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Acclaim columns overview

Columns for challenging separation needs



Broad range of HPLC and UHPLC columns



Broad range of HPLC and UHPLC columns for challenging separation needs

General-purpose RP

Separate complex samples with high surface area columns (C18, etc)

Mixed-Mode

Retain multiple types of analytes on a single column

• Application-specific

Unique columns for specific applications: surfactants, organic acids, pesticides, aminoglycosides, explosive residues

• HILIC

Designed for the separation of polar compounds

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Acclaim column families at a glance

Thermo Scientific[™] Acclaim[™] columns are designed for high-efficiency high performance liquid chromatography (HPLC) separations. Their unique functionalities set them apart for difficult and complex separations, often providing resolution of compounds not possible by other conventional HPLC columns.

Surface chemistries include standard functionalities such as Thermo Scientific[™] Acclaim[™] 120 C18, C8, Phenyl, Polar Advantage, polar embedded phases, HILIC, as well as specialty columns: Mixed-Mode functionalities (i.e. Thermo Scientific[™] Acclaim[™] Trinity P1 and P2 columns), application-specific phases (organic acids, surfactants, pesticides, pollutants, etc.) and size exclusion columns (SEC). Further, some Acclaim columns have been optimized to work under the very high pressure, ultra high performance liquid chromatography (UHPLC) requirements of Thermo Scientific[™] Vanquish[™] UHPLC instrument.

General purpose	Mixed-Mode	Application-specific
• Start here for separations based on hydrophobic separations. These surface area Acclaim columns provides outstanding resolution for complicated matrix, large number	 Turn to Mixed-Mode phases when you have a mix of ionic and hydrophobic compounds or hydrophilic compounds. They are available in both dual and 	 Take the guess-work out of your chromatography columns selection. These columns are designed and tested for specific applications.
of peaks, or purely need more retention.	triple retention modes. Included are anionic/cationic/reversed-phase/HILIC functionalities. The bonding has been	 Columns for food/beverage, consumer goods testing, and environmental testing. For those customers who run
 Included are reversed-phase for hydrophobic interaction, HILIC for the retention or separation 	optimized for spacial distancing for greater resolution.	specific types of separations, these applications specific columns are the easy choice.
of hydrophilic compounds such catecholamines other polar compounds (specific).	 Over 10 years of experience for pharmaceutical APIs and counterions, including those required in standard operating procedures. 	• Wide range of column sizes to meet your chromatographic needs
• SEC when you are looking for size exclusion separations, these unique columns have a polymer backbone eliminating secondary separation that can occur on silica media. They are robust with a wide pH range.		



Selecting an Acclaim column

Choosing the column chemistry is the first step in identifying the column of choice. This is key in achieving the resolution and selectivity for the desired separation. Particle size is important in determining the speed of a separation and column dimensions are important in optimizing resolution while also impacting solvent consumption and sensitivity requirements.

What are the goals of the separation?

- Critical peaks requiring resolution
- Speed of analysis
- Solvent consumption/minimal waste
- Sensitivity requirements

Choosing the 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 Acclaim columns. Thus, if you are separating surfactants, the Thermo Scientific[™] Acclaim[™] Surfactant Plus column is the best first choice; likewise Thermo Scientific[™] Acclaim[™] Explosives column is used for Environmental Protection Agency (EPA) Method 8330, and the Thermo Scientific[™] Acclaim[™] Organic Acid column 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 the C18 column because of its excellent peak efficiency, low silanol activity, and ability to separate many organic molecules. Other chemistries are available and can provide better chromatography based on the properties.

Type of bonding

The bonding chemistry of the column is defined by the functional groups attached to the particle substrate, as well as the substrate itself. In reversed-phase chromatography, the mode of separation is hydrophobic interaction, and the functional groups are either Thermo Scientific[™] Acclaim[™] C18, C8, C30, Polar Advantage (PA and PA2) or Phenyl. Other chemistries include HILIC, size exclusion chromatography and Mixed-Mode chromatography.

RP	Reversed-phase chromatography	Separation is based on hydrophobic interaction of the column surface chemistry and hydrophobicity of the molecules being separated. The stronger the hydrophobicity of the molecule – the more it will bind to the stationary phase
HILIC	Hydrophilic interaction liquid chromatography	Retains and separates polar, hydrophilic compounds
ММ	Mixed-Mode chromatography	The stationary phase has more than one major form of retention, i.e., reversed-phase and ion-exchange
SEC	Size exclusion chromatography	Separation is based on molecular size

Choosing particle size and column format

Particle size affects peak efficiency, which in turn impacts resolution or the amount of space between peaks and the speed of the separation. 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 2.2 μ m, 3 μ m or 5 μ m bonded silica. When using isocratic separation conditions, it can be expected that a column packed with 3 μ m material will give about 1.7 times the separation efficiency as a column of identical proportions, packed with 5 μ m material, but the backpressure will be almost three times as high on the 3 μ m column at the same linear velocity. Similarly, for going from 3 μ m to 2.2 μ m, the efficiency increases 1.4 times, the resolution 1.2 times, and the pressure 1.9 times.



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 flow rate). 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
2.1 mm internal diameter	High-sensitivity or limited volume samples. Use with low dead-volume instrumentation
3.0 mm internal diameter	Lower solvent consumption. Use with standard instrumentation
4.0-4.6 mm internal diameter	Standard formats. Use with standard instrumentation

Column diameter selection

Your system capabilities determine which size columns you are most likely to use. These suggested dimensions are for initial method development. Based on the general considerations, you might decide to use columns that are either longer or shorter these. Your optimized final method might use some other column or particle size.

System	Pressure	Particle	Column size	Flow
Classic LC	≤400 bar	3 – 5 µm	4.6 x 250 mm, 5 μm 4.6 x 150 mm, 3 μm	1 – 2 ml/min
Modern LC	≤600 bar	2 – 3 µm	3 x 150 mm, 3 μm 2.1 x 150 mm	0.3 – 0.5 ml/min
Thermo Scientific™ Vanquish™ UHPLC	≤1500 bar	≤ 2.2 µm	2.1 x 150 mm 2.1 x 250 mm	0.4 – 0.8 ml/min

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 filter that is 0.5 µm or smaller.





Acclaim reversed-phase, HILIC and SEC columns

Acclaim reversed-phase, HILIC and SEC columns

Reversed-phase chromatography

Reversed-phase chromatography is the most widely used HPLC separation mechanism. Functional groups C18, C8, C30 and Phenyl are attached to the silica substrate either via a single attachment (monomeric bonding), or via multiple attachments (multidentate bonding) such as the Acclaim Polar Advantage II (PA2) column. Monomeric bonded phases provide higher column efficiencies, than polymeric phases, but polymeric phases are very stable under pH extremes.

These functional groups are hydrophobic and attract hydrophobic molecules in the sample. For example, Catechins are a class of polyphenolic flavanols widely distributed in plants, notably tea and cacao. As can be seen in this selectivity comparison, stationary phases with a mixture of polar and revered-phase retention provide the best separation. The Acclaim PA2 column is resistant to the acidic conditions usually preferred for separating polyphenols and gives the best separation.



Carbon load and end-capping

For reversed-phase chemistries, 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 end-capped. All Acclaim column packings are end-capped.

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.



Larger pores = Smaller surface area

Smaller pores = Larger surface area

HILIC chromatography

HILIC is the recommended mode of separating polar compounds. These compounds are unretained under conventional reversed-phase conditions, but are retained using HILIC columns, without the requirement for ion-pair additives in the mobile phase. In this technique the stationary phase is polar and the aqueous portion of the mobile phase acts as the stronger solvent. Thus, the polar analytes can be retained and separated, even with 5-20% aqueous mobile phase.

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) is a major mode of HPLC that employs porous particles in the column to separate molecules by virtue of their size in solution. Using isocratic conditions, the small molecules flow into the substrate pores and move slower than the large molecules, which are too big to move into the pores. The result is a separation of the molecules based on size.

Acclaim reversed-phase and general purpose HPLC columns

Columns	Description	
Acclaim 120 C18	High-efficiency reversed-phase columns for small molecule and complex mixtures separations, including liquid chromatography mass spektrometry (LC-MS)	
Acclaim 120 C8	Reversed-phase columns for small molecule with intermediate hydrophobic retention	
Acclaim 300 C18	Wide-pore columns for LC-MS life science applications; protein and peptides	
Acclaim Polar Advantage (PA)	Polar-embedded reversed-phase columns for separating polar and nonpolar analytes with 100% aqueous mobile phase compatibility	
Acclaim Polar Advantage II (PA2)	Polar-embedded columns with enhanced hydrolytic stability pH 1.5 – 10, excellent for polar and non-polar analytes with 100% aqueous mobile phase compatibility and LC-MS	
Acclaim C30	/ery high hydrophobic retention; good for separating structurally related isomers	
Acclaim Phenyl-1	Polar reversed-phase columns with high aromatic selectivity	
Acclaim HILIC-10	Designed for unique selectivity of hydrophilic molecules	
Acclaim SEC-1000 Acclaim SEC-300	Separation of water-soluble polymers using resin based columns	

Acclaim reversed-phase and general purpose HPLC columns specifications

	Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim PA	Acclaim PA2	Acclaim C30	Acclaim Phenyl-1	Acclaim HILIC-10
Bonded phase	Octadecyl- silane	Octyl- silane	Octadecyl- silane	Embedded sulfonamide	Embedded amide	C30 alkylsilane	Phenyl	Proprietary
USP type	L1	L7	L1	L60	L60	L62	L11	-
End-capped	Yes	Yes	Yes	Yes	Yes	Proprietary	Yes	No
Starting material				Ultrapure	e silica			
Particle shape				Sphei	rical			
Particle sizes	2.2 μm 3 μm 5 μm	2.2 μm 3 μm 5 μm	3 µm	2.2 μm 3 μm 5 μm	2.2 μm 3 μm 5 μm	3 μm 5 μm	3 μm 5 μm	3 µm 5 µm
Average pore diameter	120 Å	120 Å	120 Å	120 Å	120 Å	200 Å	120 Å	120 Å
Surface area	300 m²/g	300 m²/g	100 m²/g	300 m²/g	300 m²/g	200 m²/g	300 m²/g	300 m²/g
Total carbon content	18%	11%	7%	17%	17%	13%	-	-
pH range	2–8	2–8	2–8	2–8	1.5–10	2–8	2–8	2–8

Acclaim SEC specifications

Columns	Acclaim SEC-300	Acclaim SEC-1000
Substrate	Hydrophilic polymethacrylate resin	Hydrophilic polymethacrylate resin
Particle Shape	Spherical	Spherical
Particle Size	5 µm	7 μm
Pore Size	300 Å (multi-pore)	1000 Å (multi-pore)
Separation range for PEO*	100 – 50,000 Daltons	1,000 - 1,000,000 Daltons
Exclusion limit for PEO*	50,000 – 150,000 Daltons	3,000,000 - 7,500,000 Daltons
pH Range	2–12	2–12

*PEO = polyethylene oxides

Acclaim 120 C18 columns

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

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

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. 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



Mobile phase	200 mM HOAc in 10% (v/v) MeOH
Flow rate	(A) 1.00 mL/min (B) 0.41 mL/min (C) 0.82 mL/min
Injection volume	(Α) 10 μL (Β) 1.2 μL (C) 1.2 μL
Detection	UV, 254 nm (A) 1 Hz data rate (B) 5 Hz data rate (C) 10 Hz data rate
Temperature	20 °C
Sample	Commercial vanilla extract in 40% ethanol, filtered
Reference	AOAC official method 990.25
Analytes	1. p-Hydroxybenzoic acid 2. p-Hydroxybenzaldehyde 3. Vanillic acid 4. Vanillin



LC-MS compatibility

Acclaim 120 C18 columns are LC-MS compatible with very low bleed. 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.

