acclaim E2 allows for simultaneous separation of some nitrate ester explosives

EXPLOSIVES

Separation of 18 Explosives Showing the Unique Selectivity of the Acclaim Explosives E2 Column



Physical Specifications

	Acclaim Explosives E1	Acclaim Explosives E2
Bonding	Proprietary	Proprietary
Pore size	120–140 Å	120–140 Å
Surface area	300 m^2/g	300 m^2/g
Particle diameters	4.2–4.5-µm	4.2–4.5µm

Recommended Ranges of Operation

These operating conditions follow EPA Method Conditions.

	Acclaim Explosives E1	Acclaim Explosives E2
Mobile phase	MeOH:H_2O 43:57	MeOH:H_2O 48:52
Temperature	31 ± 1 °C	29 ± 1 °C
Flow rate	1.0 mL/min	1.0 mL/min

Guaranteed Performance for EPA Method 8330

The exceptional performance of both Acclaim Explosives columns for EPA Method 8330 is guaranteed. Both products undergo extensive testing to ensure column-to-column reproducibility and are use-tested for this specific application. Both columns perform reproducibly under their optimized conditions, although separation conditions for the Acclaim Explosives E2 can be readily changed to accommodate inclusion of additional explosives compounds.

Performance Specifications

	Acclaim Explosives E1	Acclaim Explosives E2
Efficiency (N)	20,000	20,000
Asymmetry	0.95–1.32	0.95–1.32
Retention time	6.96–7.54	7.76–8.64

Example chromatographic specifications for Acclaim E1 and E2 4.6 × 250 mm. Please refer to Part 4 for a detailed description of the tests and specifications. Specifications are subject to change at the discretion of Dionex

Ordering Information: Acclaim Explosives Columns

Standard particle size is nominally 5-µm. analytical columns are stocked in 4.6–mm-diameter widths and 250-mm length. Guard cartridges in 4.3-mm sizes, packed with 5-µm particles are recommended to protect the analytical columns. Other geometries can be made to order. Enquire with your Dionex sales representative.

Please order through your local Dionex office or distributor. Refer to the following part numbers.

Acclaim Explosives Analytical Columns

5 µm 4.6 × 250 mm E1 064305
5 µm 4.6 × 250 mm E2 064309
5 µm E1 and E2 kit 064312

Acclaim Explosives Guard Columns

5 µm 4.3 × 10 mm E1 (pack of 2) 064303
5 µm 4.3 × 10 mm E2 (pack of 2) 064307
Biocompatible guard cartridge holder 059456
Guard for analytical column coupler. 059457
Acclaim holder and coupler kit 059526

These columns are designed for optimal performance using Dionex UltiMate 3000 and ICS-3000 chromatography instruments.

PART THREE: APPLICATIONS

Contents

Pharmaceutical

Isocratic Resolution of Antihistamines and Their Impurities on Acclaim 120 Å, C18 29
Separation of Four Benzodiazepine Drugs on Acclaim 120 C18 29
Separation of Five Antidepressant on Acclaim PA 29
Spectinomycin and Lincomycin on Acclaim PA with Electrochemical Detection 29
Beta Blockers on Acclaim PA2 29
Venlafaxine and Related Substances 29
Assay for Pramoxine in Topical Anesthetic Ointment on Acclaim 120 Å, C18 with SPE Cleanup on OnGuard II RP 30
Determination of Four Active Ingredients in a Multisymptom Cold Remedy Using Acclaim PA. 30
Separation of Basic Drugs on Acclaim 120 Å, C18....30
Baseline Separation of Catecholamines & Serotonin at Physiological Concentrations on Acclaim 120 Å, C18 with Electrochemical Detection..... 30
Gradient Resolution of Sulfa Drugs on Acclaim PA 30
Catecholoamines in Urine on Acclaim PA2 30
Bromide and Organic Acids in Cough Syrup Using Acclaim OA 31
Isocratic Separation of Six Cardiac Anti-Arrhythmic Drugs (Beta-Blockers) on 120 Å C8 ... 31
Sulfa Drugs on Acclaim PA2 31
Active Ingredients in Zantac 75 Acid-Reducer Medication 31
Loratidine and Pseudoephedrine on Acclaim PA 31
Propafenone and Related Substances 31
Antidepressants on Acclaim PA2 32
Active ingredients in Cough Syrup 32
Barbiturates on Acclaim 120 C18 32
Budesonide and Related Substances 32
Hydrocortisone in Skin Ointment 32
Phenytoin and Related Substances 32
Active Ingredients in Sunscreen Lotion on Acclaim 120 Å, C18 Using ASE Extraction..... 33
Glucocorticosteroids in Serum on Acclaim 120 C18.33
Nonsteroidal Anti-inflammatory Drugs 33
Fexofenadine & Pseudoephedrine on Acclaim PA...33

Environmental

LC/MS Detection of Herbicides on Acclaim 120 C18 34
LC Separation of Nitrosamines in EPA 8270 Using Acclaim PA 34
Benzidines by EPA Method 605 on Acclaim 120 C18 with Electrochemical Detection 34
Phenol Standards by EPA Method 604 on Acclaim PA 34
Separation of Phthalate Esters in EPA Method 606 Using Acclaim PA 34
Brodifacoum in Rodent Bait 34
Thirteen Carbonyl-DNPH Derivatives for CARB Method 1004 on Acclaim 120 Å, C18 35
Carbamate Insecticides in EPA 531.1 on Acclaim 120 C8 with MS Detection 35
Improved Separation of 13 Carbonyl-DNPH Derivatives by CARB Method 1004 Using Coupled Acclaim PA and Acclaim C18 Columns 35
Polynuclear Aromatic Hydrocarbon Standards by EPA Method 8310 on Acclaim PA..... 35
LC Separation of Benzidines in EPA 8270 Using Acclaim PA 36
Sensitive Detection of Triazine Herbicides on Acclaim 120 C18 with MS Detection 36
EPA Method 8330 Using Acclaim Explosives E2.. 36
EPA Method 8330 Using Acclaim Explosives E1.. 36
Four Antibiotics in Animal Feed on Acclaim 120 C18 36
Carbamate Insecticide Standards in EPA 531.1 on Acclaim PA 36

Food and Beverage

Fat-Soluble Vitamin Standards on Acclaim 120 C18 37
Amino Acids in Vitamin Premix on Acclaim OA.. 37
Organic Acids in Orange Juice on Acclaim OA 37
Organic Acids in White Wine on Acclaim OA..... 37
Carotenoids in Serum on Acclaim PA 37
Pigments in Turmeric on Acclaim PA2..... 37
Assay of Aspartame and Its Impurities on Three Formats of Acclaim 120 C18 Columns 38
Assay for Water-Soluble Vitamins in Vitamin Tablets Using Acclaim PA 38
Nitrofuran Antibiotic Residues in Animal Feed ... 38
Fat-Soluble Vitamins and Carotenoids in Vitamin Tablets..... 38
Determination of Quinine in Tonic Water..... 39
Determination of Ingredients of an Energy Drink. 39
Hops Extract on Acclaim 120 C18..... 39
Sudan Dyes in Paprika 39
Water-Soluble Vitamins Using Acclaim PA2..... 39
Parabens on Acclaim PA2..... 39
Four Aflatoxin Standards on Acclaim 120 C18..... 40
Fat-Soluble Vitamin Standards Using Acclaim PA 40
Glutamate in Beer by Automated Precolumn OPA Derivatization..... 40
Determination of Soft Drink Ingredients..... 40
Tetracycline Antibiotic Residues in Pork..... 40
Fat-Soluble Vitamins and Carotenoids in Dry Pet Food 40
Antioxidants in Edible Oils by AOAC 983.15 on Acclaim 120 C18 41
Sulfonamide Anibiotic Residues in Milk..... 41
Bitter Principles in Beer on Acclaim 120 C18..... 41

Chemical

Separation of Cationic Surfactants Using ELSD.... 42
Separation of Ethoxylated Quats..... 42
Analysis of ZONYL FSO Fluorosurfactant..... 42
Analysis of a Shampoo..... 42
Analysis of a Fabric Softener..... 42
Analysis of a Mouthwash 42
Separation Hydrotropes, Anionic, Cations, and Amphoteric Surfactiants 43
Analysis of Sodium Lauryl Sulfate Using LC-ESI-MS 43
Analysis of PEG monoethyl ether (MW-550)..... 43
Analysis of Triton X-100 Using LC-ESI-MS 43
Analysis of a Laundry Washing Detergent..... 43
Analysis of a Dishwashing Liquid..... 43
Analysis of Ammonium Laureth Sulfate Using Conductivity Detection 44
Separation of Inorganic Anion, Hydrotropes, Cations, Nonionic, Amphoteric and Anionic Surfactiants 44
Analusis of Toilet Bowl Cleaner..... 44
Analysis of a Strongly Hydrophilic Hydrotrope... 44
Analysis of Quats Using LC-ESI-MS 44
Analysis of NEODOL 25-7 44
Analysis of PEG-200 Distearate..... 45
Separation of Cationic Surfactants with Suppressed Conductivity Detection 45
Separation of Quaternary Imidazolinium Compounds..... 45
Separation of Different Polyethylene Glycols..... 45
POE(30) Ammonium Lauryl Sulfate 45
Sudan Dyes on Acclaim 120 C18..... 45

Bioscience

Tryptic Peptide Map of Cytochrome c on Acclaim 300 C18..... 46
Capillary LC for Peptide Mapping Using Acclaim 300 C18 46
Separation of Protein Standards by Micro-LC on Acclaim 300 C18 46
Tryptic Peptide Map of Bovine Serum Albumin on Acclaim 300 C18..... 46
Cytochrome c from Three Species on Acclaim 300 C18 47
Protein Mixture by Reversed-Phase HPLC on Acclaim 300 C18 47
Peptide Test Mix on Acclaim 300 and PA2 with Formic Acid 47
Comparison of Acid Additives for Peptide Analysis..... 47

Natural Products

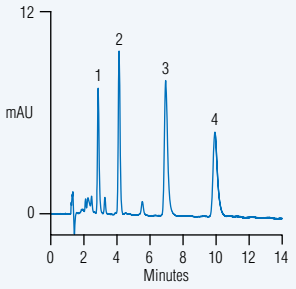
Alkaloids in Goldenseal Root on Acclaim 120 C18 with ASE Extraction 48
Isoflavones in Red Clover on Acclaim 120 C18 with ASE Extraction..... 48
Flavonoids in Green and Black Teas Using Acclaim 120 C18 48

Organic Acids

Resolution of Benzene Polycarboxylic Acids on Acclaim OA 49
Acrylic Acid Oligomers on Acclaim OA 49
Separation of Hydroxybenzoic Acids on Acclaim OA 49

Pharmaceutical

Isocratic Resolution of Antihistamines and Their Impurities on Acclaim 120 C18

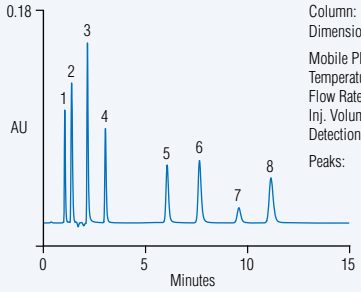


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 50 mM sodium acetate
(B) Methanol
Isocratic: (A) 20%, (B) 80%
Temperature: 25 °C
Detection: UV, 249 nm

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

17510

Beta Blockers on Acclaim PA2

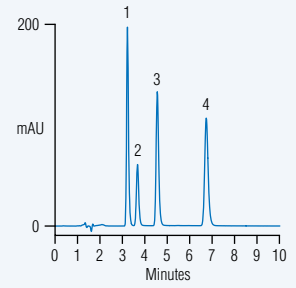


Column: Acclaim PA2, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: MeOH/0.2% NH_4OH, pH10, v/v, 60/40
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm

Peaks: (40 ppm each)
1. Maleate
2. Labetalol
3. Metaraminol
4. Atenolol
5. Acebutolol
6. Metoprolol
7. Timolol
8. Oxprenolol

21134

Separation of Four Benzodiazepine Drugs on Acclaim 120 C18

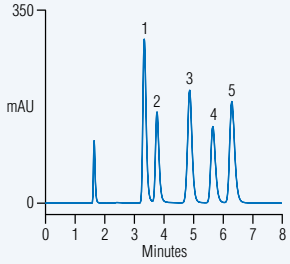


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 60% acetonitrile
Flow Rate: 1.0 mL/min
Detection: UV, 235 nm

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

17511

Separation of Five Antidepressants on Acclaim PA

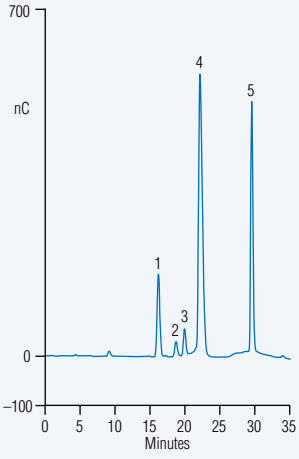


Column: Acclaim PA, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 80/20 v/v MeOH/
30 mM phosphate, pH 6.0
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 220 nm

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

19299

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:

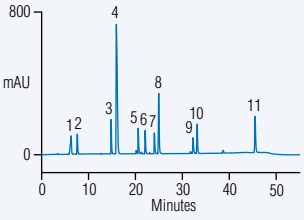
Time	*% A*	*% B*	*% C*
0.0	70	30	0
10.0	70	30	0
10.1	0	100	0
20.0	0	100	0
23.0	0	75	25
37.0	0	75	25

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

Venlafaxine and Related Substances



Data courtesy of: V. Bhate of Analytical Solutions

Column: Acclaim 120 C18, 5 µm,
4.6 × 250 mm
Pump: Summit P680A
Mobile Phase: (A) 10% acetonitrile; 90% water;
1.0 mL/L triethylamine,
adj. to pH 3.5 with phosphoric acid
Mobile Phase: (B) 75% acetonitrile; 25% water;
1.0 mL/L triethylamine,
adj. to pH 3.5 with phosphoric acid
Flow: 1.0 mL/min

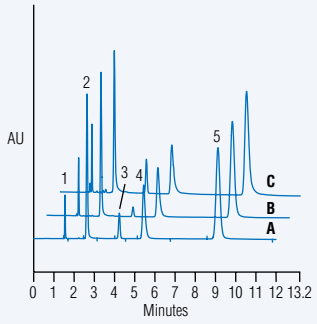
Gradient Times:	0	35	40	45	47	55
%A:	95	25	10	10	95	95
%B:	5	75	90	90	5	5

Temperature: TCC100 thermostat at 35 °C
Injection: ASI-100 autosampler; 20 µL
Detector: UVD 340U; UV at 226 nm
Sample: "Resolution" test mix

Peaks:			
1.	Impurity 1	10	µg/mL
2.	Impurity A	10	
3.	FL II	10	
4.	Venlafaxine	1000	
5.	Impurity III	10	
6.	Impurity C	10	
7.	Impurity B1	10	
8.	PMPA	10	
9.	Impurity IV	10	
10.	FL I	10	
11.	Impurity V	10	

22660

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

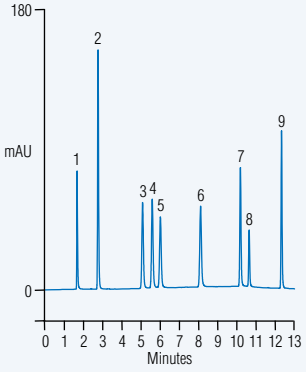
Amitriptyline Mass:
Trace A: 1200 ng
Trace B: 400 ng (normalized peak height)
Trace C: 94 ng (normalized peak height)

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

Gradient Resolution of Sulfa Drugs on Acclaim PA

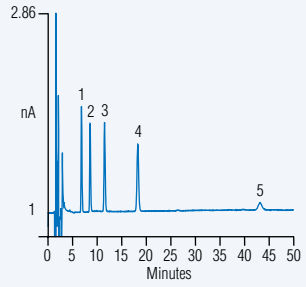


Column: Acclaim PA, 3 µm
Dimensions 4.6 × 150 mm
Mobile Phase: (A) 90/10 methanol/ 20 mM phosphate, pH 2.7
(B) 10/90 methanol/ 20 mM phosphate, pH 2.7
Inj. Volume: 10 µL
Gradient: (A) 15–30% at 5 min, to 75% at 10 min, to 85% at 12 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Detection: UV, 254 nm

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



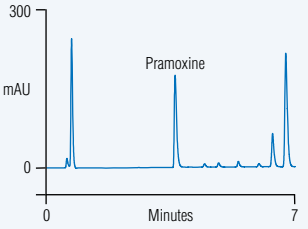
Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 57 mM citric acid, 43 mM sodium acetate, 0.1 mM EDTA, 1 mM sodium octanesulfonate, 10% methanol
Flow Rate: 1 mL/min
Detection: dc amperometry, Glassy Carbon at 700 mV, Ag/AgCl Referemce electrode