Reproducible results of Peptide Map overlay of five chromatograms for the separation of digested infliximab	Reproducible results of Peptide Map overlay of five chromatograms for the separation of digested inf	fliximab
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Mobile phase	(B	·	% formic acio onitrile (10:90 id		12.94 6.29 11.73 16.4 11.17 7.25	18.53 19.80 21.88	Column A (n=5)
Flow rate	0.4	4 mL/min			3.75 4.74		30.85
Detection	U١	/, 214 nm					~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Temperature	60) °C, forced a	air		5.76 4.75 7.26 6.31 11.19 11.75 13.52 16.50		30.88 Column B (n=5)
Time (min)	0	40	40.1	43	12.97	18.56 19.83 21.91	
A%	99	55	99	99	10.02	20 25	30 3
B%	1	45	1	1	5 10 15	20 25 Minutes	30 3

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Isocratic resolution of antihistamines and their impurities on Acclaim 120 C18 column

Acclaim 120 C18 column, 5 μm, 4.6 × 150 mm			
Mobile phase	(A) 50 mM sodium acetate (B) Methanol		
Isocratic	(A) 20% (B) 80%		
Detection	UV, 249 nm		
Temperature	25 °C		
Peaks	 Thenyldiamine HCI Phenothiazine Promethazine HCI Pyrrobutamine phosphate 		



Separation of basic drugs on Acclaim 120 C18 column in various concentrations

Acclaim 120 C18 column, 5 μm, 4.6 × 150 mm			
Mobile phase	80/20 methanol/30 mM phosphate, pH 6		
Flow rate	1 mL/min		
Injection volume	5 μL		
Detection	UV, 220 nm		
Temperature	30 °C		
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



Nitrofuran antibiotic residues in animal feed on Acclaim 120 C18 column

Acclaim 120 C18 column, 5μm, 4.6 × 150 mm			
Mobile phase	Acetonitrile:10 mM ammonium acetate pH 5.0, 20:80 v/v; isocratic		
Flow rate	1.0 mL/min		
Injection volume	20 µL		
Detection	UV, 365 nm		
Temperature	30 °C		
Sample	 3.0 g guinea pig feed in a 50 mL centrifuge tube Add 9 mL water and let stand 5 min Add 21 mL methanol:acetonitrile 1:1 and extract for 30 min. Pass through cleanup cartridge containing 1.7 g of neutral alumina; discard first 1.7 mL, retain next 3.5 mL 		
Peaks	 Nitrofurazone Nitrofurantoin Furazolidone Furaltadone 		



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

Bitter principles in beer on Acclaim 120 C18 column

Acclaim 120 C18	column, 5µm, 4.6 × 150 mm		
Mobile phase	75% methanol, 24% water, 1% phosphoric acid (v/v/v)		
Flow rate	1.2 mL/min		
Injection volume	25 µL		
Detection	UV, 270 nm		
Temperature	TCC-100 thermostat, 35 °C		
Sample	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 × A275 For HPLC • Evaporate dry and reconstitute in mobile phase <i>Obtained from Am. Soc. Brewing chemists</i>		
Peaks	 Isocohumulone Mixed isohumulone congeners Isoadhumulone 		



Acclaim 120 C8 columns

High performance reversed-phase columns with intermediate hydrophobic retention

Thermo Scientific 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.

Acclaim 120 C8 columns employ the same bonding chemistry and substrate as C18, and therefore features the same high standards of efficiency, coverage, and silanol activity. Acclaim 120 C8 column features:

- Similar selectivity to C18 columns, but with reduced retention
- 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

Triclosan in toothpaste

Acclaim RSLC C8 column, 2.2 $\mu m,$ 50 x 2.1 mm Isocratic, 15% buffer, (2 mM Ammonium acetate Mobile phase pH 5), 85% methanol (v/v) Flow rate 0.2 mL/min Injection volume 1.0 µL Diode array detector, 281 nm, 10 Hz, 0.1 s resp. Detection time and spectra 200-400 nm Temperature 50 °C Toothpaste containing 0.3% triclosan Sample 1. Saccharin Analytes 2. Triclosan





Isocratic separation of six cardiac antiarrhythmic drugs (beta-blockers) on Acclaim 120 C8 column

Acclaim 120 C8 cc	Acclaim 120 C8 columns, 3 μm, 4.6 × 150 mm			
Mobile phase	51/49 w/w MeOH/ 25 mM phosphate, pH 7.0			
Flow rate	1.0 mL/min			
Injection volume	5 µL			
Detection	UV, 214 nm			
Temperature	40 °C			
Analytes	 Maleic acid (-) Acebutolol (50 μg/mL) Metoprolol (50) Timolol (100) Oxprenolol (50) 7. Labetalol diasteromers (50) Propranolol (20) 			



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Hydrocortisone in skin ointment

Acclaim 120 C8 column, 3 μm, 4.6 × 150 mm		
Mobile phase	Acetonitrile:water 49:51	
Flow rate	1.0 mL/min	
Injection volume	1 µL	
Detection	UV, 245nm	
Temperature	30 °C	
Sample	 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%	



Acclaim 300 C18 columns

High-resolution reversed-phase separation of proteins and peptides

Thermo Scientific 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.

Acclaim 300 series columns are designed for small protein (up to 150 kDa) and peptide separations. Acclaim 300 columns are also useful for general-purpose, reversed-phase chromatography of small molecules.

Acclaim 300 C18 columns feature:

- Technology designed for high-resolution peptide 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
- Minimal secondary interactions for repeatable results day-to-day and column-to-column
- LC-MS compatible

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.

Budesonide and related substances

Acclaim 300 C18 column, 3 μm, 150 x 4.6 mm			
Mobile phase(A) Acetonitrile:ethanol 15:1(B) 0.1% phosphoric acid isocratic 66% B			
Flow rate	1.0 mL/min		
Injection volume	15 μL		
Detection	UV, 240nm		
Temperature	30 °C		
Sample	Budesonide, 500 μ g/mL after three days		
Analytes	7, 8. Budesonide epimers, 99%		
Deference: Hou & Hind	Ile M. Buren DD. I. Dharm. Biomad. Apoly, 2001 24:271-90		

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





Tryptic Peptide Map of bovine serum albumin on Acclaim 300 C18 column

Acclaim 300 C18 column, 3 μm, 4.6 × 50 mm			
Mobile phase	(A) 95/5/0.1 H2O/acetonitrile/TFA (B) 5/95/0.1 H2O/acetonitrile/TFA		
Flow rate	1.0 mL/min		
Injection volume	40 µL		
Detection	UV, 214 nm		
Gradient	5% B to 40% B in 35 min		



Comparison of acid additives for peptide analysis on Acclaim 300 C18 column.....

Acclaim 300 C18 column, 3 µm, 4.6 × 150 mm							
Mobile phase	(B) Water	(A) 70% acetonitrile v/v(B) Water(C) Acid stock solution					
Flow rate	1.0 mL/min						
Injection volume	5 µL						
Detection	UVD 340U; UV, 2	14 nm					
Temperature	TCC100 thermos	TCC100 thermostat at 30 °C					
Acid additive	B. Trifluoroacetic	A. Phosphoric acid, 0.15%B. Trifluoroacetic acid, 0.1%C. Formic acid, 0.1%					
Sample	Sigma H2016 Pe	Sigma H2016 Peptide Mix					
Peaks	1. Gly-Tyr 2. Val-Tyr-Val 3. Met-Enkephalin 4. Angiotensin-II 5. Leu-Enkephalin						
Gradient	-8	-8 0 16					
A%	7 7 75						



Protein mixture by reversed-phase HPLC on Acclaim 300 C18 column

68

25

0 25

68

25

В%

C%

Acclaim 300 C18	Acclaim 300 C18 column, 3 μm, 4.6 × 150 mm					
Mobile phase	(A) 5 mM MSA in 95/5 H ₂ O/acetonitrile (B) 5 mM MSA in 5/95 H ₂ O/acetonitrile					
Injection volume	25 µL					
Detection	UV, 214 n	m				
Flow rate	1.0 mL/m	in				
Peaks	anyhdrase 3. Impurit 4. Lysozy 5. Impurit anhydrase 6. Myoglo	y in carbonic e y in lysozyme me y in carbonic e				
Credient	0	0	10	00		

Gradient	0	3	18	23
A%	100	100	0	0
B%	0	0	100	100



Acclaim C30 columns

Columns for separating structurally related isomers

The Thermo Scientific Acclaim C30 column is designed to provide high shape selectivity for separating hydrophobic structural related isomers and unique selectivity complementary to other reversed-phase columns (e.g., C18).

- High shape selectivity
- Unique selectivity complementary to other reversed-phase columns
- Compatibility with highly aqueous mobile phase
- High-quality: low column bleed, high efficiency and rugged packing

Carotenoids in vegetables

LC system	UltiMate 3000 RS					
	(A) Acetonitrile					
Mobile phase	(B) Methanol:Ethyl acetate 1:1 (v/v)					
	(C) 200 mM acetic acid in water					
Injection volume	28 μL					
Detection	Diode Array; VIS 450 nm, spectra 260-800 nm					
Flow rate	1.50 mL/min					
Temperature	30 °C					
0	A. Carrot extract in acetone					
Sample	B. Spinach extract in acetone					
	C. Maize extract in acetone					
	1. Lutein					
	2. Zeaxanthin					
	3. Chlorophyll-b					
Peaks	4. alpha-Cryptoxanthin					
1 Ouro	5.beta-Cryptoxanthin					
	6. Chlorophyll-a					
	7. alpha-Carotene					
	8. beta-Carotene					
Time -	5 0 2 15 25					

Time	-5	0	2	15	25
A%	85.0	85.0	85.0	65.0	65.0
B%	14.5	14.5	14.5	34.5	34.5
C%	0.5	0.5	0.5	0.5	0.5

Glucocorticosteroids

Acclaim C30 colur	nn, 3 μm, 150 x 3.0 mm
LC system	UltiMate 3000 RS system
Mobile phase	Methanol:tetrahydrofuran:water 3:25:72(v/v)
Injection volume	2 μL
Detection	Diode Array, UV, 240 nm
Flow rate	0.50 mL/min
Temperature	30 °C
	A. Carrot extract in acetone
Samples	B. Spinach extract in acetone
	C. Maize extract in acetone
	1. Prednisone
	2. Cortisone
	3. Prednisolone
Peaks	4. Hydrocortisone
(50 µg/mL)	5. Dexamethasone
(10)	6. 6-Methylprednisolone
	7. Corticosterone
	8. 11-Deoxyhydrocortisone





Reference: "HarvetsPlus Handbook for Carotenoid Analysis", D.B. Rodriguez- Amaya and M. Kimura, International Food Policy Research Institute, 2004.