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

18358

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

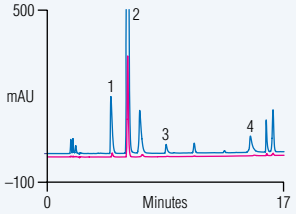
Sample Prep: 1. Disperse 0.5 g ointment in 8.5 mL MeOH
2. Add 0.5 mL conc. NH_4OH and make to 10 mL with MeOH
3. Apply 1.0 mL to a conditioned OnGuard II RP 1-cc cartridge and expel with 1 mL air
4. Elute twice with 2.0 mL of 2% acetic acid in acetonitrile and expel with 1 mL air.
5. Combine and make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

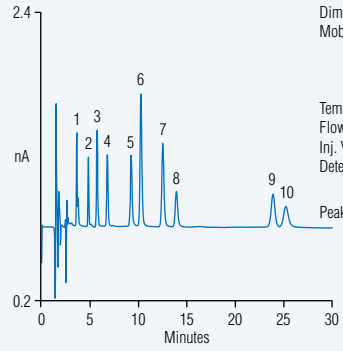
Gradient :

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

Catecholamines in Urine on Acclaim PA2

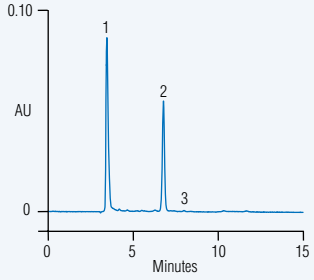


Column: Acclaim PA2, 3 µm
Dimensions: 2.1 × 150 mm
Mobile Phase: Buffer (11.98 g citric acid, 3.53 g NaOAc, 37.2 mg EDTA, 10 mL of 0.1 M methanesulfonic acid in 1 L D.I. water)/MeOH v/v 90/10
Temperature: 30 °C
Flow Rate: 0.2 mL/min
Inj. Volume: 2 µL
Detection: DC amperometry [GC electrode, 800 mV]

Peaks: (1 nM each)
1. 4-Hydroxy-3-methoxymandelic acid
2. 4-Hydroxy-3-methoxyphenylglycol
3. Norepinephrine
4. Epinephrine
5. 3,4-digydroxybenzlamine
6. Normetanephrine
7. Metanephrine
8. Dopamine
9. 4-Hydroxy-3-methoxyphenylacetic acid
10. 5-Hydroxyindoleacetic acid

20466

Bromide and Organic Acids in Cough Syrup Using the Acclaim OA



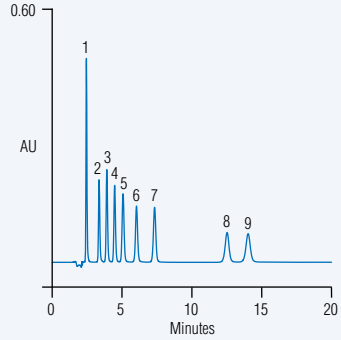
Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methane-sulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample Prep: OnGuard II P
Sample: NyQuil®

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

NyQuil is a registered trademark of Proctor and Gamble.

20025

Sulfa Drugs on Acclaim PA2

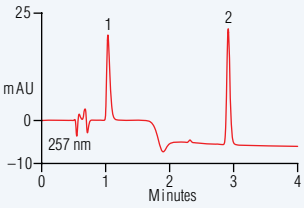


Column: Acclaim PA2, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: CH_3CN/0.1 M NH_4OAc, pH 5.4, v/v, 25/75
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: UV, 265 nm

Peaks: (50 ppm each)
1. Sulfanilamide
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethazine
5. Sulfisoxazole
6. Sulfapyridazine
7. Sulfamethoxazole
8. Sulfadimethoxine
9. Sulfaquinoxaline sodium

21132

Loratidine and Pseudo-ephedrine in a Time-Release Tablet on Acclaim PA



Column: Acclaim PA C16, 5 µm, 4.6 × 50 mm
Pump: Summit P680A DGP-6
Mobile Phases: (A) 15:85:0.050 acetonitrile:water:TFA
(B) 40:60:0.050 acetonitrile:water:TFA
Flow: 1.25 mL/min
Temperature: 35 °C

Gradient:

Times	0.00	0.40	0.41	4.00
%A	100	100	0	0
%B	0	0	100	100

Injection: Summit ASI-100, 10 µL
Detector: Summit UVD 340U diode array, UV at 206, 240 and 257 nm

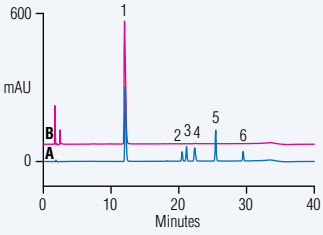
Peaks:

1.	Pseudo-ephedrine	240 µg/mL
2.	Loratidine	10

Sample Preparation:
– Finely grind a weighed tablet.
– Weigh an aliquot containing 1 mg loratidine (120 mg) into a dry 100 mL flask.
– Add 10 mL methanol and disperse.
– Add 3 mL of 1N HCl with mixing.
– Dilute to 100 mL with water.
– Filter through 0.45 µm membrane filter.

21785

Propafenone and Related Substances



Column: Acclaim PA C16, 5 µm, 4.6 × 150 mm
Pump: Summit P680A LPG
Mobile Phase: (A) 0.010 M ammonium acetate, adj. to pH 2.4 with phosphoric acid
(B) Methanol
Flow: 1.0 mL/min

Gradient Times:

	0	5	10	15	30	33
%A:	80	50	40	30	5	80
%B:	20	50	60	70	95	20

Temperature: TCC100 thermostat at 25 °C
Injection: ASI-100 autosampler; 20 µL
Detector: UVD 340U; UV at 249 nm
Sample: (A) "Resolution" test mix
(B) Formulation

Peaks:

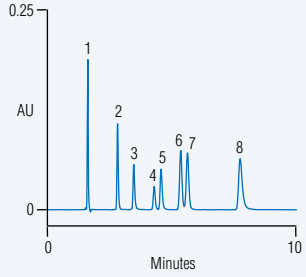
1.	Propafenone	10 µg/mL
2.	Epoxyefenone	22
3.	Chlorohydrine	82
4.	Tertiary amine	44
5.	HBAH	34
6.	Dimer	34

Sample Preparation:
Weigh accurately about 230 mg of sample in 100 ml standard volumetric flask. Add 60 ml of methanol and sonicate for 20–25 min and dilute up to the mark with methanol. Centrifuge the above solution for 2–3 min and further dilute 1 ml of clear supernatant solution to 25 ml with diluent. Diluent is mobile phases A:B 40:60.

Data courtesy of: V. Bhate of Analytical Solutions

22663

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



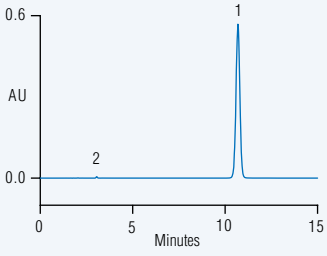
Column: Acclaim 120 C8, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 51/49 w/w MeOH/25 mM phosphate, pH 7.0
Flow Rate: 1.0 mL/min
Temperature: 40 °C
Inj. Volume: 5 µL
Detection: UV, 214 nm

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

Active Ingredient in Zantac® 75 Acid-Reducer Medication



Column: Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Pump: GP50 micro
Mobile Phase: 40 mM KH_2PO_4: acetonitrile:methanol: triethylamine 345:20:35:0.7 v:v:v:v isocratic
Flow: 1.0 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detector: AD20 UV/VIS at 320 nm
Sample Prep.: One tablet dissolved in 100 mL water; filtered

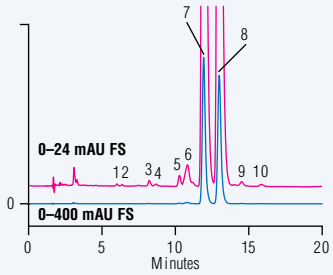
Peaks:
1. Ranitidine
2. Ranitidine S-oxide (impurity)

Reference: Ho C, Huang HM, Hsu SY, Shaw CY, Chang BL, *Drug Dev. Ind. Pharm.* **1999** *25*:379-385.

Zantac is a registered trademark of Warner-Lambert Co.

21535

Budesonide and Related Substances

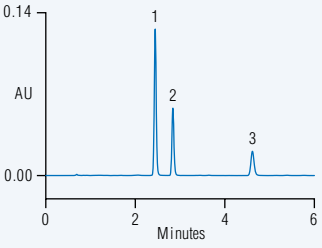


Column:	Acclaim 300 C18, 3 µm, 4.6 x 150 mm
Pump:	Summit P580 HPG/4
Mobile Phase:	(A) Acetonitrile:ethanol 15:1 (B) 0.1% phosphoric acid Isocratic 66% B
Flow:	1.0 mL/min
Temperature:	30 °C
Injection:	ASI-100 autosampler, 15 µL
Detector:	UVD 340U; UV at 240 nm
Sample:	Budesonide, 500 µg/mL after three days
Peaks:	7, 8. Budesonide epimers, 99%

Reference: Hou S, Hindle M, Byron PR; *J. Pharm. Biomed. Analy.* **2001** 24:371-80.

21523

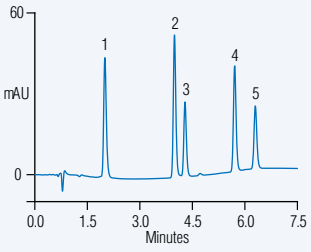
Hydrocortisone in Skin Ointment



Column:	Acclaim 120 C8, 3 µm, 4.6 × 150 mm
Pump:	Dionex GP50 microbore
Mobile Phase:	Acetonitrile:water 49:51
Flow:	1.0 mL/min
Temperature:	30 °C
Injection:	1 µL
Detector:	AD-20; UV at 245nm
Sample Prep.:	– Disperse 100 mg of ointment in 7.0 mL denatured ethanol – Mix in 3.0 mL hexane then 1.0 mL water – Centrifuge to separate emulsion – Filter ethanol layer
Peaks:	1. Hydrocortisone 1.05% (label 1.0%) 2. Methyl paraben 0.32% 3. Propyl paraben 0.21%

21526

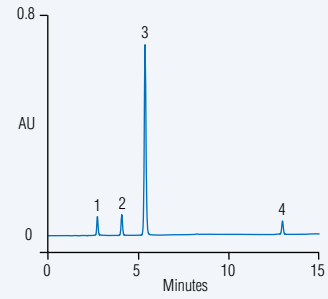
Barbiturates on Acclaim 120 C18



Column:	Acclaim 120 C18 5µm, 4.6 x 50 mm			
Pump:	Summit P580 HPG/4			
Mobile Phase:	(A) Acetonitrile:methanol 4:1 v:v (B) 0.1% phosphoric acid			
Gradient:	Time	0.0	5.0	7.5
	%A	20	45	45
	%B	80	55	55
Flow:	1.0 mL/min			
Temperature:	30 °C			
Injection:	ASI-100 autosampler, 3 µL			
Detector:	UVD 340U, UV at 210 nm			
Sample:	25 µg/mL each in methanol			
Peaks:	1. Barbital 2. Phenobarbital 3. Butabarbital 4. Amobarbital 5. Secobarbital			

21521

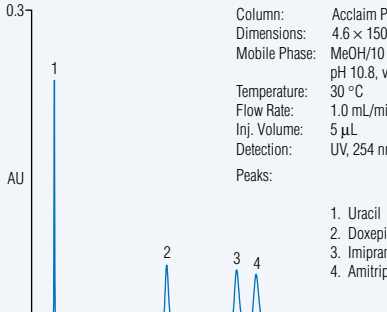
Active Ingredients in Cough Syrup



Column:	Acclaim PA2, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	Acetonitrile/ 50 mM phosphate buffer, pH 1.9 from 10/90 to 40/60 (v/v) in 15 min
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
Detection:	UV, 210 nm
Peaks:	1. Doxylamine 2. Pseudo-ephedrine 3. Acetaminophen 4. Dextromethorphan

21136

Antidepressants on Acclaim PA2

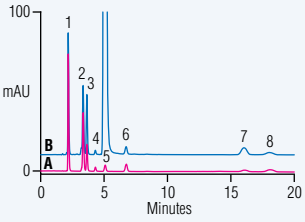


Column:	Acclaim PA2, 5µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	MeOH/10 mM triethylamine, pH 10.8, v/v 75/25
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Detection:	UV, 254 nm

Peaks:	Concentration (µg/mL)	Asymmetry (EP)
1. Uracil	10	1.08
2. Doxepin	100	1.14
3. Imipramine	80	1.15
4. Amitriptyline	100	1.11

20465

Phenytoin and Related Substances



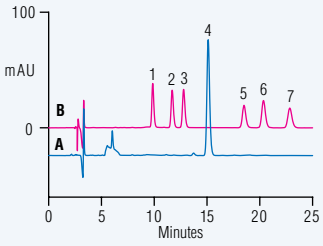
Data courtesy of: V. Bhate of Analytical Solutions

Column:	Acclaim 120, C18 5 µm, 4.6 × 150 mm
Pump:	Summit P680A
Mobile Phase:	20% methanol; 30% acetonitrile; 50% 0.05 M $NH_4H_2PO_4$ adj. to pH 2.5
Flow:	1.5 mL/min
Temperature:	TCC100 thermostat at 60 °C
Injection:	ASI-100 autosampler; 25 µL
Detector:	UVD 340U; UV at 220 nm
Samples:	(A) "Low load" test mix (B) "Resolution" test mix

Peaks:		
1. Impurity A	1	µg/mL
2. Impurity B	1	
3. Benzoic acid	9	
4. Benzaldehyde	9	
5. Phenytoin sodium	1000 (B) or 3 (A)	
6. Benzoin	9	
7. Benzophenone	1	
8. Benzil	9	

22664

Glucocorticosteroids in Serum on Acclaim 120 C18



Reference: McWhinney B C, Ward G, Hickman P E; *Clin. Chem*, **1996,** 42:979-981.

Column: Acclaim 120 C18 5 µm, 4.6 × 250 mm
Pump: Summit P580 HPG/4
Mobile Phase: Methanol:tetrahydrofuran:water 3:25:72 v:v:v, isocratic
Flow: 1.0 mL/min
Temperature: 30 °C
Injection: ASI-100 autosampler, 60 µL
Detector: UVD 340U; UV at 240 nm
Samples: (A) Extract of Bovine Serum extracted with ethyl acetate from serum alkalized with NaOH
(B) Six steroid standards, 5 µg/mL in mobile phase

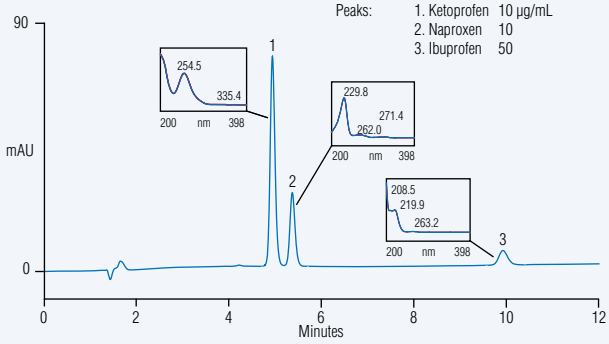
Peaks:
1. Prednisone
2. Prednisolone
3. Cortisol
4. Fludrocortisone (I.S.)
5. Methylprednisolone
6. 11-Deoxycortisol
7. Dexamethasone

21532

Nonsteroidal Anti-inflamatory Drugs on Acclaim PA2 C18

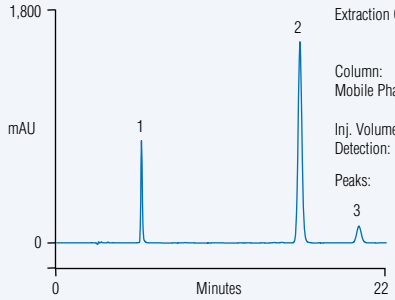
Column: Acclaim PA2 C18, 5 µm, 4.6 × 150 mm
Pump: Summit P 680A DGP-6
Mobile Phase: Acetonitrile:0.1% phosphoric acid, 55:45 v/v; isocratic
Flow: 1.0 mL/min
Temperature: 30 °C
Injection: Summit ASI-100, 20 µL
Detector: Summit UVD340U diode array, 265nm, and spectra 200–400 nm

Peaks:
1. Ketoprofen 10 µg/mL
2. Naproxen 10
3. Ibuprofen 50



21557

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

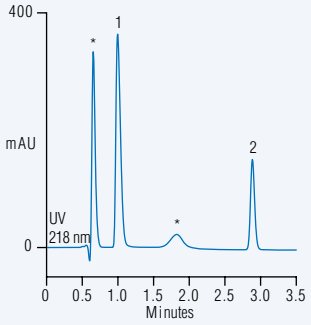


Extraction Conditions: ASE 200 with 11 mL extraction cell 50/50 MeOH/CH_2Cl_2
Column: Acclaim 120 C18
Mobile Phase: Isocratic 85/14/0.75 methanol/water/HOAc
Inj. Volume: 20 µL
Detection: UV, 310 nm

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

20398

Fexofenadine and Pseudoephedrine on Acclaim PA



Column: Acclaim PA C16, 5 µm, 4.6 × 50 mm
Pump: Summit P 680A DGP-6
Mobile Phases: A. 15:85:0.047 acetonitrile:water:TFA
B. 44:66:0.050 acetonitrile:water:TFA
Flow: 1.25 mL/min
Temperature: 35 °C

Gradient Times:

	0.00	0.80	0.81	3.50
%A	100	100	0	0
%B	0	0	100	100

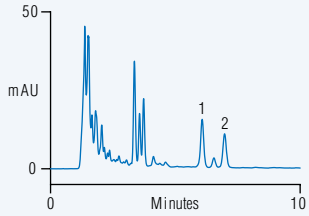
Injection: Summit ASI100, 20 µL
Detector: Summit UVD340U diode array, UV at 207, 218 and 257 nm

Peaks:
1. Pseudo-ephedrine 200 µg/mL
2. Fexofenadine 20
*. Artifacts

21559

Environmental

Brodifacoum in Rodent Bait



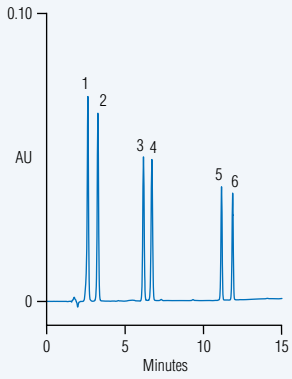
Column: Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Pump: Summit P580 HPG/4
Mobile Phase: (A) Acetonitrile
(B) 0.10% conc. phosphoric acid in water
Isocratic A:B 82.5:17.5
Flow: 1.20 mL/min
Temperature: 30 °C
Injection: Summit ASI-100, 10 µL
Detector: Summit UVD 340U diode array, 265 nm, and spectra 200–400 nm

Sample Prep.:
- Mutually saturate ethyl acetate and water for use in the next step
- Shake 1.0g of finely ground bait with 10.0 mL ethyl acetate, 5.0 mL water and 0.10 mL acetic acid
- Centrifuge to separate the emulsion
- Evaporate 3.0 mL of ethyl acetate fraction to dryness
- Reconstitute in 0.60 mL acetonitrile
- Filter

Peaks: 1. Brodifacoum isomer 1
2. Brodifacoum isomer 2

21522

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:

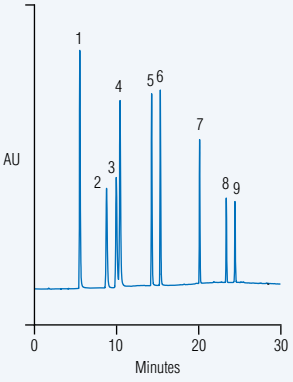
Time (min)	*% B*
0	25
10	100
15	100

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

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

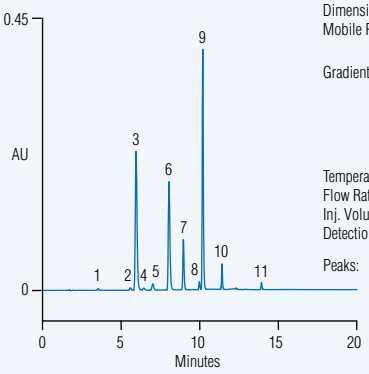
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 15 µL
Detection: UV, 230 nm

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

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

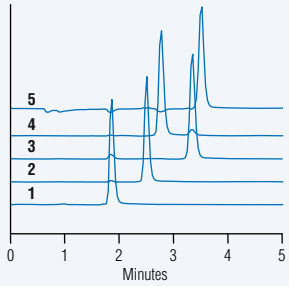
Temperature: 25 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 285 nm

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

LC/MS Detection of Herbicides on Acclaim 120 C18



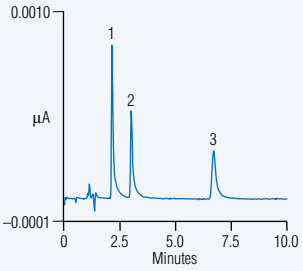
Column: Acclaim 120 C18, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phase: 70/30% methanol/ 0.1% formic acid
Flow Rate: 0.25 mL/min
Detection: AQA™ MS, +ESI, 30 V, 200 °C, SIM as indicated

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

17922

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

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

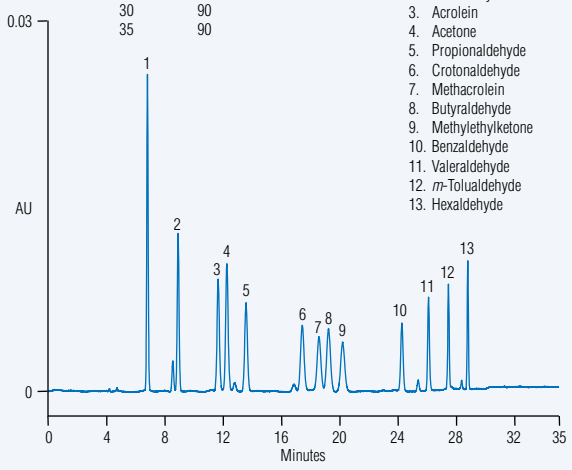
Columns: Acclaim PA, 5 µm, 4.6 × 150 mm, coupled with Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Mobile Phase: (A) H_2O
(B) CH_3CN

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

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

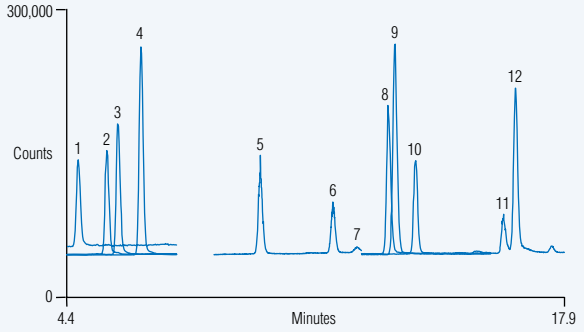
Gradient:

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

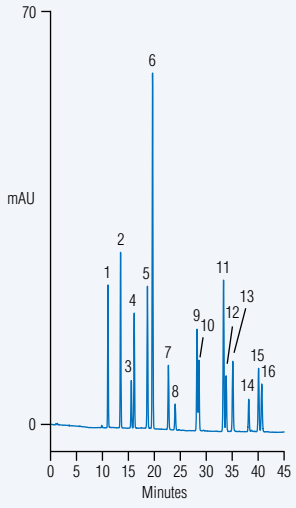
Detection: MSQ™ MS +ESI
MS Settings: Probe 400 °C
Needle 2.0 kV
Cone voltage set per ion

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



20399

Polynuclear Aromatic Hydrocarbon Standards 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

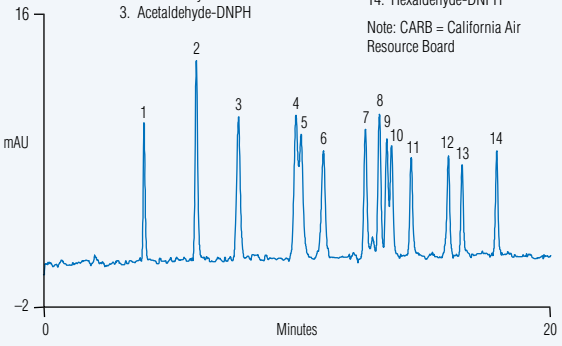
19305

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

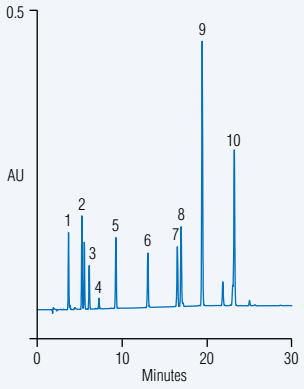
Peaks (DNPH and 13 carbonyl derivatives) 0.3 µg/mL:
1. 2,4-DNPH
2. Formaldehyde-DNPH
3. Acetaldehyde-DNPH
4. Acrolein-DNPH
5. Acetone-DNPH
6. Propionaldehyde-DNPH
7. Crotonaldehyde-DNPH
8. Methacrolein-DNPH
9. 2-Butanone-DNPH
10. Butyraldehyde-DNPH
11. Benzaldehyde-DNPH
12. Valeraldehyde-DNPH
13. *m*-Tolualdehyde-DNPH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

Note: CARB = California Air Resource Board



20111

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:

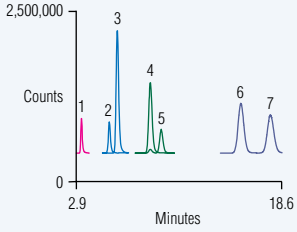
Time (min)	*% B*
0	10
30	70

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

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



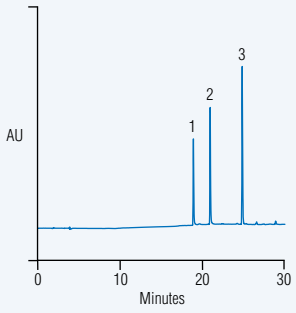
Background Information: Seven herbicides at 1 ng on column. Seven simultaneous single-ion chromatograms optimized for positive identification.

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

LC Separation of Benzidines in EPA 8270 Using Acclaim PA



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

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

Gradient:

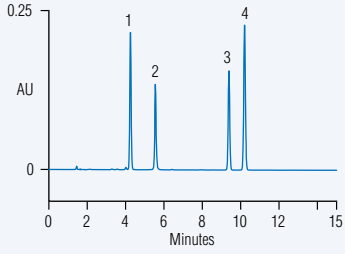
Time (min)	*% B*
0	1
5	1
25	90
30	90

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

20109b

Four Antibiotics in Animal Feed on Acclaim C18



Column: Acclaim 120 C18, 5µm, 4.6 × 150 mm
Pump: GP50 microbore
Mobile Phase A: 20 mM formic acid
Mobile Phase B: Acetontirile

Gradient Times:

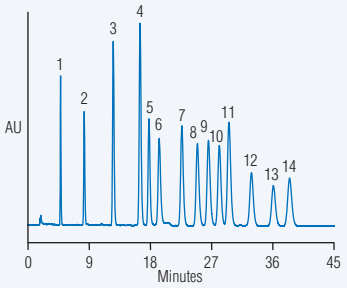
	0	15
%A:	85	50
%B:	15	50

Flow: 1.00 mL/min
Temperature: 30 °C
Injection: 5 µL
Detector: UV, 280 nm

Peaks: (100 µg/mL each)
1. Sulfamerazine
2. Oxytetracycline
3. Chloramphenicol
4. Sulfadimethoxine

22662

EPA Method 8330 Using Acclaim Explosives E1 Column

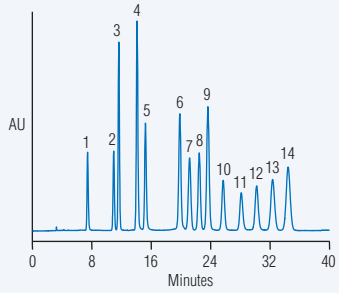


Column: Acclaim Explosives E1, 5 mm, 4.6 × 250 mm
Mobile Phase: 43/57 v/v MeOH/H_2O
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 254 nm

Peaks:
1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
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

22784

EPA Method 8330 Using Acclaim Explosives E2 Column



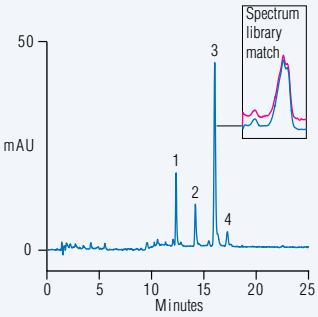
Column: Acclaim Explosives E2, 5 mm, 4.6 × 250 mm
Mobile Phase: 50/50 v/v MeOH/H_2O
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 254 nm

Peaks: (EPA 8330 mix, 50 ppm each)
1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. Tetryl
8. 2,6-Dinitrotoluene
9. 2,4-Dinitrotoluene
10. 2-Nitrotoluene
11. 4-Nitrotoluene
12. 3-Nitrotoluene
13. 4-Amino-2,6-Dinitrotoluene
14. 2-Amino-4,6-Dinitrotoluene

22779

Food and Beverage

Carotenoids in Serum on Acclaim PA



Column: Acclaim PA C16, 3 µm, 4.6 × 150 mm
Pump: Summit P580 HPG/4
Mobile Phase: (A) Water
(B) 54:44:2 methanol:acetonitrile:isopropanol

Gradient:

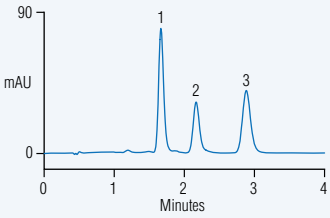
Time	0	8.0	8.1	25
%A	5	5	0	0
%B	95	95	100	100

Flow: 1.25 mL/min
Temperature: 30 °C
Injection: Summit ASI-100, 25 µL
Detector: Summit UVD 340U diode array, 450 nm and spectra 200–595 nm
Sample: Extract of bovine serum, fortified with lycopene equivalent to 300 ng/mL

Peaks:
1. Unidentified carotenoid
2. *trans*-lycopene
3. *trans*-β-carotene
4. *cis*-β-carotene

21524

Pigments in Turmeric on Acclaim PA2



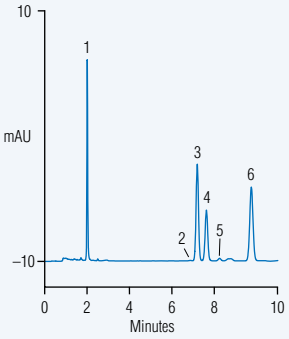
Column: Acclaim PA2, 3 µm, 4.6 × 50 mm
Pump: Summit P680 DGP
Mobile Phase: (A) 10 mM phosphoric acid
(B) Methanol
Isocratic: 78% B
Flow: 1.50 mL/min
Temperature: Summit TCC-100, 30 °C
Injection: Summit ASI-100, 10 µL
Detector: Summit UVD340U diode array, 428 nm, and spectra 200–595 nm

Peaks:
1. Curcumin
2. Demethoxycurcumin
3. *Bis*-demethoxycurcumin

Sample Preparation:
– Extraction solvent: 70% ethanol, 29.5% water, 0.5% phosphoric acid
– Extract 100 mg of ground turmeric with 20 mL of solvent in a capped vial at 65 °C for 15 min
– Centrifuge
– Dilute 10× in 75% methanol
– Filter

22659

Fat-Soluble Vitamin Standards on Acclaim 120 C18

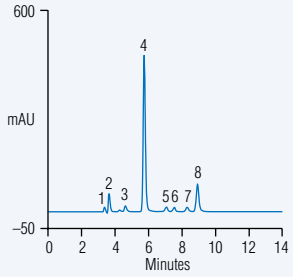


Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 95/5 v/v acetonitrile/methanol
Flow Rate: 2.0 mL/min
Temp.: 30 °C
Inj. Volume: 5 µL
Detection: UV, 280 nm

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

Amino Acids in Vitamin Premix on Acclaim OA

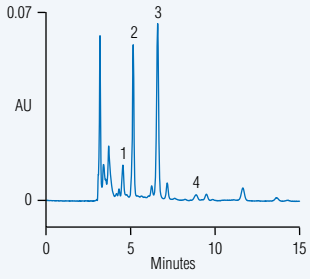


Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 40 mM Na_2SO_4 adjusted to pH 3.05 with methanesulfonic acid
Flow Rate: 0.60 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: UV, 210 nm

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

20412

Organic Acids in Orange Juice on Acclaim OA

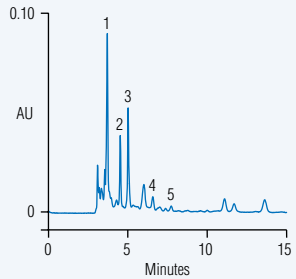


Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample Prep.: OnGuard II P, diluted 2× with D.I. H_2O

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

20023

Organic Acids in White Wine on Acclaim OA

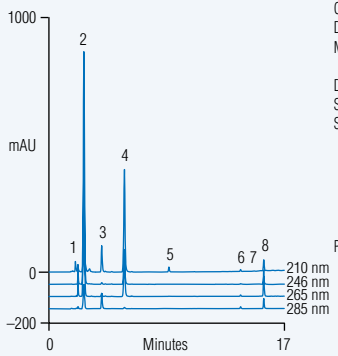


Column: Acclaim OA, 5 µm
Dimensions: 4 × 250 mm
Mobile Phase: 0.1 M Na_2SO_4, pH 2.68 (adjusted with methanesulfonic acid)
Temperature: 30 °C
Flow Rate: 0.6 mL/min
Inj. Volume: 5 µL
Detection: UV, 210 nm
Sample prep: OnGuard II P

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

20024

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



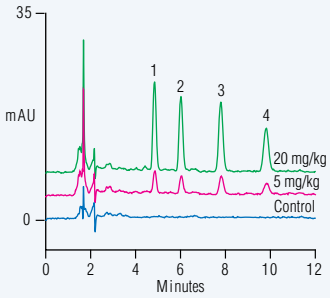
Column: Acclaim PA, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: Phosphate pH 3.4, gradient 0–20% CH_3CN
Detection: UVD 340 at listed wavelengths
Sample: One-A-Day® brand "Active Formula"
Sample Prep.:
1. Grind a 1500 mg tablet
2. Extract 150 mg with 100 mL of pH 3.4 buffer
3. Sonicate 10 min
4. Filter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

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

19350

Nitrofuran Antibiotic Residues in Animal Feed on Acclaim 120 C18



Reference:
R.J. McCracken, D.G. Kennedy;
J. Chromatogr. A, **1997,** *771*, 349–354.