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

Analysis of cooking oils

Acclaim C30 column, 5 μm, 150 x 4.6 mm		
LC system	UltiMate 3000 RS system	
Mobile phase	Acetinitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH5.0) (Buffer)	
Injection volume	2 µL	
Detection	Corona <i>ultra</i> (Gain = 100 pA; Filter = medium; Neb. temp = 25 °C)	
Flow rate	1.0 mL/min	
Temperature	40 °C	
Sample	Peanut, olive, or wheat germ oil (5 mg/mL in iso-propanol)	

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
80	0	95	5



Profile of egg lecitin

Acclaim C30 column, 5 μm, 150 x 4.6 mm		
LC system	UltiMate 3000 RS system	
Mobile phase	Acetinitrile (MeCN)/Iso-propanol (IPA)/ Ammonium Acetate (0.1 M, pH5.0) (Buffer)	
Injection volume	2 μL	
Detection	Corona <i>ultra</i> (Gain = 100 pA; Filter = medium; Neb. Ttemp = 25 °C)	
Flow rate	1.0 mL/min	
Temperature	40 °C	
Sample	Peanut, olive, or wheat germ oil (5 mg/mL in iso-propanol)	

Time (min)	MeCN	IPA	Buffer
-15	70	0	30
0	70	0	30
0.1	70	0	30
10	90	0	10
35	10	80	10
50	0	95	5
60	0	95	5



Acclaim Polar Advantage (PA) columns

Novel polar-embedded reversed-phase columns with unique selectivity

The Thermo Scientific 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 minimizes both residual silanols and amines, thus making the Acclaim PA column suitable for acidic, basic, or neutral analytes.

The Acclaim PA column 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
- LC-MS compatible

EPA method 604 Phenols separation

Acclaim RSLC Pola	r Advantage column, 2.2 µm, 50 x 3.0 mm	
Mobile phase	(A) 10mM formic acid + 10 mM ammonium formate, pH 3.75 ± 0.05 (B) Acetonitrile	
Flow rate	1.25 mL/min	
Injection volume	0.5 µL	
Detection	UV, 280nm, 10Hz, 0.5 s resp. time	
Temperature	30 °C	
Peaks	 Phenol 2,4-Dinitrophenol 4-Nitrophenol 2-Chlorophenol 2-Nitrophenol 2,4-Dimethylphenol 4,6-Dinitro-2-methylphenol 4-Chloro-3-methylphenol 2,4-Dichlorophenol 2,4,6-Trichlorophenol Pentachlorophenol 	

Gradient	-1.5	0.0	0.3	2.6	3.0
A%	70	70	70	10	10
В%	30	30	30	90	90





Resistance to dewetting example

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 column 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 column remains wetted through many cycles.

Resistance to dewetting

Acclaim PA column, 5 μm, 4.6 × 150 mm			
Mobile phase	2.5 mM methanesulfonic acid, pH 2.6		
Flow rate	1 mL/min		
Injection volume	5 μL		
Detection	UV, 254 nm		
Temperature	30 °C		
Peaks	1. Cytosine 2. Uracil 3. Thymine		

Each cycle consists of two steps:

- 1. Equilibrate columns for 20 min before testing for 10 min.
- 2. Stop flow for 30 min before next cycle begins.



Separation of five antidepressants on Acclaim PA column

Acclaim PA column, 5 µm, 4.6 × 150 mm		
Mobile phase	80/20 v/v MeOH/30 mM phosphate, pH 6.0	
Flow rate	1 mL/min	
Injection volume	5 µL	
Detection	UV, 220 nm	
Temperature	30 °C	
Peaks	 Protriptyline 50 Nortriptyline 25 Doxepin 50 Imipramine 40 Amitriptyline 50 	



Acclaim Polar Advantage II (PA2) columns

Complementary selectivity and enhanced hydrolytic stability

Thermo Scientific Acclaim Polar Advantage II (PA2) columns, like the Acclaim PA2 column, 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.

Acclaim PA2 column 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 column provides selectivity that is complementary to conventional C18 columns, and to our Acclaim PA column. This column is fully compatible with 100% aqueous mobile phases and provides symmetrical peaks for both polar and non-polar analytes.

- 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

Turmeric separation

Acclaim 120 C18 column, 2.2 μm, 100 x 2.1 mm Acclaim PA2 column, 2.2 μm, 100 x 2.1 mm		
Mobile phase	(A) 15mM H ₃ PO ₄ (B) Methanol	
Flow rate	0.41 mL/min	
Isocratic	C18: 70% B (v/v) PA2: 80% B (v/v)	
Detection	UV, 428 nm	
Sample	Turmeric extract	
Temperature	30 °C	
Analytes	 Curcumin Demethoxycurcumin Bis-demethoxycurcumin 	

Com City Spin 120A 4.6 X 250 mm is particular to a spin to a spin





Beta blockers on Acclaim PA2 columns

Acclaim PA2 column, 5 μm, 4.6 × 150 mm				
Mobile phase	MeOH/0.2% NH ₄ OH	MeOH/0.2% NH ₄ OH, pH10, v/v, 60/40		
Flow rate	1.0 mL/min	1.0 mL/min		
Injection volume	5 µL			
Detection	UV, 210 nm			
Temperature	30 °C	30 °C		
Peaks (40 ppm each)	1. Maleate 2. Labetalol 3. Metaraminol 4. Atenolol	5. Acebutolol 6. Metoprolol 7. Timolol 8. Oxprenolol		



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Catecholamines in urine on Acclaim PA2 columns

Acclaim PA2 colu	ımn, 3 μm, 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	
Flow rate	0.2 mL/min	
Injection volume	2 µL	
Detection	DC amperometry [GC electrode, 800 mV]	
Temperature	30 °C	
Peaks (1 nM each)	 4-Hydroxy-3-methoxymandelic acid 4-Hydroxy-3-methoxyphenylglycol Norepinephrine Epinephrine S,4-digydroxybenzlamine 3,4-digydroxybenzlamine Normetanephrine Metanephrine Dopamine 4-Hydroxy-3-methoxyphenylacetic acid 5-Hydroxyindoleacetic acid 	



Separation of water-soluble vitamins on Acclaim PA2 columns

Acclaim RSLC Polar Advantage PA2 column, 2.2 µm, 150 × 2.1 mm				
Mobile phase	(A) 25 mM potassium dihydrogen phosphate, pH3.8 (adjusted with phosphoric acid)(B) 70:30 acetonitrile/25 mM potassiumdihydrogen phosphate, pH 3.8			
Flow rate	0.4 mL/min	0.4 mL/min		
Injection volume	2 µL			
Detection	UV, 210 nm			
Temperature	25 °C (with passive pre-h	25 °C (with passive pre-heater)		
Peaks	 Ascorbic acid Thiamine Pyridoxal Pyridoxin Nicotinamide 	 Pantothenic acid Folic acid Cyanocobalamine Riboflavinm 		

Time	0	5	7	10
В%	0	36	10	0



Acclaim Phenyl-1 column

A unique reversed-phase column with high aromatic selectivity

Thermo Scientific Acclaim Phenyl-1 columns are designed to provide unique selectivity distinguished from other reversed-phase HPLC columns, resulting in superior separations for analytes that cannot be resolved well on typical alkyl phases (C18 and C8) or other phenyl-type columns.

- High aromatic selectivity
- High hydrophobic retention
- Unique and complementary selectivity compared to any other phenyl type column
- Compatibility with highly aqueous mobile phase
- High efficiency and rugged packing



Acclaim Phenyl-1 column has a higher pi-pi interaction than other phenyl phases and provides unique selectivity for aromatic compounds while maintaining sufficient hydrophobic interaction and aqueous compatibility for superior chromatographic performance.

Acclaim Phenyl-1 column can be used in a wide range of applications in pharmaceutical, environmental, food testing and product-quality testing. This column is ideally suited for the analysis of aromatic analytes; some examples include glucocorticosteroids, estrogens, fat-soluble vitamins and phospholipids.

Separation of fat-soluble vitamins

Acclaim Phenyl-1 column, 3 μm, 150 x 3.0 mm			
Mobile phase	Methanol/water v/v 90/10		
Flow rate	0.5 mL/min		
Injection volume	2 μL		
Detection	UV, 220nm		
Temperature	30 °C		
Peaks (100 ppm each)	 Retinol acetate (vitamin A acetate) Vitamin D2 Vitamin D3 delta-Tocopherol gamma-Tocopherol alpha-Tocopherol (vitamin E) Impurity (unknown) Vitamin E acetate Vitamin K2 Vitamin K1 		



Separation of glucocorticosteroids

Acclaim Phenyl-1 column, 3 µm, 3.0 × 250 mm					
Mobile phase	Methanol/water v/v 46/54				
Flow rate	0.5 mL/min				
Injection volume	5 µL				
Detection	UV, 254 nm				
Temperature	40 °C				
Peaks (50 ppm each)	 Prednisone Cortisone Prednisolone Hydrocortisone Dexamethasone 6-Methylprednisolone Corticosterone Deoxyhydrocortisone 				



Analysis of soybean and egg lecithin

Acclaim Phenyl-1 column, 3 μm, 3.0 × 150 mm			
Mobile phase Acetonitrile/2-propanol/ammonium acetate (100 mM, pH5.0) v/v/v 45/45/10			
Flow rate	0.45 mL/min		
Injection volume	2 µL		
Detection	Corona ultra (Gain = 100 pA; Filter = low; Nebulizer temp. = 25 °C)		
Temperature	25 °C		
Samples	1. Soybean Lecithin (1 mg/mL in 2-propanol) 2. Egg lecithin (2.5 mg/mL in 2-propanol)		

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Phospholipids in soybean lecithin

Acclaim Phenyl-1 column, 3 μm, 3.0 × 150 mm			
Mobile phase	Acetonitrile/2-propanol/ammonium acetate (100 mM, pH 5.0) v/v/v 70/10/20		
Flow rate	0.45 mL/min		
Injection volume	5 µL		
Detection	Corona ultra (Gain = 100 pA; Filter = low; Nebulizer temp. = 25 °C)		
Temperature	25 °C		
Sample	Soybean lecithin (1 mg/mL in 2-propanol)		



Acclaim HILIC-10 column

Designed with unique selectivity for hydrophilic molecules

The Thermo Scientific Acclaim HILIC-10 column is designed for separating highly hydrophilic molecules by hydrophilic interaction liquid chromatography (HILIC). This column is based on high-purity spherical porous silica covalently modified with a proprietary hydrophilic layer.

HILIC is a complementary technique to reversed-phase liquid chromatography (RPLC) with several benefits. Polar analytes that cannot be retained using RP columns can be retained and separated using the Acclaim HILIC-10 column. The advantage of the HILIC phases is that they allow the use of 5–20% aqueous mobile phase, while maintaining affinity for polar analytes.

The Acclaim HILIC-10 column is ideally suited for analysis of polar analytes as demonstrated its use in a wide variety of applications including pharmaceuticals, metabolites, fat-soluble vitamins, oils, industrial applications, etc.