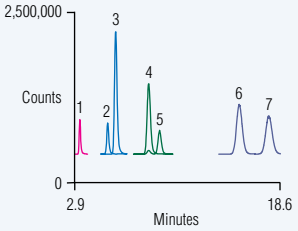
Column: Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Pump: Summit P680A DGP-6
Mobile Phase: Acetonitrile: 10 mM ammonium acetate pH 5.0, 20:80 v/v; isocratic
Flow: 1.0 mL/min
Temperature: 30 °C
Injection: Summit ASI100, 20 µL
Detector: Summit UVD340U diode array, 365 nm, and spectra 200–500 nm

Sample Preparation:
1. 3.0 g guinea pig feed in a 50 mL centrifuge tube
2. Add 9 mL water and let stand 5 min.
3. Add 21 mL methanol:acetonitrile 1:1 and extract for 30 min.
4. Pass through cleanup cartridge containing 1.7g of neutral alumina; discard first 1.7 mL, retain next 3.5 mL

Peaks:
1. Nitrofurazone
2. Nitrofurantoin
3. Furazolidone
4. Furaltadone

21558

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



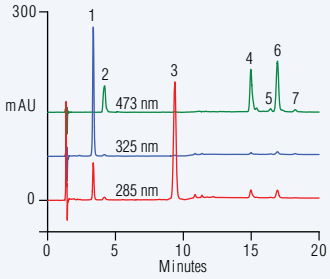
Background Information: Seven herbicides at 1 ng on column. Seven simultaneous single-ion chromatograms optimized for positive identification.

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

Fat-Soluble Vitamins and Carotenoids in a Vitamin Tablet



Sample Preparation:
– Finely grind a weighed tablet.
– Weigh an aliquot of about 1/10 tablet (150 mg) into a screw-cap vial.
– Partition between 1.0 mL water and 5.0 mL ethyl acetate with vigorous shaking.
– Warm to 60 °C for 10 min.
– Shake vigorously again for 0.5 min.
– Centrifuge to separate phases.

Column: Acclaim PA C16, 3 µm, 4.6 × 150 mm
Pump: Summit P680A DGP-6
Mobile Phase: (A) Water
(B) 54:44:2 methanol:acetonitrile: isopropanol

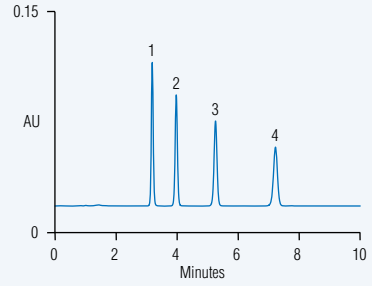
Gradient:	*Times*	0	8.0	8.1	25
	%A	5	5	0	0
	%B	95	95	100	100

Flow: 1.25 mL/min
Temperature: 30 °C
Injection: Summit ASI-100, 15 µL
Detector: Summit UVD 340U diode array, 473, 325 and 285 nm, and spectra 200–595 nm

Peaks:
1. *trans*-Retinol acetate
2. *trans*-Lutein
3. α-Tocopherol acetate
4. *trans*-Lycopene
5. *cis*-Lycopene
6. *trans*-β-Carotene
7. *cis*-β-Carotene

21786

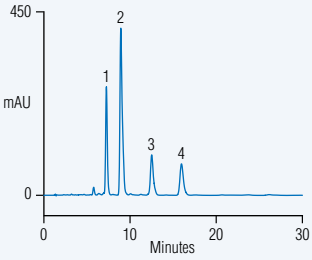
Parabens on Acclaim PA2



Column:	Acclaim PA2, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	CH_3CN/H_2O v/v 60/40
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	2 µL
Detection:	UV, 254 nm
Peaks:	(0.1 mg/mL each) 1. Methylparaben 2. Ethylparaben 3. Propylparaben 4. Butylparaben

20464

Hop Extract on Acclaim 120 C18



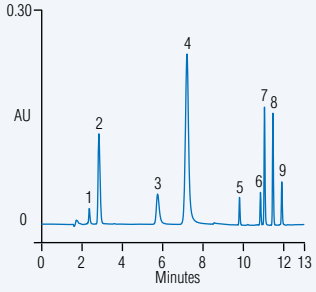
*Obtained from Am. Soc. Brewing Chemists

Column:	Acclaim 120 C18, 5µm, 4.6 × 150 mm
Pump:	Summit P680A DGP-6
Mobile Phase:	Methanol, water, phosphoric acid; 85:17:0.25 (v/v/v)
Flow:	1.2 mL/min
Temperature:	TCC-100 thermostat, 35 °C
Injection:	ASI100 autosampler, 25 µL
Detector:	UVD-340U, UV 314 nm; spectra 200–400 nm
Standard*:	ICE-2 certified hop extract, 986 µg/mL in acidified methanol

Peaks:		
1. Cohumulone	142	µg/mL
2. Humulone + adhumulone	344	
3. Colupulone	127	
4. Lupulone + adlupulone	118	

21997

Water-Soluble Vitamins Using Acclaim PA2

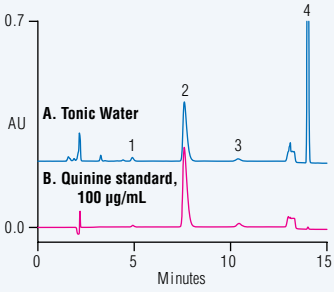


Column:	Acclaim PA2, 5 µm
Dimensions:	4.6 150 mm
Mobile Phase:	(A) CH_3CN (B) 25 mM phosphate buffer, pH 3.31
Gradient:	Hold B for 5 min B to A/B (40/60) in 7 min then to A/B (75/25) in 1 min Hold A/B (75/25) for 4 min
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	50 µL
Detection:	UV, 210 nm

Peaks:		
1. Thiamine	2.5	µg/mL
2. Ascorbic acid	60	
3. Pyridoxine	2.5	
4. Niacinamide	20	
5. Pantothenic acid	10	
6. Vitamin B12	1.25	
7. Folic acid	5	
8. Riboflavin	5	
9. Biotin	12.5	

20281

Determination of Quinine in Tonic Water Using Acclaim PA



Reference: *U.S. Pharmacopeia,* 22nd Ed., **1990,** 1209.

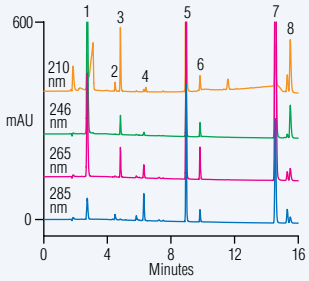
Column:	Acclaim PA C16, 5 µm, 4.6 × 150 mm
Pump:	GP50 microbore
Mobile Phase:	(A) 896 mL water, 100 mL acetonitrile, 1.40 mL methanesulfonic acid, 0.80 mL acetic acid, 2.00 mL diethylamine (B) 70:30 v:v acetonitrile:water

Gradient:					
Time	0.0	10.5	10.6	15.0	
%A	100	100	50	50	
%B	0	0	50	50	

Flow:	1.0 mL/min
Temperature:	30 °C
Injection:	25 µL
Detector:	AD20, UV 235 nm
Sample Prep.:	Filter
Peaks:	1. Cinchonidine 2. Quinine 3. Dihydroquinine 4. Benzoic acid

21528

Determination of Energy Drink Ingredients with Acclaim PA2



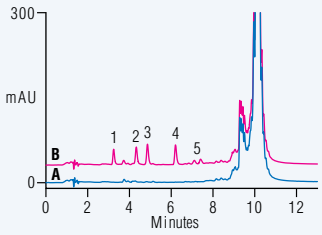
Column:	Acclaim PA2, 3 µm, 4.6 × 150 mm
Pump:	Summit P680 DGP6
Mobile Phases:	(A) Acetonitrile (B) 30 mM phosphate buffer, pH 3.28 (C) 30 mM phosphate buffer, pH 2.54

Gradient:			
Times	0.0	14.0	16.0
%A	0	40	40
%B	100	0	0
%C	0	60	60

Flow:	1.0 mL/min
Temperature:	30 °C
Injection:	ASI-100 autosampler, 5 µL
Detector:	UVD 340U, UV 210, 246, 265, 285 nm; spectra 200–400 nm
Sample Prep:	Filter
Peaks:	1. Ascorbic acid 2. Pyridoxine 3. Niacinamide 4. Pantothenic acid 5. Caffeine 6. Riboflavin 7. Sorbic acid 8. Benzoic acid

21784

Sudan Dyes in Paprika



Sample Preparation:
- Finely grind dried peppers
- Take an aliquot of about 400 mg
- Partition between 2.0 mL water and 6.0 mL ethyl acetate with vigorous shaking for 2 min
- Centrifuge to separate phases
- Evaporate 1.0 mL of ethyl acetate phase and reconstitute in 0.33 mL ethanol

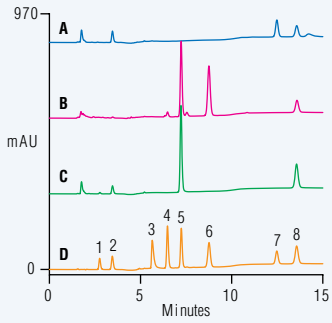
Column:	Acclaim 120 C18, 5 µm, 4.6 × 150 mm
Pump:	Summit P680 DGP
Mobile Phase:	(A) Water (B) 1:1 tetrahydrofuran:methanol

Gradient:				
Times	0	7.0	7.1	13
%A	20	8	0	0
%B	80	92	100	100

Flow:	1.25 mL/min
Temperature:	30 °C
Injection:	Summit ASI-100, 15 µL
Detector:	Summit UVD340U diode array, 500 nm, and spectra 200–595 nm
Samples:	(A) Control paprika without artificial coloring (B) Paprika spiked with approx. 25 mg/kg of each dye
Peaks:	1. Sudan I 2. Sudan II 3. Sudan III 4. Sudan IV 5. Oil Red O (2 peaks) (Natural pigments 8-12 min.)

21787

Determination of Soft Drink Ingredients with Acclaim OA



Note: For alternate calibration mixes, use ascorbic acid instead of erythorbic acid, or use saccharin instead of aspartame.

Minute-Maid and Diet Coke are registered trademarks of Coca-Cola. Mountain Dew is a registered trademark of Pepsico.

Column: Acclaim OA, 5 µm, 4.0 × 150 mm
Pump: Summit P580 HPG/4
Mobile Phase: (A) 14.2 g/L Na_2SO_4 + 0.550 mL/L CH_3SO_3H in water
(B) Methanol

Gradient:

Time	0.0	1.0	2.0	6.5	7.5	15.0
%A	100	100	69	69	60	60
%B	0	0	31	31	40	40

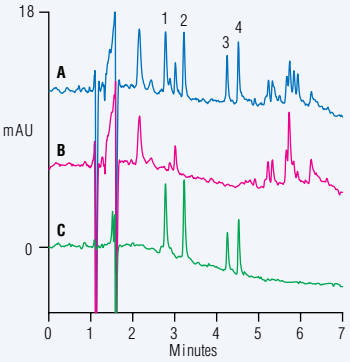
Flow: 0.8 mL/min
Temperature: 30 °C
Injection: ASI-100 autosampler, 10 µL
Detector: UVD 340U, UV 210 nm; optional 230, 262 nm
Samples: (A) Minute-Maid® Orange Soda
(B) Diet Coke®
(C) Mountain Dew®
(D) Standards
Sample Prep.: Filter and dilute 5× with mobile phase A

Peaks:

1. Erythorbic acid	50 µg/mL
2. Citric acid	500
3. Acesulfame-K	50
4. Aspartylphenylalanine	50
5. Caffeine	12
6. Aspartame	50
7. Sorbate potassium	50
8. Benzoic acid	50

21531

Tetracyclines in Pork on Acclaim PA



Sample Prep.: - 2.0 g pork muscle tissue
- Grind with mortar and pestle in sequence with
0.1 g of citric acid
1.0 mL of 3.6 M HNO_3
4.0 mL of methanol
1.0 mL of water
- Centrifuge

Reference: Farrington et. al., "Food Additives and Contaminants", 1991, Vol. 8, No. 1, 55-64.

Column: Acclaim PA C18, 5 µm, 4.6 × 150 mm
Pump: Summit P580 HPG/4
Mobile Phase: (A) 22 mM H_3PO_4, 0.5 mM $Na_4P_2O_7$
(B) Acetonitrile

Gradient:

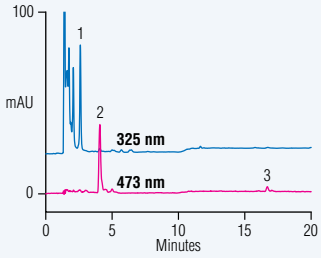
Time	0.0	0.5	7.0
%A	82	82	45
%B	18	18	55

Flow: 1.5 mL/min
Temperature: 30 °C
Injection: ASI-100 autosampler, 60 µL
Detector: UVD 340U, UV at 360 nm
Samples: (A) Spiked pork muscle, 1.0 µg/g each
(B) Control pork
(C) Tetracycline standards, 0.33 µg/mL in water

Peaks:
1. Oxytetracycline
2. Tetracycline
3. Chlortetracycline
4. Doxycycline

21534

Fat-Soluble Vitamins and Carotenoids in Dry Pet Food



Sample Preparation:
Extraction by AOAC Official Method 970.64
– 4.0 g of finely ground feed
– Add 30 mL of hexane-acetone-ethanol-toluene, 10:7:6:7, v/v/v/v
– Add 4 mL of 40% KOH in methanol
– Saponify at 56 °C for 20 min
– Cool for 1 hr
– Add 30 mL hexane
– Dilute to 100 mL with 10% sodium sulfate solution
– Shake vigorously
– For HPLC, evaporate 1.0 mL of hexane phase and reconstitute in 0.10 mL of acetone

Column: Acclaim PA C16, 3 µm, 4.6 × 1 50 mm
Pump: Summit P680A DGP-6
Mobile Phase: (A) Water
(B) 54:44:2 methanol:acetonitrile: isopropanol

Gradient Times:

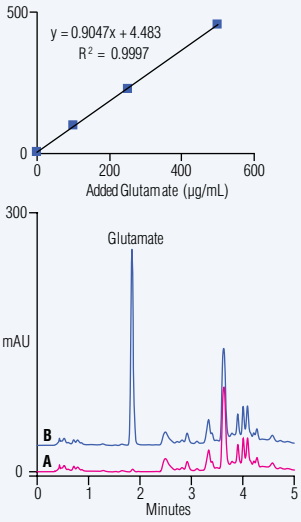
	0	8.0	8.1	25
%A	5	5	0	0
%B	95	95	100	100

Flow: 1.25 mL/min
Temperature: 30 °C
Injection: Summit ASI-100, 15 µL
Detector: Summit UVD 340U diode array, 473, 325 and 285 nm, and spectra 200–595 nm

Peaks:
1. *trans*-Retinol
2. *trans*-Lutein
3. *trans*-β-Carotene

22661

Glutamate in Beer by Automated Precolumn OPA Derivatization



Column: Acclaim PA, C16 5 µm, 4.6 × 50 mm
Pump: Summit P680A
Mobile Phases: (A) Methanol
(B) 20 mM phosphate, pH 7.0

Gradient Times:

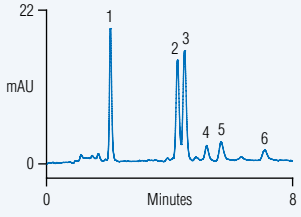
	0.0	2.0	3.0	5.0	5.1	6.5
%A:	10	30	75	75	10	10
%B:	90	70	25	25	90	90

Flow: 1.5 mL/min
Temperature: TCC100 thermostat at 30 °C
Injection: ASI-100 autosampler; 20 µL
Detector: UVD 340U; UV at 330nm and spectra 200–400 nm
Samples: (A) Beer
(B) Beer + 250 µg/mL glutamic acid

Derivatizing Conditions: (Reagent A) 2 mg/mL o-phthalaldehyde in 0.5M borate buffer, pH 10.4
(Reagent B) 6 mg/mL $(CH_3)_2NCH_2CH_2SH$ HCl in water
Program: 75 µL reagent A + 75 µL reagent B + 15 µL sample, mixed in round-bottom glass vial
Reaction Time: 5 min at room temperature

23065

Fat-Soluble Vitamin Standards Using Acclaim PA

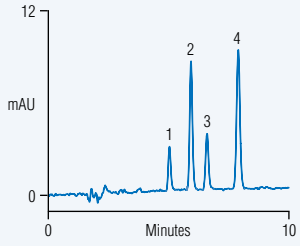


Column: Acclaim PA, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phase: 97/3 acetonitrile/NH_4HCO_2, pH 3.4
Flow Rate 0.25 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: UV, 285 nm

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

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

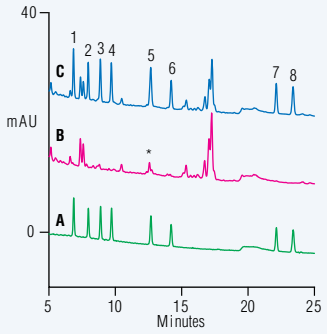
Peaks:

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

20403

APPLICATIONS

Sulfonamide Antibiotic Residues in Milk on Acclaim 120 C18



Column: Acclaim 120 C18, 5 µm, 4.6 × 250 mm
Pump: Summit P580 HPG/4
Mobile Phase: (A) 30 mM acetic acid adjusted to pH 4.2 with NaOH
(B) Methanol

Gradient Times:

0.0	20	25
%A	88	60	60
%B	12	40	40

Flow: 1.5 mL/min
Temperature: 35 °C
Injection: ASI-100 autosampler, 100 µL
Detector: UVD 340U, UV at 265 nm with spectral confirmation
Sample Prep.: AOAC 993.32; refer to procedure for important notes
Samples: (A) Spiked extract of skim milk, 1.0 µg/g each
(B) Milk extract
(C) Sulfonamide standards, 0.20 µg/mL in water

Peaks:
1. Sulfadiazine (SDZ)
2. Sulfathiazole (STZ)
3. Sulfapyridine (SPD)
4. Sulfamerazine (SMR)
5. Sulfamethazine (SMZ)
6. Sulfachloropyridazine (SCP)
7. Sulfadimethoxine (SDM)
8. Sulfaquinoxzline (SQX)
*. Theobromine

Note: Most interferences are organic acids that may be moved out of the way by small adjustements to the pH of the mobile phase.

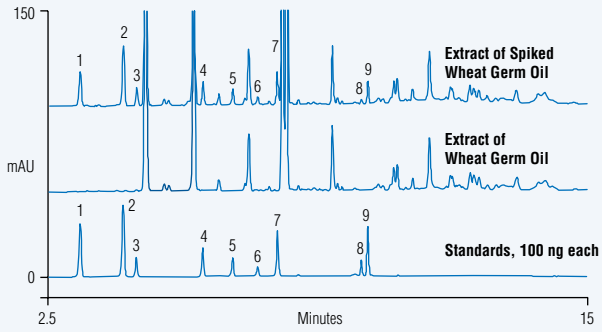
21533

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

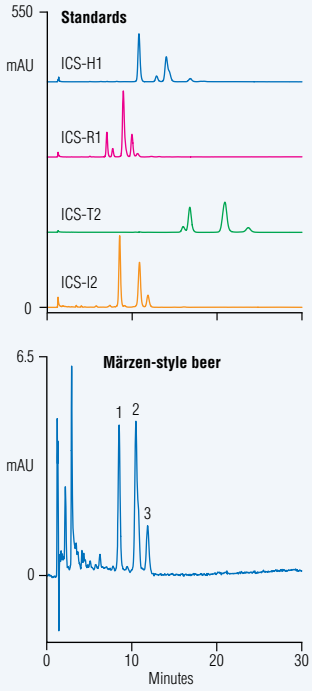
Peaks:
1. Propyl gallate
2. THBP
3. TBHQ
4. NDGA
5. BHA
6. Ionox-100
7. Octyl gallate
8. BHT
9. Dodecyl gallate



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

20404

Bitter Principles in Beer on Acclaim 120 C18



Column: Acclaim 120 C18 5µm, 4.6 × 150 mm
Pump: Summit P680A DGP-6
Mobile Phase: 75% methanol, 24% water, 1% phosphoric acid (v/v/v)
Flow: 1.2 mL/min
Temperature: TCC-100 thermostat, 35 °C
Injection: ASI100 autosampler, 25 µL
Detector: UVD-340U, UV 270 nm; spectra 200–400 nm

Standards*:

ICS-I2: isohumulones	205 µg/mL
ICS-T2: tetrahydroisohumulones	135
ICS-R1: rho-isohumulones	200
ICS-H1: hexahydroisohumulones	210

Peaks:
1. Isocohumulone
2. Mixed isohumulone congeners
3. Isoadhumulone

Sample Preparation:
Extraction per ASBC
- 10 mL beer + 1 mL 3N HCl + 50 µL 1-octanol + 20 mL iso-octane
- Shake vigorously
- Centrifuge to separate phases
- Bitterness Units = 50 × A_{275}

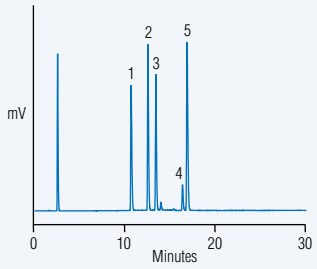
For HPLC
- Evaporate dry and reconstitute in mobile phase

*Obtained from Am. Soc. Brewing Chemists

22027

Surfactants

Separation of Cationic Surfactants Using ELSD

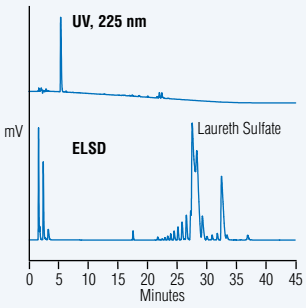


Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25–85% A in 30 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 25 µL
Detection: ELSD

Peaks:
1. Lauryl pyridinium chloride
2. Lauryldimethylbenzyl ammonium chloride
3. Octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride
4. Cetyltrimethylammonium chloride
5. Cetylpyridinium chloride

21082

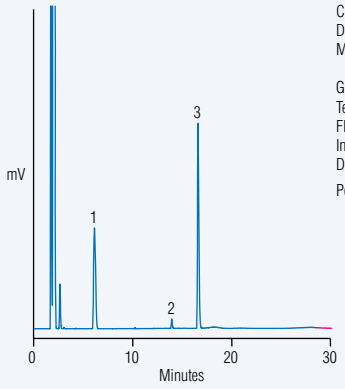
Analysis of a Shampoo



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25–80% A in 30 min, then hold at 80% A for 15 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELSD, UV

21083

Analysis of a Mouthwash

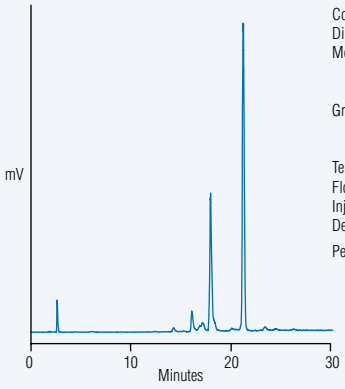


Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25–80% A in 30 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 20 µL (direct injection)
Detection: ELSD

Peaks:
1. Sodium sacchrin and benzoate
2. Domiphen bromide
3. Cetylpyridinium chloride

21120

Analysis of a Fabric Softener

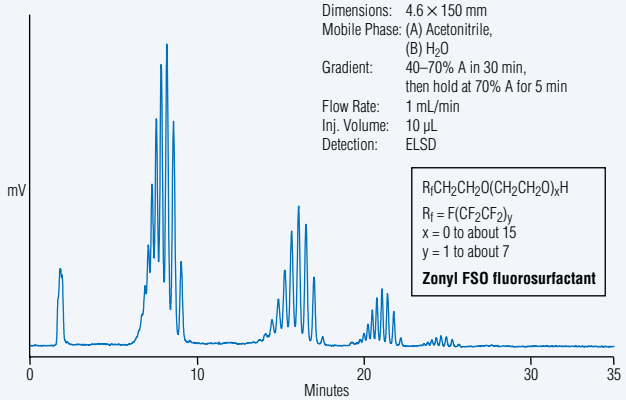


Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) THF
(C) 0.1 M NH_4OAc, pH 5.4
Gradient: 40% A/10% B/50% C to 75% A/10% B/75% C in 20 min, then hold for 10 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 20 µL
Detection: ELSD

Peaks: Methyl bis[ethyl(tallowate)]-2-hydroxyethyl ammonium methyl sulfate (500×)

21121

Analysis of ZONYL FSO Fluorosurfactant

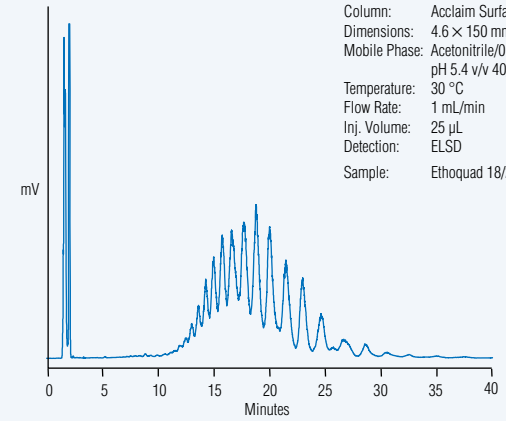


Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile,
(B) H_2O
Gradient: 40–70% A in 30 min, then hold at 70% A for 5 min
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELSD

$R_fCH_2CH_2O(CH_2CH_2O)_xH$
$R_f = F(CF_2CF_2)_y$
x = 0 to about 15
y = 1 to about 7
Zonyl FSO fluorosurfactant

21081

Separation of Ethoxylated Quats



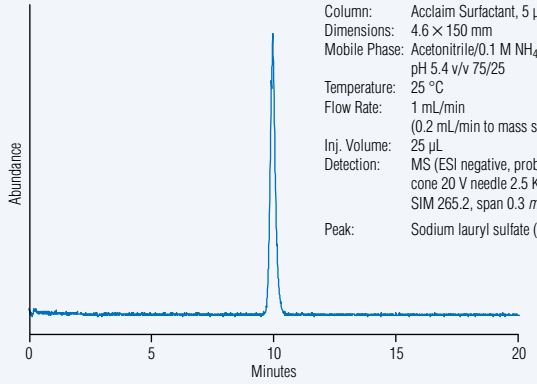
Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: Acetonitrile/0.2 M NH_4OAc, pH 5.4 v/v 40/60
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 25 µL
Detection: ELSD

Sample: Ethoquad 18/25 (500×)

21116

Surfactants

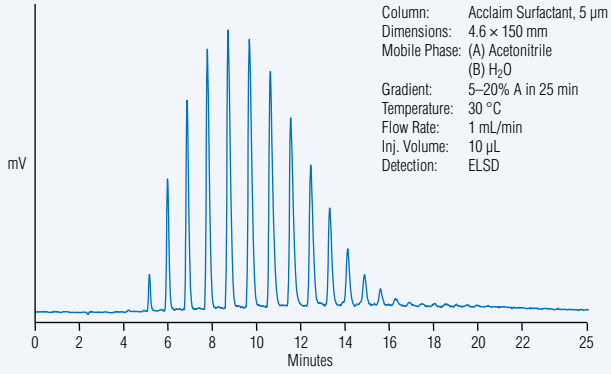
Analysis of Sodium Lauryl Sulfate Using LC-ESI-MS



Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	Acetonitrile/0.1 M NH_4OAc, pH 5.4 v/v 75/25
Temperature:	25 °C
Flow Rate:	1 mL/min (0.2 mL/min to mass spectrometer)
Inj. Volume:	25 µL
Detection:	MS (ESI negative, probe 300 °C, cone 20 V needle 2.5 KV, SIM 265.2, span 0.3 *m/z*)
Peak:	Sodium lauryl sulfate (1 ppm)

21126

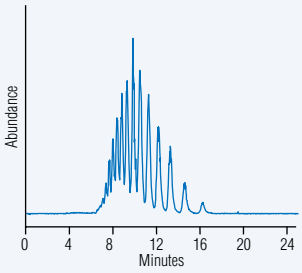
Analysis of PEG Monoethyl Ether (MW-550)



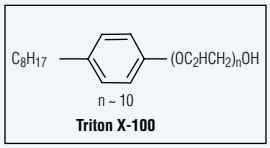
Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) Acetonitrile (B) H_2O
Gradient:	5–20% A in 25 min
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	ELSD

21118

Analysis of Triton X-100 Using LC-ESI-MS

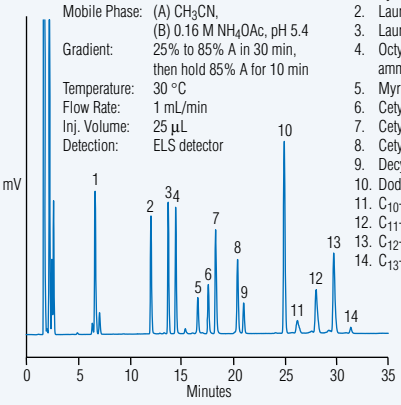


Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	Acetonitrile/50 mM NH_4OAc, pH 5.4 v/v 45/55
Temperature:	25 °C
Flow Rate:	1 mL/min (0.2 mL/min to mass spectrometer)
Inj. Volume:	25 µL
Detection:	MS (ESI positive, probe 300 °C, cone 20 V, needle 2.5 kV, SIM 312.2, 356.2, 400.4, 444.4, 488.4, 532.4, 576.5, 620.6, 664.6, 708.6, 752.5, 796.6, 840.7, 884.7, 928.7, 972.7, 1016.8, 1060.7, 1104.7, span 0.5 *m/z*)
Peak:	Triton X-100 (100 ppm)



21128

Separation of Hydrotrope, Anionic, Cationic, and Amphoteric Surfactants

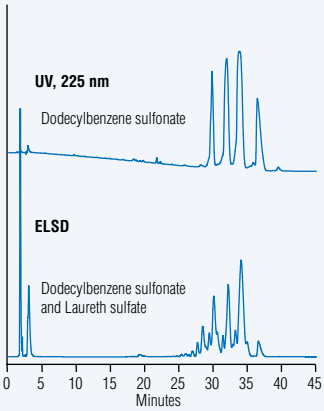


Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) CH_3CN, (B) 0.16 M NH_4OAc, pH 5.4
Gradient:	25% to 85% A in 30 min, then hold 85% A for 10 min
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	25 µL
Detection:	ELS detector

Peaks:
1. Xylene sulfonate
2. Laurylpyridinium chloride
3. Lauryldimethylbenzyl ammonium chloride
4. Octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride
5. Myristamidopropyl betaine
6. Cetyltrimethyl ammonium chloride
7. Cetylpyridinium chloride
8. Cetyl betaine
9. Decyl sulfate
10. Dodecyl sulfate
11. C_{10}-LAS
12. C_{11}-LAS
13. C_{12}-LAS
14. C_{13}-LAS

22794

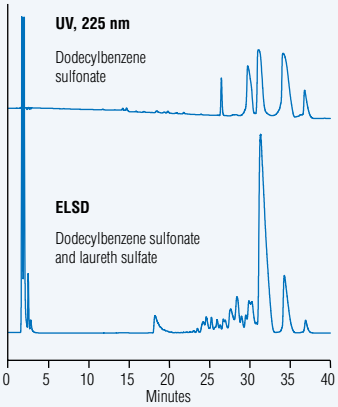
Analysis of a Laundry Washing Detergent



Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) Acetonitrile (B) 0.1 M NH_4OAc, pH 5.4
Gradient:	25–80% A in 30 min, then hold at 80% A for 15 min
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Detection:	ELSD, UV
Sample Prep:	Dilute 10× with 70% acetonitrile, then filtered through 0.2-µm membrane

21084

Analysis of a Dishwashing Liquid

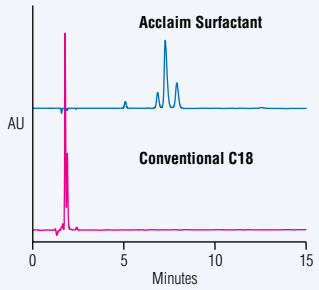


Column:	Acclaim Surfactant, 5 µm
Dimensions:	4.6 × 150 mm
Mobile Phase:	(A) Acetonitrile (B) 0.1 M NH_4OAc, pH 5.4
Gradient:	25–80% A in 30 min, then hold at 80% A for 10 min
Temperature:	30 °C
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Detection:	ELSD, UV
Sample Prep:	Dilute 10× with 70% acetonitrile, then filtered through 0.2-µm membrane

21085

Surfactants

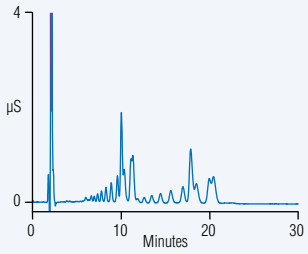
Analysis of a Strongly Hydrophilic Hydrotrope



Dimensions: 4.6 × 150 mm
Mobile Phase: Acetonitrile/0.1 M NH_4OAc, pH 5.4 v/v 30/70
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Detection: UV, 225 nm
Sample: Sodium xylene sulfonate (1000 ppm)

21074

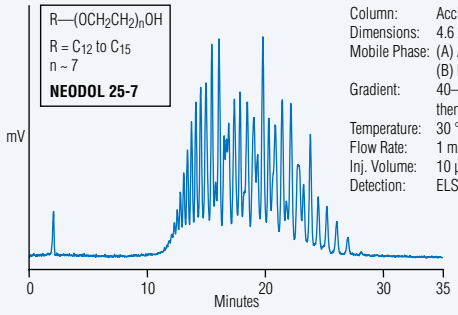
Analysis of Ammonium Laureth Sulfate Using Conductivity Detection



Column: Acclaim Surfactant, 5 µm
Dimension: 4.6 × 150 mm
Mobile Phase: Acetonitrile/0.5 M H_3BO_4 and 20 mM NaOH v/v 55/45
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: Suppressed CD (AMMS III)
Sample: Ammonium laureth sulfate