- Retains highly polar molecules that are not retained by reversed-phase chromatography
- Unique selectivity, complementary to reversed-phase columns
- Hydrolytically stable
- Rugged column packing
- Broad application range

Glycerides

Acclaim HILIC-10 column, 3 μm, 150 x 3.0 mm				
Mobile phase	(A) Heptane (B) 2-Propanol/acetic	(A) Heptane (B) 2-Propanol/acetic acid 99.5:0.5		
Flow rate	0.50 mL/min			
Injection volume	4 μL			
Detection	Corona ultra, nebulizer 15 °C			
Temperature	25 °C			
Analytes	1. Tristearin5. Distearin isomer 22. Trilaurin6. Dilaurin isomer 23. Distearin isomer 17. Monostearin4. Dilaurin isomer 18. Monolaurin			

Separation of hydrophilic pharmaceuticals

Acclaim HILIC-10 column, 3 μm, 4.6 × 150 mm			
Mobile phase $\begin{array}{l} 90/10 \text{ v/v CH}_3\text{CN}/10 \text{ mM (total)} \\ \text{NH}_4\text{OAc, pH 5} \end{array}$			
Flow rate	1 mL/min		
Injection volume	on volume 2 μL		
Detection	UV, 230 nm		
Temperature	30 °C		
Analytes	 Acetaminophen 0.1 mg/mL Salicylic acid 0.1 Aspirin 0.2 Penicillin G 0.1 Metformin 0.1 		









Separation of acrylic acid and oligomers

Acclaim HILIC-10 column, 3 μm, 4.6 × 150 mm			
Mobile phase $\begin{array}{c} 90/10 \text{ v/v CH}_3\text{CN}/10 \text{ mM (total)} \\ \text{NH}_4\text{OAc, pH 5} \end{array}$			
Flow rate	1 mL/min		
Injection volume	2 µL		
Detection	Corona CAD ultra		
Temperature	30 °C		
Analytes	1. Cyanuric acid 0.2 mg/mL 2. Melamine 0.2		



Separation of Good's buffer salts

Acclaim HILIC-10 column, 3 µm, 4.6 × 150 mm			
Mobile phase $\begin{array}{l} 85/15 \text{ v/v CH}_{3}\text{CN}/10 \text{ mM (total)} \\ \text{NH}_{4}\text{OAc, pH 5} \end{array}$			
Flow rate	1 mL/min		
Injection volume	10 µL		
Detection	Corona CAD ultra		
Temperature	30 °C		
Analytes (0.1 mg/mL in mobile phase)	1. TAPS 2. CHES 3. MOPS 4. TES 5. HEPES		



Separation of mono-, di-, and triglycerides

Mobile phase	(A) Heptane (B) 2-Propanol-acetic acid 99.5:0.5				
Flow rate	0.50 mL/min				
Injection volume	4 µL				
Detection	Corona <i>ultra</i> , nebulizer 15 °C				
Temperature	25 °C				
Analytes	 Tristearin Trilaurin Distearin isomer 1 Dilaurin isomer 1 Distearin isomer 2 Dilaurin isomer 2 Monostearin Monolaurin 				

Time	-6.0	0.0	0.5	4.0	10.0
A%	99	99	96	87	87
В%	1	1	4	13	13



Acclaim size exclusion chromatography (SEC)

High performance SEC columns for analysis of water soluble polymers

Thermo Scientific Acclaim SEC-300 and SEC-1000 columns are a family of resin based, high-performance size exclusion chromatography columns specifically designed for the separation of water soluble polymers and oligomers.

- Proprietary mono-dispersed multi-pore hydrophilic resin: no inflection points in calibration curve
- SEC-300 columns calibrated from 100 to 50,000 Daltons
- SEC-1000 columns calibrated from 1,000 to 1,000,000 Daltons
- Availability of small particle sizes packed in 300 x 4.6 mm dimension allows for high-resolution analysis at reduced solvent consumption
- Stable surface bonding with low column bleed and compatibility with UV, RI, MS, ELSD and Thermo Scientific[™] Dionex[™] Corona[™] Charged Aerosol Detectors

Acclaim SEC-300 column, 5 μm, 300 x 4.6 mm Acclaim SEC-1000 column, 7 μm, 300 x 4.6 mm		100,000,000 - 10,000,000 -	1				SEC-10	
Mobile phase	10 mM sodium perchlorate	(() ,,,,,,,,		N N			SEC-30	10
Flow rate	0.35 mL/min	(a) 1,000,000 - <u>H6</u> (a) 100,000 -	1	No.				
Injection volume	50 µL							
Detection	RI	900,00 - 000,00 -		A.	×,			
Temperature	25 °C	≥ 1000 -			N.	X		
	(0.03% - 0.1% in mobile phase) dextran (MW 5,000,000-40,000,000), PEO (MW 895,000, 580,000, 272,000, 185,000,	100 -			×.	N. N		
Analytes	80,000, 43,000, and 20,000), PEG (MW 8,300, 3,000, 1,500, 600, 400 and 200),	10	2.00	2.50	3.00	3.50	4.00	4.50
	diethylene glycol (MW 106 and ethylene glycol MW 62)				ion volun			

Separation of polyethylene glycols on Acclaim SEC-300 columns vs. Acclaim SEC-1000 columns

Acclaim SEC-300 column, Acclaim SEC-1000 column, 4.6 x 300 mm			
Mobile phase	10 mM sodium perchlorate		
Flow rate	0.35 mL/min		
Injection volume	5 μL		
Temperature	30 °C		
Detection	RI		
Sample	(5 mg/mL in mobile phase) 1. PEG MW 35,000 2. PEG MW 12,000 3. PEG MW 3,400 4. PEG MW 2,000 5. PEG MW 300		





Particle size 5 µm, 7 µm

Polyacrylic acid using SEC with charged-aerosol detection

Mobile phase	(A) Acetonitrile (B) Water		- F			0.450 Area [pA*min]
Flow rate	0.35 mL/min		-			0.200-
Injection volume	35 µL	٨d		Λ	400µg/L	
Detection	Corona III; evaporator 55 °C, Engine 40 °C, 2 Hz, filter 5, power function 1.20		- 	Λ	200µg/L 100µg/L	0.00c0 Concentration [µg/L] 0 125 250 375 500
Temperature	30 °C		-	∧	50µg/L	
Analyte	PAA standards in water	-0.0		^	25µg/L	
			0	5 Minut	ies 10 15	

Separation of polyacrylic acid on Acclaim SEC-300 columns vs Acclaim SEC-1000 columns

Acclaim SEC-300 column, Acclaim SEC-1000 column, 4.6 x 300 mm			
Mobile phase	10 mM sodium perchlorate		
Flow rate	0.35 mL/min		
Injection volume	5 µL		
Temperature	30 °C		
Detection	RI		
Sample	Dextran, MW 10,000 (5 mg/mL in mobile phase)		



Maltodextrin separation using the Acclaim SEC-1000 columns

Acclaim SEC-1000 column, 4.6 x 300 mm		
Mobile phase	100 mM ammonium acetate pH 5.0	
Flow rate	0.35 mL/min	
Injection volume	5 µL	
Temperature	25 °C	
Detection	Corona ultra Charged Aerosol Detector	
Sample	MALTRINs, 5 mg/mL each	





Acclaim Mixed-Mode columns

Acclaim Mixed-Mode HPLC columns

Mixed-Mode chromatography

Mixed-Mode chromatography provides multiple functionalities on a single chromatographic support. For example, combining both reversed-phase and ion-exchange retention mechanisms or reversed-phase and HILIC or even three mechanisms (Trinity). One major advantage of this approach is that column selectivity can easily be modified for optimal selectivity by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As a result, not only is the selectivity of a Mixed-Mode column complementary to that of reversed-phase columns, but it also allows for the development of multiple complementary selectivities on the same column under different appropriate conditions. Mixed-Mode chromatography is well-suited to retaining ionic analytes, whether hydrophobic (e.g. Naproxen) or hydrophilic (e.g. Na⁺ and Cl⁻ ions) and requires no ion-pairing agents in the method, significantly improving the MS compatibility. Most importantly, Mixed-Mode chromatography column chemistry can be customized to a desired selectivity during stationary phase design.^{1,2}

- Excellent performance: selectivity, resolution and retention
- Good for separations of active pharmaceutical ingredients (APIs), mixtures, formulations, ions
- Good when retention requirements are contradictory for a single-mode column
- Offers flexibility in method development

Bi-modal Mixed-Mode phases

The Acclaim family contains three bi-modal Mixed-Mode columns and two tri-modal columns. As shown here, bi-modal columns have both a hydrophobic arm – providing reversed-phase retention, and an ion-exchange or diol group at the tip – providing ion-exchange or HILIC retention.



Tri-modal Mixed-Mode phases

Nanopolymer Silica Hybrid technology: Tri-modal Mixed-Mode phases

Acclaim Trinity P1 and P2 columns are based on Thermo Scientific[™] Nanopolymer Silica Hybrid (NSH[™]) technology. These are high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner pores have a bimodal functionality and the outer nanopolymers have a differing ion-exchange functionality. The spatial separation of the two ion-exchange regions allows both retention mechanisms to function simultaneously and be controlled independently.



Acclaim Mixed-Mode HPLC columns

Columns	Description
 Reversed-phase, anion-exchange, and cation exchange functionalities For superior resolution of active phamaceutical ingredients (API) and counter ions, as mixtures 	
 Acclaim Trinity P2 HILIC, anion-exchange, and cation exchange functionalities For complex mixtures including separation pharmaceutical counterion; mono- and and anions 	
Acclaim Mixed-Mode WAX-1	Reversed-phase and anion-exchange combined functionalityFor separating anionic molecules with powerful adjustable selectivity control
Acclaim Mixed-Mode WCX-1	Reversed-phase and cation-exchange combined functionalityFor separating cationic molecules with powerful adjustable selectivity control
Acclaim Mixed-Mode HILIC-1	Combined capability to operate in either reversed-phase or HILIC mode

Acclaim Mixed-Mode HPLC column specifications

	Acclaim Trinity P1	Acclaim Trinity P2	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	
Functionality	Reversed-phaseWeak anion exchangeStrong cation exchange	HILICStrong anion exchangeWeak cation exchange	Reversed-phaseWeak anion exchange	Reversed-phaseWeak cation exchange	Reversed-phaseHILIC	
USP type	_	_	L78	L85	_	
Starting material	Ultrapure silica					
Particle shape	Spherical					
Particle sizes	3 µm	3 µm	3 μm 5 μm	3 μm 5 μm	3 μm 5 μm	
Average pore diameter	300 Å	300 Å	120 Å	120 Å	120 Å	
Surface area	100 m²/g	100 m²/g	300 m²/g	300 m²/g	300 m²/g	
pH range	2.5–7.5	2.5–7.5	2.5–7.5	2.5–7.5	2.5–7.5	

Acclaim Mixed-Mode WAX-1 columns

Designed for separating anionic molecules with powerful adjustable selectivity control

The Thermo Scientific Acclaim Mixed-Mode WAX-1 column is a novel, highefficiency silica HPLC column that combines hydrophobic and weak anion exchange characteristics. Its unique chemistry results in a multimode separation mechanism that includes reversed-phase, anion exchange, and HILIC interactions. Selectivity can be adjusted by changing ionic strength, pH or organic solvent content.

- Adjustable selectivity
- Selectivity orthogonal to reversed-phase (RP) columns
- Ideal selectivity for anionic molecules
- Excellent column efficiency and peak asymmetry
- Multimode retention mechanisms: reversed-phase, weak anion exchange, and HILIC modes

Pain relief medicine

Acclaim Mixed-Mode WAX-1 column, 5 µm, 150 x 4.6 mm

Mobile phase	40/60 v/v Acetonitrile/buffer (6.8 g potassium monophosphate and 0.5 g pyrophosphate in 1000 g D.I. H_2O , pH is adjusted to 6.0 with NaOH)
Flow rate	1 mL/min
Injection volume	1 µL
Detection	UV, 220 nm
Temperature	30 °C
Analytes	 Caffeine Acetaminophen Salicylamide Acetyl salicylic acid (Aspirin) Salicylic acid

Analysis of soft drinks

Acclaim Mixed-M	Acclaim Mixed-Mode WAX-1 column, 5 μm, 150 x 4.6 mm				
Mobile phase	(A) 55/45 v/v Acetonitrile/ 0.2 M phosphate buffer, pH 6.0 (B) 57/43 v/v Acetonitrile/ 0.12 M phosphate buffer, pH 2.9				
Temperature	30 °C				
Flow rate	1 mL/min				
Injection volume	2.5 μL				
Detection	UV, 210 nm				
Peaks	 Caffeine 100 μg/ml Aspartame 100 Acesulfame, potassium 100 Saccharin 100 Sorbate, poassium 100 Benzoic acid 100 Citrin acid 300 				



















1 Caffeine

2. Aspartame 3. Acesulfame, potassium

m 4. Saccharin

rin 5. Sorbate, potassium

m 6. Benzoic acid

7. Citric acid

Acclaim Mixed-Mode WCX-1 columns

Designed for separating cationic molecules with adjustable selectivity control

Thermo Scientific Acclaim Mixed-Mode WCX-1 is a novel, high-efficiency, silica-based column, with a proprietary ligand with both hydrophobic and weak cation exchange properties. Selectivity of ionizable and neutral compounds can be controlled independently or simultaneously by tuning mobile phase ionic strength, pH or organic modifier. This column therefore can separate using multiple separation modes: reversed-phase, cation exchange, and HILIC and is recommended for a variety of industrial applications, including pharmaceutical, chemical, consumer products, foods and beverages.

- Adjustable selectivity
- Ideal selectivity for separating basic molecules
- Selectivity complementary to C18 RP columns
- Multimode separation mechanism: reversed-phase, weak cation exchange, anion-exclusion and HILIC

Pharmaceutical counterions

Acclaim Mixed-Mode WCX-1 column, 5 µm, 150 x 4.6 mm				
Mobile phase	40/60 v/v CH ₃ CN/NH4OAc, p	oH 5.2 (20 mM total)		
Flow rate	1 mL/min			
Injection volume	5 µL			
Detection	UV, 225 nm			
Temperature	30 °C			
Analytes	 Maleate 50 μg/mL Ketoprofen 30 μg/mL Naproxen 30 μg/mL Hydrocortisone 60 μg/mL 	5. Dexamethasone 60 μg/mL 6. Oxprenolol 300 μg/mL 7. Timolol 250 μg/mL		



Separation of catecholamines

Acclaim Mixed-Mode WCX-1 column, 5 μm, 150 x 4.6 mm			
Mobile phase	2/98 v/v CH2CN/sodium phosphate, pH 6.2 (10 mM total concentration)		
Temperature	30 °C		
Flow rate	1 mL/min		
Injection volume	5 μL		
Detection	UV, 215 nm		
Peaks	(0.25 nM each) 1. NE 2. E 3. DHBA 4. DA		




Acclaim Mixed-Mode HILIC-1

Uniquely designed for both reversed-phase and HILIC operations

The Thermo Scientific Acclaim Mixed-Mode HILIC-1 column features a unique, high-efficiency, silica-based HPLC Mixed-Mode stationary phase that combines both reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) properties. This combination allows both hydrophobic and hydrophilic interactions to be utilized to optimize separations.

The functional group is of a hydrophobic alkyl chain with a diol group at the terminus. This unique combination results in the adjustable selectivity, making Acclaim Mixed-Mode HILIC-1 column separate mixtures that would be impossible for a C18 column. This column is suitable for a broad range of applications, including non-ionic ethoxylated surfactants, drug metabolites, lipids, polyethylene glycols (PEGs), ethoxylated surfactants, and more.

- Can operate in both RP and HILIC modes
- Retains highly polar molecules
- Unique selectivity complementary to RP columns
- Broader application range compared with conventional diol-based columns
- High-efficiency column for high-resolution separations

Cytosine and naphthalene

Acclaim Mixed-Mode HILIC-1 column, 5 µm, 150 x 4.6 mm				
Mobile phase	CH3CN/0.1 M NH₄OAc, pH 5.2 v/v 52/48 for RP mode v/v 92/8 for HILIC mode			
Flow rate	1 mL/min			
Injection volume	10 µL			
Detection	UV, 254 nm			
Temperature	30 °C			
Analytes	1. Cytosine (100 ppm) 2. Naphthalene (100 ppm)			



This surfactant separation below shows two modes of separation: on top, in the reversed-phase mode, the ethoxylated surfactant elutes as one peak, for easy total content measurements. The same column, when eluted with greater organic solvent concentration in the mobile phase (the HILIC mode), separates all the ethoxylated components individually which can be used to determine the degree of ethoxylation of the surfactant.

Separation dependency of organic solvent

Acclaim Mixed-Mode HILIC-1 column, 5 μm, 150 x 4.6 mm				
Mobile phase	(A) CH ₃ CN (B) 0.1 M NH ₄ OAc, pH 5.2			
Temperature	30 °C			
Flow rate	1 mL/min			
Injection volume	10 µL			
Detection	UV, 225 nm			
Sample	IGEPAL CA-630 (0.1%)			

 $G_{H_{17}} \longrightarrow (OCH_2CH_2)_nOH$ IGEPAL CA-630



pH range		
0	2.5 to 7.5	14
Hydrophobicit	ty	
Low		 High
Pore size		
0	120Å	300



Acclaim Trinity P1 columns

Mixed-Mode column technology combining reversed-phase, anion exchange and cation exchange functionality on a single support

The Thermo Scientific Acclaim Trinity P1 HPLC column is designed with nanopolymer silica hybrid (NSH) technology, which results in a multimode surface chemistry ideal for the simultaneous separation of drugs and their counterions. The surface chemistry concurrently provides reversed-phase, cation exchange, and anion exchange functionalities. The result is maximum flexibility in method development. Separations can be optimized easily by adjusting the chromatographic parameters (mobile phase pH, ionic strength, and organic strength).

- Ideal selectivity for simultaneous separation of API and counterion
- Adjustable selectivity by mobile phase ionic strength, electrolyte type, pH, and organic solvent
- Low bleed; compatible with MS, CAD and ELSD
- Retention of hydrophilic ionic and ionizable analytes without ion-pairing reagents
- Greater flexibility in method development: each retention mechanisms can be controlled independently

The Acclaim Trinity P1 column is able to separate both pharmaceutically related cations and anions on one column. The selectivity is ideal and peaks are symmetrical. The column is designed such that cations elute before anions. No other columns can do this separation, and in fact, this separation is part of the production qualification test.

Simultaneous separation of pharmaceutical counterions

Acclaim Trinity P1	column, 3 µm, 100 x	3.0 mm		
Mobile phase	60/40 v/v CH ₃ CN/2	0 mM (total) NH₄OAc, pH 5		
Flow rate	0.5 mL/min			
Injection volume	2 µL			
Detection	Corona ultra (Gain = 100 pA; Filter = med; Neb temp = 30 °C)			
Temperature	30 °C	30 °C		
Analytes	(50 to 100ppm) 1. Choline 2. Tromethamine 3. Sodium 4. Potassium 5. Meglumine	6. Mesylate 7. Nitrate 8. Chloride 9. Bromide 10. lodide		





Simultaneous separation of pharmaceutical counterions (gradient method)

Acclaim Trinity	P1 colu	mn, 3 µm, 50) x 3.0 mi	m		
Mobile phase	(B	(A) CH2 ₂ N (B) DI H ₂ O (C) 0.2 M NH ₄ OAc, PH 4				
Temperature	30) °C				
Flow rate	0.	5 mL/min				
Injection volume	2	μL				
Detection		Corona <i>ultra</i> (Gain = 100 pA; Filter = med; Neb temp = 30 °C)				
Peaks	3. 4. 5. 6. 7.	. ,		11. 12. 13. 14. 15.	Tartrate	9
Gradient	-10	0	2		7	15
A%	60	60	60		10	10
В%	35	35	35		0	0

5

C%

5

5

90



Liquid chromatography with tandem mass spektrometry (LC-MS-MS) analysis of ethanol amines (SCX mode)

90

Acclaim Trinity P1	column, 3 µm, 100 x 2.1 mm				
Chromatography of	Chromatography conditions				
System	Dionex RSLC LC LCi system				
Temperature	20 °C				
Mobile phase	90% CH ₃ CN, 10% NH ₄ OOCH buffer				
Flow rate	600 µL/min				
Injection volume	20 µL				
Mass spectrometric conditions					
System	LC-MS-MS QTRAP				
Interface	TurboSpray with Electrospray ionization				
Curtain gas	15				
Collision gas	Medium				
IonSpray voltage	4500 V				
Temperature	700 °C				
lon source gas 1	50				
lon source gas 2	20				
Detection mode	Multiple reaction monitoring (MRM)				

Analyte	Q1MS	Q3MS	DP	CE	СХР
EA	62	44	46	15	6
DEA	106	88	66	19	6
DEA-IS	114	78	53	24	6
MDEA	120	102	46	19	8
EDEA	134	116	51	21	8
TEA	150	132	61	19	0



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Acclaim Trinity P2 column

Mixed-Mode column technology; hydrophilic interaction combining HILIC, anion exchange and cation exchange functionalities

The Thermo Scientific Acclaim Trinity P2 is a unique, high-efficiency, silica-based column specifically designed for separation of pharmaceutical counterions, including monovalent and divalent cations or anions. This column is based on nanopolymer silica hybrid (NSH) technology, which consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. The innerpore area of the silica bead is modified with a covalently bonded organic layer that provides cation-exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads.

The Acclaim Trinity P2 column is aimed to complement Acclaim Trinity P1 columns to provide a total solution for pharmaceutical counter ion analysis by HPLC.