21076

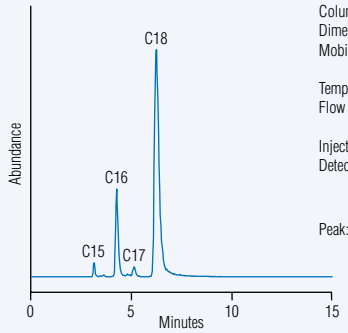
Analysis of NEODOL 25-7



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile (B) H_2O
Gradient: 40–65% A in 30 min, then hold at 65% A for 5 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELSD

21079

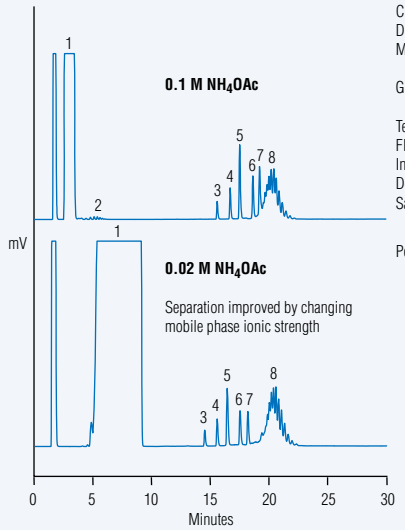
Analysis of Quats Using LC-ESI-MS



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: Acetonitrile/50 mM NH_4OAc, pH 5.4 v/v 60/40
Temperature: 25 °C
Flow Rate: 1 mL/min (0.2 mL/min to mass spectrometer)
Injection Vol.: 25 µL
Detection: MS (ESI positive, probe 300 °C, cone 30 V, needle 2.5 kV, SIM 332.4, 360.4, 374.4, 388.5, span 0.5 *m/z*)
Peak: Benzyl dimethyl hydrogenated tallow ammonium chloride (10 ppm)

22127

Analysis of Toilet Bowl Cleaner



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) CH_3CN (B) NH_4OAc, pH 5.4
Gradient: 25% to 85% A in 25 min, hold for 5 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELS detector
Sample: Toilet bowl cleaner (20× dilution)

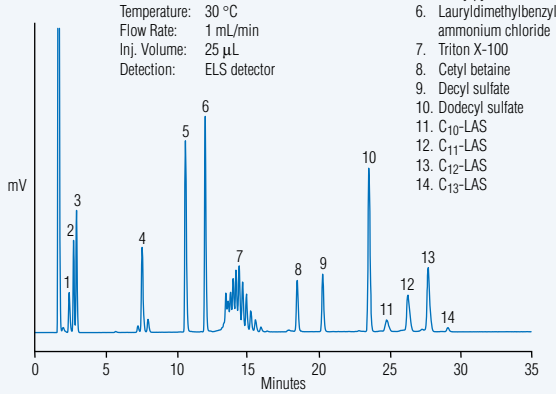
Peaks:
1. Chloride
2. PEG
3–7. Cationic surfactants
8. Nonionic surfactant

22797

Inorganic Anions, Hydrotropes, Cationic, Nonionic, Amphoteric, and Anionic Surfactants

Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) CH_3CN, (B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25% to 85% A in 25 min, then hold 85% A for 10 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 25 µL
Detection: ELS detector

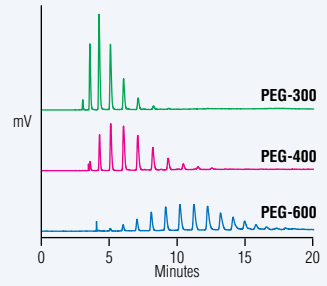
Peaks:
1. Chloride
2. Bromide
3. Nitrate
4. Xylene sulfonate
5. Laurylpyridinium chloride
6. Lauryldimethylbenzyl-ammonium chloride
7. Triton X-100
8. Cetyl betaine
9. Decyl sulfate
10. Dodecyl sulfate
11. C_{10}-LAS
12. C_{11}-LAS
13. C_{12}-LAS
14. C_{13}-LAS



22793

Surfactants

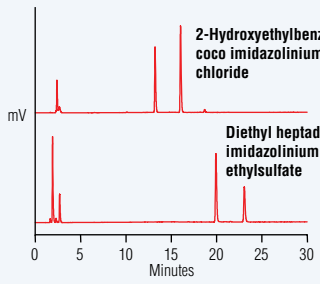
Separation of Different Polyethylene Glycols



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) H_2O
Gradient: 3–15% A in 20 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELSD

21077

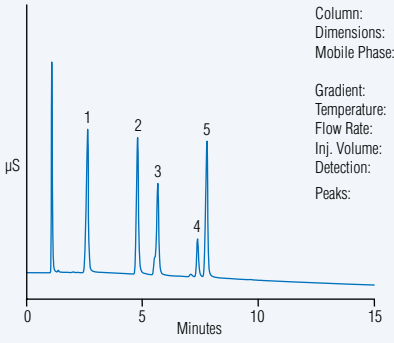
Separation of Quaternary Imidazolinium Compounds



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25–85% A in 30 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: ELSD

21117

Separation of Cationic Surfactants with Suppressed Conductivity Detection

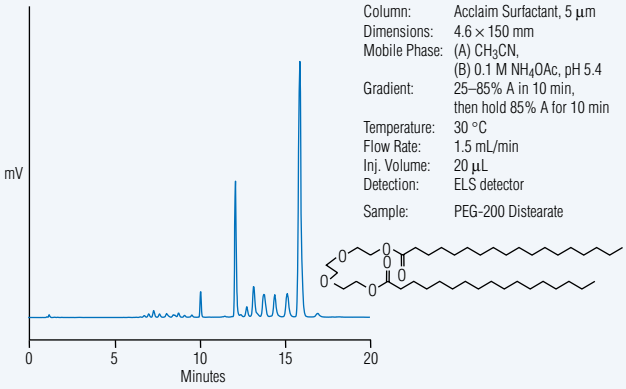


Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) Acetonitrile
(B) 20 mM acetic acid
Gradient: 20–50% A in 15 min
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Detection: Suppressed CD (AMMS III)

Peaks:
1. Lauryl pyridinium chloride
2. Lauryldimethylbenzyl ammonium chloride
3. Octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride
4. Cetyltrimethylammonium chloride
5. Cetylpyridinium chloride

21086

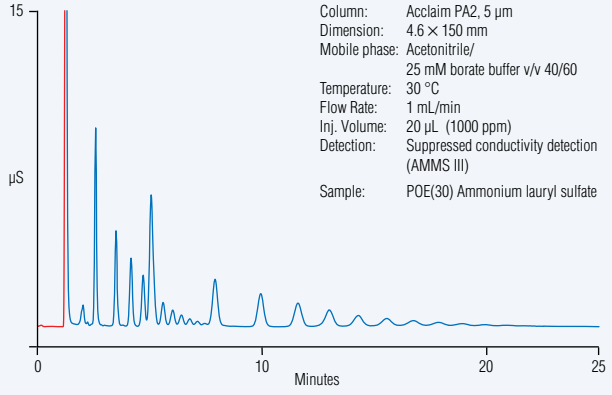
Analysis of PEG-200 Distearate



Column: Acclaim Surfactant, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) CH_3CN,
(B) 0.1 M NH_4OAc, pH 5.4
Gradient: 25–85% A in 10 min,
then hold 85% A for 10 min
Temperature: 30 °C
Flow Rate: 1.5 mL/min
Inj. Volume: 20 µL
Detection: ELS detector
Sample: PEG-200 Distearate

22796

POE(30) Ammonium Lauryl Sulfate

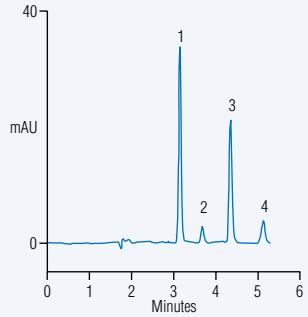


Column: Acclaim PA2, 5 µm
Dimension: 4.6 × 150 mm
Mobile phase: Acetonitrile/
25 mM borate buffer v/v 40/60
Temperature: 30 °C
Flow Rate: 1 mL/min
Inj. Volume: 20 µL (1000 ppm)
Detection: Suppressed conductivity detection (AMMS III)
Sample: POE(30) Ammonium lauryl sulfate

21137

Dyes

Sudan Dyes on Acclaim 120 C18



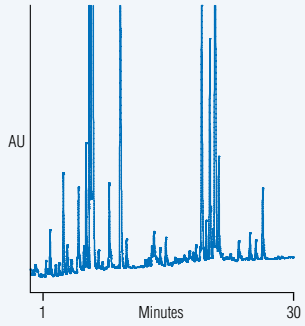
Column: Acclaim 120 C18, 3 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 50/50/0.5 MeOH/acetonitrile/HOAc
Flow Rate: 1.0 mL/min
Inj. Volume: 20 µL
Detection: UV, 280 nm

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

20411

Proteins and Peptides

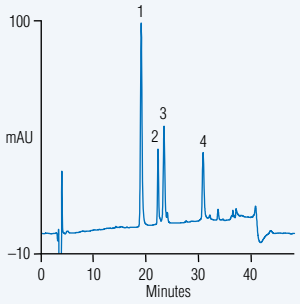
Tryptic Peptide Map of Cytochrome c on Acclaim 300 C18



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

Separation of Protein Standards by Micro LC on Acclaim 300 C18

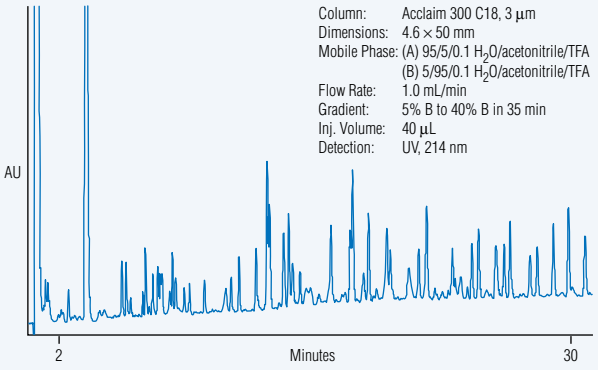


Column: Acclaim 300 C18, 3 µm
Dimensions: 300-µm i.d. × 15 cm
Mobile Phase: (A) Water. 0.05% TFA
(B) 8/2 CH_3CN/ water, 0.04 % TFA
Gradient: 15–60% B in 30 min
Flow Rate: 4 µL/min
Detection: UV, 214 nm

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

20409

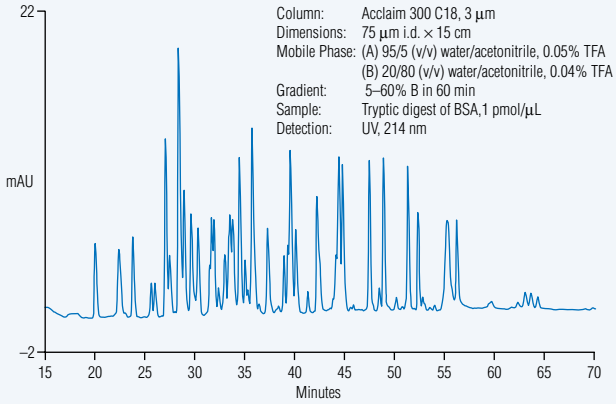
Tryptic Peptide Map of Bovine Serum Albumin on Acclaim 300 C18



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

Capillary LC for Peptide Mapping Using Acclaim 300 C18

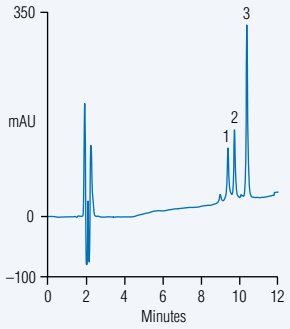


Column: Acclaim 300 C18, 3 µm
Dimensions: 75 µm i.d. × 15 cm
Mobile Phase: (A) 95/5 (v/v) water/acetonitrile, 0.05% TFA
(B) 20/80 (v/v) water/acetonitrile, 0.04% TFA
Gradient: 5–60% B in 60 min
Sample: Tryptic digest of BSA,1 pmol/µL
Detection: UV, 214 nm

20408

Proteins and Peptides

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:

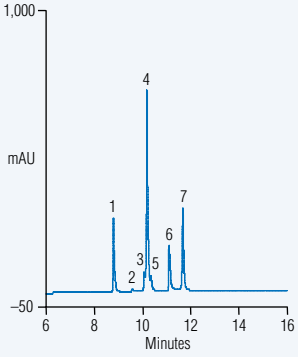
Time (min)	*% A*	*% B*
–5	80	20
0	80	20
15	45	55
20	45	55

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

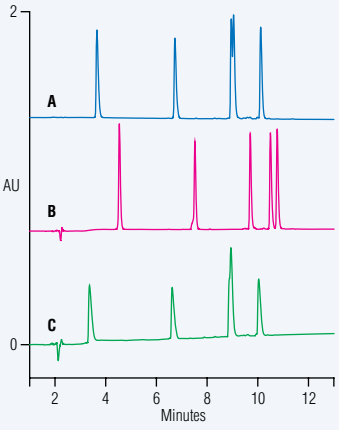
Gradient:

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 anhydrase
3. Impurity in lysozyme
4. Lysozyme
5. Impurity in carbonic anhydrase
6. Myoglobin
7. Carbonic anhydrase

20407

Comparison of Acid Additives for Peptide Analysis on Acclaim 300



Column: Acclaim 300 C18, 3 µm, 4.6 × 150 mm
Pump: Summit P680A
Mobile Phases: A: 70% acetonitrile v/v
B: Water
C: Acid stock solution

Gradient Times:

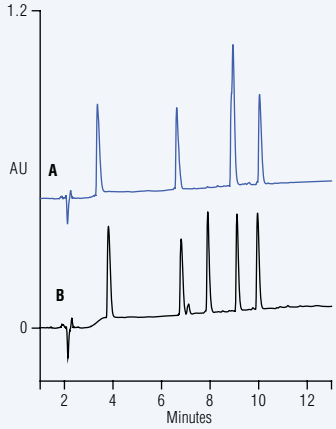
	–8	0	16
%A	7	7	75
%B	68	68	0
%C	25	25	25

Flow: 1.0 mL/min
Temperature: TCC100 thermostat at 30 °C
Injection: ASI-100 autosampler; 5 µL
Detector: UVD 340U; UV at 214 nm
Acid Additive: A. Phosphoric Acid, 0.15%
B. Trifluoroacetic Acid, 0.1%
C. Formic Acid, 0.1%
Sample: Sigma H2016 Peptide Mix

Peaks:
1. Gly-Tyr
2. Val-Tyr-Val
3. Met-Enkephalin
4. Angiotensin-II
5. Leu-Enkephalin

23064

Peptide Test Mix on Acclaim 300 and PA2 with Formic Acid



Columns: (A) Acclaim 300 C18, 3 µm, 4.6 × 150 mm
(B) Acclaim PA2, 3 µm, 4.6 × 150 mm
Pump: Summit P680A
Mobile Phases: (A) 70% acetonitrile v/v
(B) Water
(C) 0.4% formic acid v/v

Gradient Times:

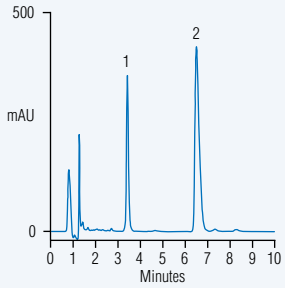
	–8	0	16
%A	7	7	75
%B	68	68	0
%C	25	25	25

Flow: 1.0 mL/min
Temperature: TCC100 thermostat at 30 °C
Injection: ASI-100 autosampler; 5 µL
Detector: UVD 340U; UV at 214 nm
Sample: Sigma H2016 Peptide Mix

Peaks:
1. Gly-Tyr
2. Val-Tyr-Val
3. Met-Enkephalin
4. Angiotensin-II
5. Leu-Enkephalin

23066

Alkaloids in Goldenseal Root on Acclaim 120 C18 with ASE Extraction

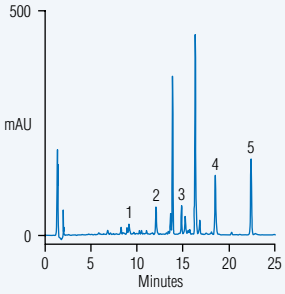


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: 73/27 0.1% H_3PO_4 in D.I. H_2O/ acetonitrile
Flow Rate: 1.8 mL/min
Inj. Volume: 10 µL
Detection: UV, 235 nm

Peaks:
1. Hydrastine
2. Berberine

17630

Isoflavones in Red Clover on Acclaim 120 C18 with ASE Extraction

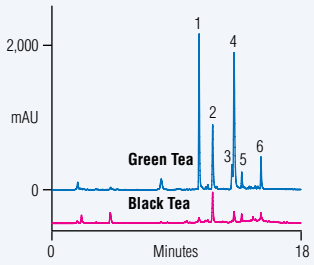


Column: Acclaim 120 C18, 5 µm
Dimensions: 4.6 × 150 mm
Mobile Phase: (A) 0.1% H_3PO_4 in D.I. H_2O
(B) Acetonitrile
Gradient: 10% B to 76% B over 35 min
Flow rate: 1.2 mL/min
Inj. Volume: 10 µL
Detection: UV, 260 nm

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:

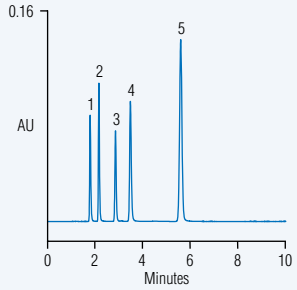
Time	*% A*	*% B*	*Flow*
0	5	95	1.00
5	5	95	
8	15	85	
10	15	85	
15	40	60	
25	60	40	

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

Separation of Hydroxybenzoic Acids on Acclaim OA

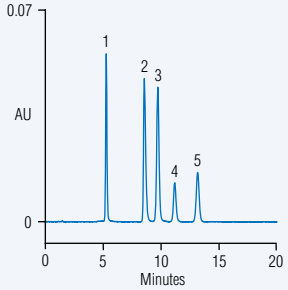


Column: Acclaim OA, 5 µm
Dimensions: 4 × 150 mm
Mobile Phase: 30/70 v/v CH_3CN/5 mM methanesulfonic acid
Temperature: 30 °C
Flow Rate: 0.75 mL/min
Inj. Volume: 5 µL
Detection: UV, 230 nm

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

Resolution of Benzene Polycarboxylic Acids on Acclaim OA

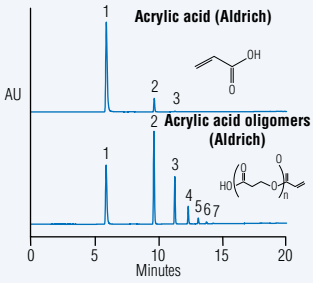


Column: Acclaim OA, 5 µm,
Dimensions: 4 × 150 mm
Mobile Phase: 12.5/87.5 v/v CH_3CN/ 5 mM methanesulfonic acid
Temperature: 30 °C
Flow Rate: 0.75 mL/min
Inj. Volume: 5 µL
Detection: UV, 230 nm

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

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

PART FOUR: APPENDIX

Appendix 1: Lot Qualification Tests for Acclaim-Bonded Silica

Column Performance Test, Acclaim C18, C8, PA, PA2

Polar Selectivity

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

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	
In. 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. 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.