- Ideal for separating pharmaceutical counterions, including monovalent and divalent cations or anions
- Selectivity complementary to the Acclaim Trinity P1 column
- Low column bleed, compatible with CAD and MS
- Hydrolytically stable
- High-efficiency

Pharmaceutical-related anions and cations

Acclaim Trinity P2	column, 3 µm, 100	x 3.0 mm			
Mobile phase	D.I. water and 100	mM NH ₄ OFm, pH 3.65 gradient			
Flow rate	0.60 mL/min				
Injection volume	2 µL				
Detection	Corona Veo Charg	Corona Veo Charged Aerosol			
Temperature	30 °C	30 °C			
Sample	0.02 – 0.10 mg/mL	each in D.I.			
Analytes	 Phosphate Sodium Potassium Chloride Malate Bromide 	 7. Nitrate 8. Citrate 9. Fumarate 10. Sulfate 11. Magnesium 12. Calcium 			

Time (min)	H₂O	0.1 M Ammonium formate, pH 3.65
-10	0.760	1.474
0	80	20
2	80	20
22	0	100
30	0	100





A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. It is highly desirable to separate both pharmaceutically important anions and cations within the same analysis and in a reasonable amount of time. This figure illustrates that Acclaim Trinity P2 column provides desired selectivity for the separation of mono- and multi-valent anions and cations – a total of twelve ions.

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Electrolytes in sports beverages

Acclaim Trinity P2	column, 3 µm, 50 x 3.0mm		
Mobile phase	(A) Water (B) 100 mM Ammonium formate, pH 3.65		
System	Dionex UltiMate 3000 RS		
Temperature	30 °C		
Flow rate	0.60 mL/min		
Injection volume	2 μL		
Detection Corona Veo (evaporator 55 °C, data rate 5 I filter 2 sec, power function 1.5)			
(A) Sports drink (Orange flavor) (B) Sports drink, zero-calorie (Fruit pu			
Sample prep	Decolorized with Dionex OnGuard-II P cartridge		
Peaks	 Sugars Ascorbic acid Phosphate Sodium Potassium Chloride Citrate Acesulfame Unknown Magnesium Calcium 		

45 -	1	4			
PA 1	3	5	7	910	11 A
0	l	5	Minutes	8 10	——————————————————————————————————————

Gradient	-8.0	0.0	1.0	11.0	20.0
A%	90	90	90	0	0
В%	10	10	10	100	100



Acclaim application-specific HPLC columns

Acclaim application-specific HPLC columns

Innovative chemistries tailored for challenging and critically important applications

Application-specific columns utilize novel and unique chemistries to provide superior resolution with ease of use for key pharmaceutical, environmental and food/beverage applications. These columns are designed and tested for specific application.

Columns	Description
Acclaim Organic Acid	Fast organic acid analysis; including both aromatic and aliphatic organic acids
Acclaim Surfactant	Designed for the separation of surfactants including anionic, non-ionic, cationic, and amphoteric surfactants in various matrices
Acclaim Surfactant Plus	Designed for the separation of surfactants including anionic, non-ionic, cationic, and amphoteric surfactants in various matrices. The Surfactant Plus column is compatible with LC-MS instrumentation, and recommended for new method development
Acclaim Explosives E2	Specialty columns to comply with EPA Method 8330; baseline resolution of all 14 explosive residues targeted by the EPA
Acclaim Trinity Q1	Excellent for trace analysis of diquat and paraquat
Acclaim Carbonyl C18	Separation of DNPH derivatives of aldehydes and ketones; including regulated methods U.S. EPA 554, EPA 8315, EPA 1667, EPA TO-11, and California Air Resources Board (CARB) Method 1004
Acclaim Carbamate	Separation of carbamate pesticides specified in U.S. EPA Method 531.2
Acclaim AmG C18	Ion-pairing reversed-phase separation of aminoglycoside antibiotics

Specifications for Acclaim application-specific HPLC columns

	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Surfactant Plus	Acclaim Explosives E2	Acclaim Trinity Q1	Acclaim Carbonyl C18	Acclaim Carbamate	Acclaim AmG C18
End-capped	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Particle shape		Spherical						
Particle size	3 μm 5 μm	3 μm 5 μm	3 μm 5 μm	2.2 μm 3 μm 5 μm	3 µm	2.2 μm 3 μm 5 μm	2.2 μm 3 μm 5 μm	3 µm
Average pore diameter	120 Å	120 Å	120 Å	120 Å	300 Å	120 Å	120 Å	120 Å
Surface area	300 m²/g	300 m²/g	300 m²/g	300 m²/g	100 m²/g	300 m²/g	300 m²/g	300 m²/g
pH range	2–8	2–8	2.5–7.5	2–8	2.5–7.5	2.5–7.5	2.5–7.5	0.5–10

Acclaim Organic Acid columns

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

The Thermo Scientific Acclaim Organic Acid (OA) is a silica-based reversed-phase column designed for high-efficiency, high-throughput organic acids analysis. It offers unparalleled performance for separating hydrophilic aliphatic and aromatic organic acids at low pH with UV detection.

Acclaim Acid column 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, and manufacturing chemicals, chemical intermediates, and environmental samples.

- Tested to guarantee consistent hydrophilic organic acid separations
- Compatible with 100% aqueous mobile phases
- Hydrolytic stability at low-pH conditions
- Ideal selectivity for separating a wide spectrum of organic acids
- Excellent column efficiency and peak shapes for organic acids

Hydrophilic organic acids

Acclaim OA colu	mn, 5 µm, 4 × 250 mm		
Mobile phase	100mM Na ₂ SO ₄ , pH 2.65 (adjusted with methanesulfonic acid)		
Flow rate	0.60 mL/min		
Injection volume	5 µL		
Detection	UV, 210nm		
Temperature	30 °C		
Analytes	 Oxalic acid 15 mg/L (ppm) Tartaric acid 120 Formic acid 180 Malic acid 120 iso-Citric acid 120 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 	

Flexible methods development

Since the Acclaim OA can be operated at low pH with 100% aqueous buffers, as well as with organic solvents, many mobile phase options are available to optimize your organic acid separations. Modifying the pH of the mobile phase, as illustrated below, allows resolution and retention control.







White wine analysis

Acclaim OA colum	n, 5 µm, 250 x 4 mm
Mobile phase	0.1 M $\mathrm{Na_2SO_4},$ pH 2.68 (adjusted with MSA*)
Flow rate	0.60 mL/min
Injection volume	5 µL
Detection	UV, 210 nm
Sample	OnGuard II P
Temperature	30 °C
Peaks	 Tartaric acid Malic acid Lactic acid Citric acid Succinic acid

*Methanesulfonic acid



Separation of seven food additives

Mobile phase	(A) 100 mM KH ₂ PO ₄ pH 3 (adjusted with MSA) (B) Acetonitrile
Flow rate	0.6 mL/min
Injection volume	5 μL
Detection	UV, 210 nm
Columns temperature	50 °C (still air mode)
Pre-heater temperature	50 °C
Peaks	 Citrate Acesulfame Saccharin Caffeine Aspartame Sorbate Benzoate

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Gradient	0	5	7	7.1	10
A%	95	75	75	95	End
В%	5	26	25	5	



Acclaim Surfactant and Surfactant Plus columns

Column of choice for surfactant analysis using higher sensitivity detection: performance, versatility, throughput

The Thermo Scientific Acclaim Surfactant and Surfactant Plus columns are highefficiency, silica-based columns designed specifically for separating a wide variety of surfactants, including anionic, cationic, nonionic, ethoxylated and amphoteric surfactants. The surface chemistry is optimized for improved performance and higher throughput.



Acclaim Surfactant column can be used with evaporative light scattering detectors (ELSD), UV-Vis detectors (UV) or refractive index (RI) detection.

The Acclaim Surfactant Plus column is similar to the original Acclaim Surfactant column and exhibits exceptionally low bleed making it ideal for use with charged aerosol detectors (CAD) and mass spectrometers (MS). The Acclaim Surfactant Plus column, like the Acclaim Surfactant columns can be used to separate a wide variety of surfactants including isomers of xylene sulfonate. Additionally, these columns can be used with suppressed conductivity detectors (SCD); non-metallic PEEK hardware is available for best compatibility with Dionex ion chromatography systems.

Surfactants are widely used in industrial, agricultural, and pharmaceutical markets, in products as diverse as pesticides, detergent powders, petroleum products, cosmetics, and pharmaceuticals. The Acclaim Surfactant and Surfactant Plus columns are designed specifically for HPLC separation of these surfactants.

- Ideal selectivity for simultaneous separation of anionic, nonionic, cationic, and amphoteric surfactants
- Surfactant Plus compatible with multiple detectors including MS, CAD, ELSD and UV
- Excellent peak shapes, especially for cationic surfactants
- Compatible with highly aqueous mobile phases
- Improved resolution for ethoxylated surfactants
- Rugged separations under a variety of conditions

Inorganic anion, hydrotropes, cationic, nonionic, amphoteric, and anionic surfactants

Acclaim Surfactant column, 5 µm, 150 x 4.6 mm (A) CH₃CN Mobile phase (B) 0.1 M NH₄OAc, pH 5.4 25% to 85% A in 25 min, then hold 85% A for 10 min Gradient Flow rate 1 mL/min Injection volume 25 µL Detection ELS detector Temperature 30 °C 1. Chloride 2. Bromide 3. Nitrate 4. Xylene sulfonate 5. Laurylpyridinium chloride 6. Lauryldimethylbenzyl-ammonium chloride 7. Triton X-100 Analytes 8. Cetyl betaine 9. Decyl sulfate 10. Dodecyl sulfate 11. C₁₀-LAS 12. C₁₁-LAS 13. C₁₂-LAS

14. C₁₃-LAS



Cationic surfactants

Acclaim Surfactan	t Plus column, 3 μ	m, 150 x 3.0 mm			
Mobile phase	(A) Acetonitrile (B) 100 mM form (C) Water	(B) 100 mM formic acid			
Flow rate	0.50 mL/min				
Injection volume	5μL				
Detection	Conductivity with	blank subtraction			
Temperature	25 °C				
Analytes	,	Imonium nonium Imonium /lammonium			
	0/ 8	0/ 10	0/ 0		

	2	3 4
5-	6 - I	7
S I	5	8
0		
0	5 Minutes	10 15

.....

Time (min)	%A	%B	%C
-12	5	5	90
0	5	5	90
12	40	5	55
20	40	5	55

Simultaneous analysis of cationic, nonionic, amphoteric and anionic surfactants by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS)

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Acclaim Surfactant Plus column, 3 µm, 150 x 3.0 mm		
Flow rate	0.6 mL/min	
Injection volume	2 µL	
Detection	Corona <i>ultra</i> (gain = 100 pA; Filter = med; Neb temp = 20 °C)	
Temperature	30 °C	
Sample	Shampoo (40 x dilution with D.I. water and filtered)	

Time (min)	Acetonitrile	0.05 M Ammonium Acetate, pH 5.2
-8	25	75
0	25	75
10	80	20
20	80	20



Simultaneous analysis of cationic, nonionic, amphoteric and anionic surfactants by LC-ESI-MS

Acclaim Surfactant Plus column, 3 µm, 150 x 2.1 mm		
Chromatographic of	conditions	
System	UltiMate 3000 RSLC	
Temperature	30 °C	
Mobile phase	(A) D.I. water (B) 100 mM ammonium acetate, pH 5 (C) Acetonitrile	
Flow rate	0.3 mL/min	

Time (min)	%A	%B	%C
-10	65	5	30
0	65	5	30
1	65	5	30
8	10	5	85
20	10	5	85

MS conditions	
System	MSQ Plus single quadrupole MS
Interface	Electrospray ionization (ESI)
Probe temp.	450 °C
Needle vol.	3 kV
Nebulizer gas	Nitrogen at 85 psi
Scan mode	Polarity switching full scan 100–1000 m/z

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Acclaim Explosives E2 column

The best solution for explosives analysis (EPA Method 8330)

Thermo Scientific Acclaim Explosives E2 columns are specifically designed to resolve all 14 explosives listed in EPA SW-846 Method 8330: nitroaromatics and nitramines by HPLC. The novel and unique chemistries of these columns provide superior resolution with complementary selectivities.

Acclaim Explosives E2 columns may be used as either a primary or a confirmatory column. The unique selectivity and versatility of this column provides a wider application range, including the analysis of explosives beyond United States Environmental Protection Agency (U.S. EPA) Method 8330 (ISO 22478).

- Acclaim E2 columns provide baseline resolution of all 14 compounds targeted by EPA Method 8330
- Columns available in 2.2 µm, 3 µm and 5 µm particle size
- Simple isocratic elution conditions
- Rugged columns with good lot-to-lot reproducibility

Rapid determination of EPA 8330A explosives

Acclaim RSLC Ex	olosives E2, 2.2 μι	m, 100 x 2.1 mm		
Mobile phase	Methanol:water	Methanol:water 48:52 (v/v)		
Flow rate	0.34 mL/min (29	3 bar)		
Injection volume	1 µL			
Detection	UV, 254nm			
Temperature	31 °C	31 °C		
Sample	Calibration mix,	Calibration mix, 25µg/mL in 50% acetontrile		
Analytes	1. HMX 2. RDX 3. 1,3,5-TNB 4. 3,5-DNB 5. NB 6. 2,4,6-TNT 7. Tetryl	8. 2,6-DNT 9. 2,4-DNT 10. 2-NT 11. 4-NT 12. 3-NT 13. 4-Am-2,6-DNT 14. 2-Am-4,6-DNT		



O₂N

Degradation products of TNT Manufacturing impurities of TNT Internal standard



NO,

1,2-DNB

NO,

TNT

60





2-NT

2,6-DNT

O₂N

-NO



NO.

4-NT

CH

NO₂ 2,4-DNT





NB

1,3-DNB



Acclaim AmG C18 column

Designed to provide rugged and reproducible reversed-phase chromatography of aminoglycoside antibiotics

Aminoglycoside antibiotics are commonly used as clinical and veterinary medicines to treat bacterial infections. HPLC using ion-pairing reversed-phase separations is an effective technique for simultaneous qualitative and quantitative determination of aminoglycosides.



- Excellent selectivity for the HPLC of aminoglycosides
- Superior resistance to acidic conditions for long column lifetime
- Easy-to-use with only aqueous mobile phase; TFA only, or TFA/HFBA or when PFPA is needed
- Compatible with simple rugged methods; no solvents are required
- High-efficiency and throughput

Mobile phase

Injection volume

Flow rate

Detection

Sample

Temperature

Acclaim AmG C18 column, 3 µm, 150 x 3.0 mm

2 µL

30 °C

100 mM TFA

0.425 mL/min

Gentamicin (1 mg/mL)



Isocratic separation of gentamicin sulfate using 100 mM TFA as the mobile phase

Corona Veo RS (Filter = 5.0 s; Evaporation Temp =

35 °C; Data Rate = 5 Hz; Power Function = 1.00)

Excellent low pH stability

The ion-pairing reversed-phase HPLC (IP-RPLC) separation of aminoglycoside antibiotics is generally performed under low pH conditions and therefore the stationary phase/column low pH stability is vital for these applications. The Acclaim AmG C18 column is specifically designed for analysis of aminoglycoside antibiotics, and compatible with these low pH conditions. The Acclaim AmG C18 columns are packed with a polymer encapsulated silica covalently bonded with C18 ligands. The polymer layer protects the siloxane linkage on the silica surface from hydrolysis when exposed to the low pH environment.



Hydrolytic stability is illustrated here, using low pH volatile perfluorinated carboxylic acids as the ion-pairing reagent, 100 mM trifluoroacetic acid (TFA) at high temperature.

Excellent low pH stability					
Acclaim AmG C18	column and Typical C18 column, 3.0 × 150 mm	140% 7			
Acid stress protoco	I	120% – Acclaim AmG C18			
Mobile phase	100 mM TFA				
Flow rate	0.425 mL/min				
Temperature	80 °C				
Performance test					
Mobile phase	Acetonitrile/10 mM NH ₄ OAc, 10/90 (v\v)				
Flow rate	0.425 mL/min	2 = 40% - 1			
Injection volume	2 µL	20% -			
Temperature	80 °C				
Detection	UV, 220 nm	0%			
Sample	Acetanilide	Exposure time (h)			

Shown below is the separation of structurally related aminoglycosides include Sisomicin, netilmicin, and etimicin. Other examples (Amikacin, Kanamycin, Tobbramycin, Arbekacin, Streptomycin, Ribostamycin, Paromyomycin, Neomycin, Spectoinomycin, Apramycin) of can be found in the product specification sheet.

Analysis of sisomicin, netilmicin, and etimicin

Acclaim AmG C18 column, 3 $\mu\text{m},$ 3.0 \times 150 mm		
Mobile phase	100 mM TFA	
Flow rate	0.425 mL/min	
Injection volume	2 µL	
Temperature	30 °C	
Detection	Corona Veo RS (Filter = 5.0 s; Evaporation temp = 35 °C; Data rate = 5 Hz; Power function = 1.00)	
Sample	1. Sisomicin (0.2 mg/mL) 2. Netilmicin (1 mg/mL) 3. Etimicin (1 mg/mL) 4. Mixture (0.25 mg/mL)	



Acclaim Trinity Q1 columns

For trace analysis of diquat and paraquat

Thermo Scientific Acclaim Trinity Q1 columns are unique, high-efficiency, silica-based columns designed for the separation of the herbicides diquat and paraquat. These herbicides are toxic and residues are monitored in drinking water, wastewater and agricultural products. The Acclaim Trinity Q1 column is a tri-mode (WCX, WAX, RP) column based on nanopolymer silica hybrid (NSH) technology. It offers unmatched high-resolution and high-throughput trace analysis of the herbicides diquat and paraquat by LC-MS/MS and LC-UV (liquid chromatography with ultraviolet detection) methods.

- Excellent resolution of diquat and paraquat
- Good peak shape
- Fast analysis
- LC-MS compatible
- No ion-pairing reagent needed

Diquat and paraquat

Acclaim Trinity Q1 colu	mn, 3μm, 50 x 3.0mm
Mobile phase	25% ammonium acetate (100mM, pH 5.0); 75% acetonitrile
Flow rate	0.5 mL/min
Injection volume	5 μL
Detection	Show Mass Spectrometric conditions and the scan events etc. table underneath are the peaks section
Temperature	Ambient
Mass spectrometric	Thermo Scientific [™] TSQ Quantiva [™] Access MAX Triple Quadrupole Mass Spectrometer
Interface	Heated Electrospary Ionization with HESI II probe
Spray voltage	1500 V
Vaporizer temp	400 °C
Sheath gas pressure	70
Aux gas pressure	10
Capillary temp	350 °C
Quantitation mode (SRM)	Selected reaction monitoring

Scan events	Precursor	Quantitative	Confirmative
		SRM (CID)	SRM (CID)
Paraquat	185	169 (27)	170 (17)
Paraquat-d ₆	193	178 (17)	
Diquat	183	157 (22)	130 (31)
Diquat-d ₃	186	158 (22)	







Method development

The Acclaim Trinity Q1 column is designed for applications using volatile buffers, such as ammonium acetate, which are compatible with MS and UV at (>225 nm). The column may be used with phosphate buffers when required. Ammonium acetate buffer is found to be effective for this application. The performance of the Acclaim Trinity Q1 column is based on reverse-phase and ion-exchange Mixed-Mode retention mechanism. The chromatography method can be optimized by adjusting mobile phase buffer concentration, solvent content, and pH.

Analysis of sisomicin, netilmicin, and etimicin

Acclaim Trinity Q1 column, 3 µm, 3.0 × 50 mm		
Mobile phase	75/25 v/v CH ₃ CN/ various conc. NH ₄ OAc, pH 5	
Flow rate	0.60 mL/min	
Injection volume	2 µL	
Temperature	30 °C	
DetectionUV, 290 nmSamplesDq and Pq (0.1 mg/mL each)		



Buffer concentration affects retentions of both diquat and paraquat. Running the separation using various buffer concentrations are shown above. Higher buffer concentration shortens retention times. If using lower buffer concentration, the retention is longer with a better the separation. Note that the resolutions were all very good for all the tested buffer concentration. For fast analysis, the 25 mM would be recommended.

Analysis of sisomicin, netilmicin, and etimicin

Acclaim Trinity Q1 column, 3 µm, 3.0 × 50 mm		
Mobile phase	MeCN/ 25 mM (total) NH₄OAc, pH 5	
Flow rate	0.60 mL/min	
Injection volume	2 µL	
Temperature	30 °C	
Detection	UV, 290 nm	
Samples	Dq and Pq (0.1 mg/mL each)	



Mobile phase organic solvent content affects retention and resolution of both diquat and paraquat. At 25 mM ammonium acetate, higher acetonitrile contents give better resolution. Typically, mobile phases containing 50 to 75% acetonitrile give excellent resolution and sufficient retention times. The retention time can be adjusted depending on sample matrix and interference.

Mobile phase pH has significant effect on the resolution of diquat and paraquat. It has been determined that pH 5 \pm 0.5 is suitable pH range for this application.

Acclaim Carbamate columns

Designed for baseline separation of carbamate pesticides specified in US EPA method 531.2

Thermo Scientific Acclaim Carbamate columns are designed for baseline separation of carbamates (N-methylcarbamate and N-methylcarbamoyloxime pesticides) specified in U.S. EPA Method 531.2. Carbamate pesticides are widely used throughout the world. Drinking water and raw surface water is monitored for the presence of carbamate pesticides and related compounds applying an established U.S. EPA Method 531.2 that uses HPLC with postcolumn derivatization. LC-MS is the method of choice for the ultimate sensitivity.

- Baseline separation of carbamate pesticides specified in U.S. EPA Method 531.2
- Use with either LC/post-column derivatization/fluorescence or LC-MS detection
- Available in 2.2, 3 and 5 µm particle size
- Compatible with both binary (methanol/water) and ternary (acetonitrile/methanol/water) mobile phase gradients
- High-efficiency, extremely low column bleed, and rugged column packing

Carbamate standard – spiked rice samples

Acclaim Carbamate column, 3 μm, 150 x 3.0 mm				
Mobile phase	Methanol-H ₂ O	Methanol-H ₂ O		
Gradient	, , ,	Methanol, -4.0–0.0 min, 14%; 2.0 min, 20%; 8.0 min, 40%; 13.6–16 min, 70%		
Flow rate	0.9 mL/min			
Injection volume	250 µL	250 µL		
Detection	Excitation/330nm and E	Excitation/330nm and Emission/465nm		
Temperature	50 °C	50 °C		
Analytes	 Aldicarb sulfoxide Aldicarb sulfone Oxamyl Methomyl 3-Hydroxy carbofuran Aldicarb 	 Propoxur Carbofuran Carbaryl 10. 1-Naphthol Methiocarb BDMC (I.S.) 		