Column Performance Test, Acclaim Surfactant

Isocratic Surfactant Selectivity

Each column is tested under these conditions to guarantee that the column is well manufactured. Column pressure, efficiency, asymmetry and retention time of propylbenzene are specified for each column. This test also is used to monitor batch-to-batch reproducibility as measured by the relative retention of an anionic surfactant to a neutral marker.

Mobile Phase:	50:50 (v/v) acetonitrile : 0.1M NH_4OAc, pH 5.2	
Flow Rate:	1.0 mL/min	
Temperature:	30 °C	
Inj. Volume:	5 µL	
Detection:	UV, 220 nm	
Peaks:	1. Uracil	0.10 mg/mL
	2. 2-(4-octylphenoxy)ethoxyethyldimethylbenzyl ammonium chloride	0.10 mg/mL
	3. Sodium p-Toluenesulfonate	0.20 mg/mL
	4. Propylbenzene	0.40 mg/mL

Gradient Surfactant Resolution

An application-based test for the resolution of anionic, non-ionic and cationic surfactants.

Mobile Phase A: Acetonitrile

Mobile Phase B: 0.10 M Ammonium acetate, pH 5.4

Time	%A	%B
0.0	25	75
25.0	85	15
30.0	85	15

Flow Rate:	1.0 mL/min	
Temperature:	30 °C	
Inj. Volume:	20 µL	
Detection:	Evaporative Light Scattering	
Analytes:	1. Laurylpyridinium chloride	0.10 mg/mL
	2. Lauryldimethylbenzyl ammonium chloride	0.10 mg/mL
	3. Triton X-100	0.40 mg/mL
	4. Sodium decylsulfate	0.20 mg/mL
	5. Sodium dodecylsulfate	0.10 mg/mL

Column Performance Test, Acclaim Explosives E1 and E2

Each column is tested under these conditions to guarantee that the column is well manufactured. Column pressure, efficiency, asymmetry, and retention time of naphthalene are specified for each column.

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	
Peaks:	1. Uracil	0.05 mg/mL
	2. Naphthalene	0.5 mg/mL

Appendix 2: Topical Index

Acclaim 120 C18 4-5, 7, 10-11, 12 13, 29-41, 48, 50
120 C8 4-5, 7, 10, 14-15, 31, 35, 50
300 C18 4-5, 7, 10, 16-17, 32, 46-47, 50
PA 4-5, 7, 10-11, 18-20, 29-31, 33-40, 50
PA2 4-5, 7, 10, 20-21, 30-33, 39, 47, 50
PolarAdvantage, see Acclaim PA and PA2
OA 4-6, 22-23, 31, 37, 40, 49
Surfactant 4-6, 24-25, 42-45, 51
Explosives 4-6, 26-27, 36, 51

Acid Aromatic 22, 49
Asymmetry 10, 19, 21
Asymmetry test 50
Hydrophilic 22
Organic 4, 6, 22-23, 37

Alkaline Buffers 9, 21

Amitriptyline 10, 29, 50

Antibiotics

Asymmetry 12, 14, 16, 19, 21, 22, 24, 27
Acid 10, 19, 21, 22, 50
Base 10, 19, 21, 50

Base Asymmetry 10, 19, 21
Asymmetry test 12, 50

Basic Buffers 9, 21
Drugs 14, 29-33

Beverages 22, 37-41

Bipyridyl 11, 50

Buffers 9

Capillary 13, 15, 17, 19

CARB Method 1004 35

Carbon Load 8

Column Diameter 8
Format 6, 8
Length 8

Corrosion 12, 14, 16, 18, 20, 24

Cytochrome c 16, 46-47

Detection limit 8

De-wetting 18, 20

Efficiency 7, 12, 14, 16, 19, 21, 22, 25, 27, 50

ELSD 24, 42-45, 51

Endcapping 5, 8, 12, 14, 16, 18, 20

Environmental 34-36

EPA Methods
531 36
604 34
605 34
606 34
8270 34, 36
8310 35
8330 26-27, 36

Evaporative Light Scattering Detection (ELSD) 24, 42-45, 51

Explosives 5, 6, 26-27, 36

Flow rate 8

Food and Beverages 37-41

Guard cartridge 13, 15, 17, 19, 21, 23, 25, 27

Hydrolytic stability 4, 9, 18, 20-21

Hydrophilic 18, 20

Hydrophobic
Steric selectivity test 11, 12, 50

Hydrophobicity 6

Ion Exchange 6

k' 12, 14

Loading Capacity 8

Lot Qualification (see lot validation)

Lot Validation 10, 12, 14, 16, 19, 21, 22, 25, 27, 50-51

LC-MS 34, 36, 43
Compatibility 13, 15, 17, 19

Metal Activity test 12, 14, 16, 19, 21, 50
Contamination 11

Methanesulfonic acid 23

Mobile Phase 9

Natural products 48

Nitramine 26, 36

Nitroaromatic 26, 36

Normal-phase 18

Octadecyldimethylsilane 12, 16

Octyldimethylsilane 14

Ordering information inside front cover, 13, 15, 17, 19, 21, 23, 25, 27

Organic acids 22-24, 31, 37, 39

PA 4-5, 7, 10-11, 18-20, 29-31, 33-40, 50

PA2 4-5, 7, 10, 20-21, 30-33, 39, 47, 50

Particle Size 5, 7

PEEK 22

Peptide 4, 16, 46-47

pH 4, 9, 12, 14, 16, 18, 20, 22, 24, 50-51

Pharmaceutical 29-33

Phenanthrene 50

Polar Embedded 4-5, 18-21
Selectivity Test 50

PolarAdvantage, see PA and PA2

Polarity index 10, 20

Pore Size 5, 7

Pressure 7, 8

Protein 4, 16-17, 46-47

Resolution 22

Reversed-phase 6

Selectivity 5, 6-7, 12, 18, 20, 22, 24, 26
Base 7
Ethylbenzene/toluene 7
Peptide 16
Polar 50
Shape 6-7
Steric 50
Tripheylene/o-terphenyl 6-7, 50
Silanol 6, 8, 10, 12, 14, 18

Silica Substrate 5

Suppressed Conductivity Detection 44, 45

Surface Area 5, 7, 12, 14, 16, 18, 20, 22, 24, 27

Surfactants 6, 24-25, 42-45, 51

Tryptic digest 16, 46

USP Code 5, 12, 14, 16

Validation (see lot Validation)

Vitamins 37-40

Warranty inside front cover

Wetting 12, 14, 18, 20,

Appendix 3: Part Number Index

Part No.	Acclaim Type	Size	Page
059122	120, 3 µm, C8	2.1 × 50 mm	15
059123	120, 3 µm, C8	2.1 × 100 mm	15
059124	120, 3 µm, C8	2.1 × 150 mm	15
059125	120, 3 µm, C8	4.6 × 50 mm	15
029126	120, 3 µm, C8	4.6 × 100 mm	15
059127	120, 3 µm, C8	4.6 × 150 mm	15
059128	120, 3 µm, C18	2.1 × 50 mm	13
059129	120, 3 µm, C18	2.1 × 100 mm	13
059130	120, 3 µm, C18	2.1 × 150 mm	13
059131	120, 3 µm, C18	4.6 × 50 mm	13
059132	120, 3 µm, C18	4.6 × 100 mm	13
059133	120, 3 µm, C18	4.6 × 150 mm	13
059134	120, 5 µm, C8	2.1 × 50 mm	15
059135	120, 5 µm, C8	2.1 × 100 mm	15
059136	120, 5 µm, C8	2.1 × 150 mm	15
059137	120, 5 µm, C8	2.1 × 250 mm	15
059138	120, 5 µm, C8	4.6 × 50 mm	15
059139	120, 5 µm, C8	4.6 × 100 mm	15
059140	120, 5 µm, C8	4.6 × 150 mm	15
059141	120, 5 µm, C8	4.6 × 250 mm	15
059142	120, 5 µm, C18	2.1 × 50 mm	13
059143	120, 5 µm, C18	2.1 × 100 mm	13
059144	120, 5 µm, C18	2.1 × 150 mm	13
059145	120, 5 µm, C18	2.1 × 250 mm	13
059146	120, 5 µm, C18	4.6 × 50 mm	13
059147	120, 5 µm, C18	4.6 × 100 mm	13
059148	120, 5 µm, C18	4.6 × 150 mm	13
059149	120, 5 µm, C18	4.6 × 250 mm	13
059446	120, 5 µm, C18	4.3 × 10 mm	13
059447	120, 5 µm, C18	2 × 10 mm	13
059448	120, 5 µm, C8	4.3 × 10 mm	15
059449	120, 5 µm, C8	2 × 10 mm	15
059456	Guard Cartridge holder		13, 15, 17,19, 23, 25, 27
059457	Guard to analyical coupler		13, 15, 17,19, 23, 25, 27
059526	Guard holder & coupler kit		13, 15, 17,19, 21, 23, 25, 27
060263	300, 3 µm, C18	2.1 × 50 mm	17
060264	300, 3 µm, C18	2.1 × 150 mm	17
060265	300, 3 µm, C18	4.6 × 50 mm	17
060266	300, 3 µm, C18	4.6 × 150 mm	17
060393	300, 3 µm, C18	4.3 × 10 mm	17
060395	300, 3 µm, C18	2 × 10 mm	17
061316	PA, 3 µm, C16	2.1 × 100 mm	19
061317	PA, 3 µm, C16	2.1 × 150 mm	19
061318	PA, 3 µm, C16	4.6 × 150 mm	19
061319	PA, 5 µm, C16	4.6 × 50 mm	19
061320	PA, 5 µm, C16	4.6 × 150 mm	19
061321	PA, 5 µm, C16	4.6 × 250 mm	19
061331	PA, 5 µm, C16	2 × 10 mm	19
061332	PA, 5 µm, C16	4.3 × 10 mm	19
062902	OA, 5 µm	4.0 × 250 mm	23
062903	OA, 5 µm	4.0 × 150 mm	23
062925	OA, 5 µm Guard	4.3 × 10 mm	23
063187	PA2, 3-µm	2.1 × 150 mm	21
063189	PA2, 3-µm	4.6 × 50 mm	21
063191	PA2, 3-µm	4.6 × 150 mm	21
063193	PA2, 5 µm Guard 2.0 × 10 mm (pack of 2)		21
063195	PA2, 5 µm Guard 4.3 × 100 mm (pack of 2)		21
063197	PA2, 5-µm	4.6 × 150 mm	21
063199	PA2, 5-µm	4.6 × 250 mm	21
063201	Surfactant 5 µm	4.6 × 150 mm	25
063203	Surfactant5 µm	4.6 × 250 mm	25
063215	Surfactant 5 µm Guard 4.3 × 10 mm (pack of 2)		25
064305	Expolsives	4.6 × 250 mm E1	27
064303	Expolsives 5 µm Guard 4.3 × 10 mm E1 (pack of 2)		27
064307	Expolsives 5 µm Guard 4.3 × 10 mm E2 (pack of 2)		27
064309	Expolsives 5 µm	4.6 × 250 mm E2	27
064312	Expolsives 5 µm	E1 and E2 kit	27
161450	120, 5 µm, C18	1.0 × 50 mm	13
161451	120, 5 µm, C18	1.0 × 150 mm	13
161452	120, 5 µm, C18	1.0 × 250 mm	13
161453	120, 5 µm, C18	300 µm × 50 mm	13
161454	120, 5 µm, C18	300 µm × 50 mm	13
161455	120, 5 µm, C18	300 µm × 250 mm	13
161456	120, 5 µm, C18	75 µm × 50 mm	13
161457	120, 5 µm, C18	75 µm × 150 mm	13
161458	120, 5 µm, C18	75 µm × 250 mm	13
162204	120, 3 µm, C8	1.0 × 50 mm	15
162205	120, 3 µm, C8	1.0 × 150 mm	15
162206	120, 3 µm, C8	300 µm × 50 mm	15
162207	120, 3 µm, C8	300 µm × 150 mm	15
162208	120, 3 µm, C8	75 µm × 50 mm	15
162209	120, 3 µm, C8	75 µm × 150 mm	15
162210	120, 5 µm, C8	1.0 × 50 mm	15
162211	120, 5 µm, C8	1.0 × 150 mm	15
162212	120, 5 µm, C8	1.0 × 250 mm	15
162213	120, 5 µm, C8	300 µm × 50 mm	15
162214	120, 5 µm, C8	300 µm × 150 mm	15
162215	120, 5 µm, C8	300 µm × 250 mm	15
162216	120, 5 µm, C8	75 µm × 50 mm	15
162217	120, 5 µm, C8	75 µm × 150 mm	15
162218	120, 5 µm, C8	75 µm × 250 mm	15
162219	300, 3 µm, C18	1.0 × 50 mm	17
162220	300, 3 µm, C18	1.0 × 150 mm	17
162221	300, 3 µm, C18	300 µm × 50 mm	17
162222	300, 3 µm, C18	300 µm × 150 mm	17
162223	300, 3 µm, C18	75 µm × 50 mm	17
162224	300, 3 µm, C18	75 µm × 150 mm	17
162234	120, 3 µm, C18	1.0 × 50 mm	13
162235	120, 3 µm, C18	1.0 × 150 mm	13
162236	120, 3 µm, C18	300 µm × 50 mm	13
162237	120, 3 µm, C18	300 µm × 150 mm	13
162238	120, 3 µm, C18	75 µm × 50 mm	13
162239	120, 3 µm, C18	75 µm × 150 mm	13
162240	PA, 3 µm, C16	1.0 × 50 mm	19
162241	PA, 3 µm, C16	1.0 × 150 mm	19
162242	PA, 3 µm, C16	300 µm × 50 mm	19
162243	PA, 3 µm, C16	300 µm × 150 mm	19
162244	PA, 3 µm, C16	75 µm × 50 mm	19
162245	PA, 3 µm, C16	75 µm × 150 mm	19
162246	PA, 5 µm, C16	1.0 × 50 mm	19
162247	PA, 5 µm, C16	1.0 × 150 mm	19
162248	PA, 5 µm, C16	1.0 × 250 mm	19
162249	PA, 5 µm, C16	300 µm × 50 mm	19
162250	PA, 5 µm, C16	300 µm × 150 mm	19
162251	PA, 5 µm, C16	300 µm × 250 mm	19
162252	PA, 5 µm, C16	75 µm × 50 mm	19
162253	PA, 5 µm, C16	75 µm × 150 mm	19
162254	PA, 5 µm, C16	75 µm × 50 mm	19
162261	120, 5 µm, C8	1.0 × 5 mm	15
162262	120, 5 µm, C8	1.0 × 15 mm	15
162263	120, 5 µm, C8	800 µm × 5 mm	15
162264	120, 5 µm, C8	500 µm × 5 mm	15
162265	120, 5 µm, C8	500 µm × 15 mm	15
162266	120, 5 µm, C8	300 µm × 5 mm	15
162302	PA, 5 µm, C16	300 µm × 5 mm	19
162304	120, 5 µm, C8	300 µm × 1.0 mm	15
162306	120, 5 µm, C18	300 µm × 1.0 mm	13
162310	PA, 5 µm, C16	300 µm × 1.0 mm	19
162321	120, 5 µm, C18	1.0 × 5 mm	13
162322	120, 5 µm, C18	1.0 × 15 mm	13
162323	120, 5 µm, C18	800 µm × 5 mm	13
162324	120, 5 µm, C18	500 µm × 5 mm	13
162325	120, 5 µm, C18	500 µm × 15 mm	13
162326	120, 5 µm, C18	300 µm × 5 mm	13
162333	PA, 5 µm, C16	1.0 × 5 mm	19
162334	PA, 5 µm, C16	1.0 × 15 mm	19
162335	PA, 5 µm, C16	800 µm × 5 mm	19
162336	PA, 5 µm, C16	500 µm × 5 mm	19
132337	PA, 5 µm, C16	500 µm × 15 mm	19

Appendix 4: Analyte Index

Acebutolol 14, 29, 31
Acenaphthalene 35
Acenaphthene 35
Acesulfame-K 40
Acetaldehyde-DNPH 35
Acetaminophen 30, 32
Acetic acid 23
Acetone-DNPH 35
cis-Aconitic acid 23
trans-Aconitic acid 23
Acrolein-DNPH 35
Acrylic acid 49
Acrylic acid dimer 49
Adhumulone 39
Adlupulone 39
Aflatoxin B1 40
Aflatoxin B2 40
Aflatoxin G1 40
Aflatoxin G2 40
Aldicarb 35, 36
Aldicarb sulfone 35, 36
Aldicarb sulfoxide 35, 36
Ametryn 36
2-Amino-4,6-dinitrotoluene 26, 27, 36
4-Amino-2,6-dinitrotoluene 26, 27, 36
Amitriptyline 10, 29, 30, 32, 50
Amobarbital 32
Angiotensin-II 47
Aniline 7
Anthracene 35
Antibiotics 30, 31, 36, 38, 40, 41
Antidepressants 10, 29, 30, 32, 50
Antioxidants 41
Ascorbic acid (Vitamin C) 37, 38, 39
Aspartame 38, 40
Aspartylphenylalanine 38, 40
Aspartyl-phenylalanine methyl ester 38, 40
Atenolol 14, 29
Atrazine 13, 34, 36
Barbital 32
Benzaldehyde 32
Benzaldehyde-DNPH 35
Benzene 1,2,3-tricarboxylic acid 49
Benzene 1,2-dicarboxylic acid 49
Benzene 1,3,5-tricarboxylic acid 49
Benzene 1,3-dicarboxylic acid 49
Benzene 1,4-dicarboxylic acid 49
Benzidine 34, 36
Benzil 32
Benzo[a]anthracene 35
Benzo[a]pyrene 35
Benzo[b]fluoranthene 35
Benzo[g,h,i]perylene 35
Benzo[k]fluoranthene 35
Benzoic acid 32, 39, 40, 42, 50
Benzoin 32
Benzophenone 32
Benzophenone-3 33
Benzyl alcohol 29
5-Benzyl-3,6-dioxo-2-piperazine acetic acid 38
Benzyldimethyl hydrogenated tallow ammonium chloride 44
Berberine 48
Biochanin A 48
Biotin 38, 39
2,2′-Bipyridyl 11, 50
4,4′-Bipyridyl 11, 50
Bis(2-ethylhexyl)phthalate 34
Bisdemethoxycurcumin 37
Bovine serum albumin tryptic digest 46
Brodifacoum 34
Bromide 31, 44
Budesonide 32
Butabarbital 32
2-Butanone-DNPH 35
4-Butylbenzoic acid 10, 20
Butyl paraben 39
Butylated hydroxyanisole (BHA) 41
Butylated hydroxytoluene (BHT) 41
Butylbenzylphthalate 34
Butyraldehyde-DNPH 35
Butyric acid 23
C10-LAS 25, 43, 44
C11-LAS 25, 43, 44
C12-LAS 25, 43, 44
C13-LAS 25, 43, 44
Caffeine 39, 40, 48
Caproic acid 23
Carbamate insecticides 35, 36
Carbaryl 35, 36
Carbofuran 35, 36
Carbonic anhydrase 46, 47
beta-Carotene 37, 38, 40
cis-beta-Carotene 37, 38
trans-beta-Carotene 37, 38, 40
Carotenoids 37, 38, 40
Cetyl betaine 24, 43, 44
Cetylpyridinium chloride 24, 42, 43, 45
Cetyltrimethylammonium chloride 24, 42, 43, 45
Chloramphenicol 36
Chloride 44
Chlorohydrine 31
4-Chloro-3-methylphenol 34
2-Chlorophenol 34
Chlortetracycline 40
Cholecalciferol (Vitamin D3) 37, 40
Chyrsene 35
Cinchonidine 39
Citric acid 23, 31, 37, 40
Clonazepam 29
Cohumulone 39
Colupulone 39
Cortisol (Hydrocortisone) 32, 33
Crotonaldehyde-DNPH 35
Curcumin 37
Cytochrome-c 46, 47
Cytochrome-c tryptic digest 16, 46
Cytosine 18, 21
Decyl sulfate 24, 43, 44, 51
Demethoxycurcumin 37
11-Deoxycortisol 33
Dexamethasone 33
Dextromethorphan 30, 32
2,4-Diamino-6-nitrotoluene 27
Diazepam 29
Dibenzo[a,h]anthracene 35
Di-n-butylphthalate 34
3,3′-Dichlorobenzidine 34, 36
2,4-Dichlorophenol 34
Diethyl heptadecyl imidazolinium ethylsulfate 45
Diethylphthalate 34
Dihydroquinine 39
2,5-Dihydroxybenzoic acid 49
3,5-Dihydroxybenzoic acid 49
3,4-Dihydroxybenzylamine 30
Dimethylaniline 7, 10, 20
3,3′-Dimethylbenzidine 34, 36
2,3-Dimethyl-2,3-dinitrobutane (DMDNB) 27
2,4-Dimethylphenol 34
Dimethylphthalate 34, 50
1,3-Dinitrobenzene 26, 27, 36
2,4-Dinitrophenol 34
2,4-Dinitrophenylhydrazine (DNPH) 35
2,4-Dinitrotoluene 26, 27, 36
2,6-Dinitrotoluene 26, 27, 36
Di-n-octylphthalate 34
Diuron 34
Dodecyl gallate 41
Dodecyl sulfate 24, 43, 44, 51
Dodecylbenzene sulfonate 25, 43
Domiphen bromide 42
Dopamine 30
Doxepin 10, 29, 30
Doxycycline 40
Doxylamine 30, 32
Epicatechin gallate 48
Epichatechin 48
Epigallocatechin 48
Epigallocatechin gallate 48
Epinephrine 30
Epoxyefenone 31
Ergocalciferol (Vitamin D2) 37, 40
Erythorbic acid 40
Ethoquad 18/25 42
Ethyl 4-hydroxybenzoate 50
Ethyl benzoate 7
Ethyl paraben 39
Ethylbenzene 7
Ethyleneglycol dinitrate (EGDN) 27
Explosives 26, 27, 36
Fludrocortisone 33
Fluoranthene 35
Fluorene 35
Folic acid 38, 39
Formaldehyde-DNPH 35
Formic acid 23
Formononotin 48
Fumaric acid 23, 37
Furaltadone 38
Furazolidone 38
Genistin 48
Glutamate 40
Glutamine 37
Gly-Tyr 47
Heptanoic acid 23
Herbicides 13, 34, 36
Hexahydroisohumulone 41
Hexaldehyde-DNPH 35
HMX 26, 27, 36
Humulone 39
Hydrastine 48
Hydrocortisone 32, 33
4-Hydroxybenxoic acid 49, 50
3-Hydroxycarbofuran 35, 36
2-Hydroxyethylbenzyl coco imidazolinium chloride 45
5-Hydroxyindoleacetic acid 30
4-Hydroxy-3-methoxymandelic acid 30
4-Hydroxy-3-methoxyphenylacetic acid 30
Ibuprofen 33
Imipramine 29, 32
Indeno[1,2,3-cd]pyrene 35
Insulin 46
Ionox-100 41
Isoadhumulone 41
Isobutyric acid 23
Isocaproic acid 23
Isocitric acid 23
Isocohumulone 41
Isohumulone 41
rho-Isohumulone 41
Isoleucine 37
Isovaleric acid 23
Ketoprofen 33
Labetalol 14, 29, 31
Lactic acid 23, 37
Laureth sulfate 42-44
Lauryl sulfate 24, 43, 44, 51
Lauryldimethylbenzyl ammonium chloride 24, 42-45, 51
Laurylpyridinium chloride 24, 42-45, 51
Leucine 37
Leu-Enkephalin 47
Lincomycin 29
Linuron 13, 34
Loratidine 31
Lupulone 39
trans-Lutein 38, 40

Analyte Index

cis-Lycopene 38
trans-Lycopene 37, 38
Lysine 37
Lysozyme 47
Maleic acid 14, 29, 31
Malic acid 23, 37
Metanephrine 30
Metaraminol 14, 29, 31
Met-Enkephalin 47
Methacrolein-DNPH 35
Methiocarb 35, 36
Methomyl 35, 36
3-Methoxy-4-hydroxyphenylglycol 30
Methyl bis(ethyl(tallowate))-2-hydroxy-ethyl ammonium methylsulfate 42
2-Methyl-4,6-dichlorophenol 34
Methyl paraben 32, 39
Methylethylketone-DNPH 35
Methylprednisolone 33
Metoprolol 14, 29, 31
Monuron 13, 34
Myoglobin 46, 47
Myristamidopropylbetaine 43
N,N-Dimethylaniline 7, 10, 20
Naphthalene 35, 51
Naproxen 33
NEODOL 25-7 44
Niacinamide 38, 39
Nitrate 24, 44
Nitrobenzene 7,26, 27, 36
Nitrofurantoin 38
Nitrofurazone 38
2-Nitrophenol 34
4-Nitrophenol 34
N-Nitrosodibutylamine 34
N-Nitrosodiethylamine 34
N-Nitrosodimethylamine 34
N-Nitrosodiphenylamine 34
N-Nitrosodipropylamine 34
N-Nitrosomethylethylamine 34
N-Nitrosomorpholine 34
N-Nitrosopiperidine 34
N-nitrosopyrrolidine 34
2-Nitrotoluene 26, 27, 36
3-Nitrotoluene 26, 27, 36
4-Nitrotoluene 26, 27, 36
nor-Dehydroguiaretic acid (NDGA) 41
Norepinephrine 30
Normetanephrine 30
Nortriptyline 29
Octyl gallate 41
Octyl methoxycinnamate 33
Octyl salicylate 33
Octylphenoxyethoxyethyl dimethylbenzyl ammonium chloride 24, 42, 45, 51
Oil red O 39
Ononin 48
Oxalic acid 23
Oxamyl 35, 36
Oxazepam 29
Oxprenolol 14, 29, 31
Oxytetracycline 36, 40
Pantothenic acid 38, 39
PEG monoethyl ether 43
PEG-200 Distearate 45
PEG-300 44
PEG-400 44
PEG-600 44
Pentachlorophenol 34
Pentaethyritol tetranitrate (PETN) 27
Phenanthrene 11, 35, 50
Phenobarbital 32
Phenol 7, 10, 20, 34
Phenols 7, 10, 20, 34
Phenothiazine 29
Phenytoin 32
POE(1) Lauryl sulfate 25
POE(30) Ammonium lauryl sulfate 45
Pramoxine 30
Prednisolone 33
Prednisone 33
Promethazine HCl 29
Prometon 36
Prometryn 36
Propafenone 31
Propazine 13, 34, 36
Propionaldehyde-DNPH 35
Propionic acid 23
Propoxur 35, 36
Propranolol 10, 14, 29, 30, 31, 50
Propyl benzene 51
Propyl gallate 41
Propyl paraben 32, 39
Protryptyline 29
Pseudo-ephedrine 30, 31, 32, 33
Pyrene 35
Pyridine 7, 10, 20
Pyridoxine 38, 39
Pyrrobutamine phosphate 29
Quinine 39
Ranitidine 31
Ranitidine S-oxide 31
RDX 26, 27, 36
trans-Retinol 37, 40
Retinol acetate 38, 40
Riboflavin 38, 39
Ribonuclease 46
RNAse-A 47
Saccharin Sodium 42
Secobarbital 32
Serotonin 30
Simazine 36
Sissotrin 48
Sodium lauryl sulfate 24, 43, 44, 51
Sorbic acid 39
Spectinomycin 29
Steroids 32, 33
Succinic acid 23, 31, 37
Sudan Orange I 39, 48
Sudan Orange II 39, 48
Sudan Orange III 39, 48
Sudan Orange IV 39
Sulfachloropyridazine 41
Sulfadiazine 30, 41
Sulfadimethoxine 30, 31, 36, 41
Sulfamerazine 30, 31, 36, 41
Sulfamethazine 30, 31, 41
Sulfamethoxazole 30, 31, 41
Sulfanilamide 30, 31
Sulfanilic acid 30
Sulfapyridazine 31
Sulfapyridine 41
Sulfaquinoxaline 31, 41
Sulfathiazole 30, 31
Sulfisoxazole 30, 31
Tartaric acid 37
t-Butylhydroquinone (TBHQ) 41
Temazepam 29
Terbutryn 36
o-Terphenyl 7, 11, 50
Tetracycline 40
Tetrahydroisohumulone 41
Tetryl 26, 27, 36
Thenyldiamine HCl 29
Theobromine 41
Thiamine 38
Thymine 18, 21, 39
Timolol 14, 29, 31
alpha-Tocopherol 37, 40
alpha-Tocopherol acetate (Vitamin E) 38, 40
beta-Tocopherol 37
delta-Tocopherol 37
m-Tolualdehyde-DNPH 35
Toluene 7, 10, 11, 20, 30, 50
Toluene sulfonate 51
2,4,6-Trichlorophenol 34
3,4,5-Trihydroxybenzoic acid 49
Trihydroxybenzophenone (THBP) 41
1,3,5-Trinitrobenzene 26, 27, 36
2,4,6-Trinitrotoluene 26, 27, 36
Triphenylene 7, 11, 50
Triton X-100 ..... 24, 43, 44, 51
Uracil 10, 18, 20, 21, 30, 32, 50, 51
Valeraldehyde-DNPH 35
Valeric acid 23
Valine 37
Val-Tyr-Val 47
Venlafaxine 29
Vitamin A acetate 38, 40
Vitamin B_{12} 38, 39
Vitamin C 37, 38, 39
Vitamin D_2 ..... 37, 40
Vitamin D_3 ..... 37, 40
Vitamin E 37, 40
Vitamin E acetate 38, 40
Vitamin K_1 40
Vitamins 37–40
Xylene sulfonate 24, 43, 44
ZONYL FSO fluorosurfactant 42

SYSTEMS

Acclaim Columns—Optimal Performance with the UltiMate 3000 Intelligent LC and ICS-3000

The UltiMate 3000 Intelligent LC series delivers the full range of HPLC. Select from a wide range of modules to optimize systems for all types of applications, from sensitive nanoflow LC/MS analyses to automated semipreparative purifications. Whichever configuration you choose, you will get a highly integrated solution with optimum fluidic connections, single-point and intelligent control through Chromeleon® software, and seamless intermodule communication. The UltiMate 3000 Intelligent LC System won:

- Gold award for best new product from *Instrument Business Outlook*.

The ICS-3000 metal-free system is ideal for applications requiring chemically inert (PEEK) chromatography. The ICS-3000 has won more than five awards:

- R&D 100 Award as one of the best new products of 2006
- Two Gold Awards from *Instrument Business Outlook*
- An IDEA Bronze award for Industrial design from *Business Week*'s editorial staff.
- The Editors' Choice Silver Award as one of the best new products at Pittcon 2005

Corporate Headquarters

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA 94088-3603
Tel: (408) 737-0700
Fax: (408) 730-9403

Worldwide Sales and Service

North America

U.S.	(847) 295-7500
Canada	(905) 844-9650

South America

Brazil	(55) 11 3731 5140

Europe

Austria	(43) 1 616 51 25
Belgium	(32) 3 353 4294
Denmark	(45) 36 36 90 90
France	(33) 1 39 30 01 10
Germany	(49) 6126 991 0
Italy	(39) 02 51 62 1267
The Netherlands	(31) 20 683 9768
Switzerland	(41) 62 205 9966
U.K. and Ireland	(44) 1276 691722

Asia Pacific

Australia	(61) 2 9420 5233
China	(852) 2428 3282
India	(91) 22 28475235
Japan	(81) 6 6885 1213
Korea	(82) 2 2653 2580
Singapore	(65) 6289 1190

www.dionex.com

Dionex products are designed, developed, and manufactured under an ISO 9001 Quality System.

© 2007 Dionex Corporation
AQA and MSQ are trademarks of Thermo Electron Corporation.
Zantac is a registered trademarks of Glaxo Smith Kline, Inc.
All other trademarks and registered trademarks are the property of Dionex Corporation.

LPN 1668-03 PDF 07/07