B: with dispersive SPE using PSA



LC-MS method

Compared to fluorescence detection, mass spectrometric detection can significantly improve method selectivity and sensitivity. Figure 2 demonstrates excellent separation, high throughput (20 min. run time), and enhanced selectivity and sensitivity with MS detection achieved on a 2.1 × 150 mm Acclaim Carbamate column. This method has been successfully applied to the determination of carbamates in various types of water samples and performance was evaluated with respect to linearity, calibration range, detection limits, and recovery from a simulated ground water matrix.⁴

DNPH aldehydes and ketones

Acclaim Carbamate	e column, 3 μm, 2.1 × 150 mm	
Chromatographic conditions		
System	UltiMate 3000 HPLC system	
Mobile phase	(A) Methanol (B) 1.0 mM ammonium formate (C) Water	
Flow rate	300 µL/min	
Injection volume	20 µL	
Detector	MSQ Plus single quadrupole mass spectrometer	
Mass spectrometric	conditions	
Ionization interface	Electrospray ionization (ESI) positive mode	
Detection mode	Selected ion monitoring (SIM)	

Time (min)	%A	%B	%C
-4	10	5	85
0.0	10	5	85
2.0	10	5	85
15.0	65	5	30
15.1	90	5	5



Acclaim Carbonyl C18 columns

A silica-based, reversed-phase column designed specifically for separating DNPH derivatives of aldehydes and ketones

Thermo Scientific Acclaim Carbonyl C18 columns are silica-based reversed-phase columns designed specifically for separating DNPH derivatives of aldehydes and ketones. They exhibit superior resolution compared with other commercially available columns.

Aldehydes and ketones are common pollutants in air and water. Several standard methods have been developed to apply using dinitrophenylhydrazine (DNPH) to various environmental situations to measure these compounds. Some of the better known ones include California Air Resources Board (CARB) Method 1004 for vehicle exhaust, EPA Method 554 for drinking water, EPA Method 1667 for pharmaceutical wastewater, and EPA Method 8315 for general wastewater.

- Ideal selectivity for baseline resolution of DNPH derivatives of aldehydes and ketones regulated by various official methods, including EPA 554, EPA 8315, EPA 1667, EPA TO-11, and CARB 1004
- High-efficiency for UHPLC performance
- Rugged columns with good lot-to-lot reproducibility
- Proven robust methods

Gradient

A%

B%

-4.5

48

52

0.0

48

52

8.3

48

52

15.0

0

100

18.0

0

100

DNPH aldehydes and ketones

Acclaim Carbonyl	RSLC column, 2.2 µm, 150 x 2.1 mm
Mobile phase	(A) D.I. water (B) Acetonitrile
Flow rate	0.400 mL/min
Injection volume	1 μL
Detection	UV, 360nm
Temperature	28 °C
Samples	Calibration mixes diluted in methanol
Analytes	 Formaldehyde DNPH Acetaldehyde DNPH Propionaldehyde DNPH Crotonaldehyde DNPH Crotonaldehyde DNPH Butyraldehyde DNPH Gyclohexanone DNPH Valeraldehyde DNPH Hexanal DNPH Heptanal DNPH Heptanal DNPH Octanal DNPH Octanal DNPH Decanal DNPH Acetone DNPH Acetone DNPH Acetone DNPH Butanone DNPH Butanone DNPH Butanone DNPH Butanone DNPH Butanone DNPH Isovaleraldehyde DNPH Isovaleraldehyde DNPH Isovaleraldehyde DNPH Yalylaldehyde DNPH Xylylaldehyde DNPH





Separation of 12 carbonyl compounds regulated by EPA Method 554

Mobile phase	(A) D.I. water (B) Methanol
Flow rate	0.5 mL/min
njection volume	1 μL
Temperature	42 °C
Detection	UV, 360 nm, data collection rate at 10 Hz
Sample	Calibration mix, 50 µg/mL in methanol
Peaks	 Formaldehyde DNPH Acetaldehyde DNPH Propionaldehyde DNPH Crotonaldehyde DNPH Crotonaldehyde DNPH Butyraldehyde DNPH Cyclohexanone DNPH Valeraldehyde Hexanal DNPH Heptanal DNPH Octanal DNPH Nonanal DNPH Decanal DNPH



Gradient	-1.7	0.0	3.4	5.5	7.0
A%	30	30	30	0	0
В%	70	70	70	100	100

References

- 1. Exploring Mixed-Mode Chromatography: Column Chemistry, Properties, and Applications, Xiaodong Liu, and Christopher Pohl; Thermo Fisher Scientific, Sunnyvale, CA, USA, Thermo Publication PN21137
- 2. http://files.alfresco.mjh.group/alfresco_images/pharma//2015/03/24/17516363-2762-4507-b9a8-6487ae731afc/PN-PITTCON-MixedModeChrom.pdf
- 3. Mixed-Mode chromatography in pharmaceutical and biopharmaceutical applications, Journal of Pharmaceutical and Biomedical Analysis, K. Zhang, X. Liu, 128 (2016) 73-88
- 4. Wang, L., Liu, X., Henday, S.M. and Schnute, W.C. Improved LC-MS Method for the Determination of Carbamates in Water Samples (http://www.dionex.com/ en-us/ webdocs/77385-POHPLC-Carbamates-01Jul2009- LPN2295-01.pdf).

Acclaim column selection guide

	Acclaim column selection guide																						
										N	lived	-Mod	~	HIL		SEC	٨٣	oplica	tion	0000	ifio		
			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim C30	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Trinity P2	Acclaim Mixed-Mode WAX-1		Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim SEC-300 and SEC-1000	Acclaim Organic Acid	Acclaim Surfactant/Plus	Acclaim Carbonyl	Acclaim Explosives E2		Acclaim AmG	Example Aapplications
	Neutral molecules	High hydrophobicity Intermediate hydrophobicity Low hydrophobicity																					Fat-soluble vitamins, PAHs, glycerides Steroids, phthalates, phenols, polyphenols Acetaminophen, urea, polyethylene glycols
	Anionic molecules	High hydrophobicity Intermediate hydrophobicity Low hydrophobicity																					NSAIDs, phospholipids Aspirin, alkyl acids, aromatic acids Small organic acids, e.g. acetic acids
General applications	Cationic molecules	High hydrophobicity Intermediate hydrophobicity Low hydrophobicity																					Antidepressants Beta blockers, benzidines, alkaloids Antacids, pseudoephedrine, biogenic amines
Genera	Amphoteric/ zwitterionic molecules	High hydrophobicity Intermediate hydrophobicity Low hydrophobicity																					Phospholipids Amphoteric surfactants, peptides Amino acids, aspartame, small peptides
	Mixtures of neutral, anionic, cationic	Neutrals and acids Neutrals and bases Acids and bases																					Artificial sweeteners Cough syrup Drug active ingredient with counterion
	molecules	Neutrals, acids, and bases Anionic Cationic																					Combination pain relievers, PEG SDS, LAS, laureth sulfates Quats, benzylalkonium in medicines
	Surfactants	Nonionic Amphoteric Hydrotropes																					Triton X-100 in washing tank Cocoamidopropyl betaine Xylenesulfonates in handsoap
	Organic acids	Surfactant blends Hydrophobic Hydrophilic																					Nonionic and anionic surfactants Aromatic acids, fatty acids Organic acids in soft drinks, pharmaceuticals
tions		Explosives Carbonyl compounds Phenols Chlorinated/Phenoxy acids																					U.S. EPA Methods 8330, 8330B U.S. EPA Methods 1667, 555, 0T-11; CA CARB 1004 Compounds regulated by U.S. EPA 604 U.S. EPA Method 555
Specific applications	Environmental contaminants	Triazines Nitrosamines Benzidines Perfluorinated acids																					Compounds regulated by U.S. EPA 619 Compounds regulated by U.S. EPA Method 8270 U.S. EPA Method 605 Dionex TN73
S		Microcystins Isocyanates Carbamate insecticides																					US. OSHA Methods 42, 47 U.S. PA Methods 531.2
	Vitamins	Water-soluble vitamins Fat-soluble vitamins Anions																					Vitamins in dietary supplements Vitamin pills Inorganic anions and organic acids in drugs
	Pharmacutical counterions	Cations Mixture of Anions and Cations API and counterions																					Inorganic cations and organic bases in drugs Screening of pharmaceutical counterions Naproxen Na ⁺ salt, metformin Cl ⁻ salt, etc.
	Industry	Hydrophilic cations Water soluble Polymers																					Antibiotics, aminoglycosides PEG, PVP, PAA, PEI

Ordering information

Particle size (µm)	Format	Length (mm)	ID (mm)	120 C18	120 C8	Polar Advantage	Polar Advantage II	Phenyl-1	C30	HILIC-10	Mixed- Mode HILIC-1	Mixed-Mode WAX-1	Mixed-Mode WCX-1
Acclaim	analytical	columns											
		30	2.1	071400	072614		071402						
			3.0			072625							
		50	2.1	068981	072615	072622	068989						
	UHPLC		3.0	071605			071608						
	column	75	3.0	075697									
2.2		100	2.1	068982	072616	072623	068990						
			3.0	071604	072620		071607						
		150	2.1	071399	072617	072624	071401						
		250	2.1	074812	074811	074813	074814						
	Vanguish	150	2.1	071399-V			071401-V						
		250	2.1	074812-V			074814-V						
		33	3.0	066272	-		066276						
			2.1	059128	059122	063174	077999		078666				
		50	3.0		-		068973		078663		071912	071908	071910
			4.6	059131	059125	-	063189		078661				
		75	3.0	066273	-	066275	066277						
			2.1	059129	059123	061316	077998		078665				
3.0	HPLC	100	3.0	076186		076214	078000	074693	078662				
0.0	column		4.6	059132	059126		078001		078660				
			2.1	059130	059124	061317	063187	071971	075725	074259	070091	070089	070093
		150	3.0	063691	068970	063693	063705	071970	075724	074258	070090	070088	070092
			4.0										
			4.6	059133	059127	061318	063191	071969	075723	074257			
		250	2.1	076187			077997		078664				
		200	3.0	070077		070079	070080		075726				
		50	2.1	059142	059134	-	-						
			4.6	059146	059138	061319	-						
		100	2.1	059143		-	-						
		100	4.6	059147	059139	-	-						
			2.1	059144	059136	-	-				066847	067084	068371
	HPLC	150	4.0										
5.0	column		4.6	059148	059140	061320	063197		075719		066843	064984	068353
			7.8										
		250	2.1	059145	059137	-	-						
			4.0										
			4.6	059149	059141	061321	063199	079697	075718		066844	064985	068352
			4.6										
			7.8										
	HPLC	150	7.8										
7.0	column	300	4.6										
			7.8										
Acclaim gua	ards												
	Guard		2.1							074263			
3.0	cartridge	10	3.0					071974		074261			
			4.6					071973		074262			
	Guard		2.1	069689	069688	069691	069692		075722		069694	069686	-
5.0	cartridge	10	3.0	071981	071979	071983	071985		075721		071913	071909	071911
5.0			4.6	069695	069696	069698	069699		075720		069706	069704	069705
	Guard column	33	4.6										
7.0	Guard column	33	4.6										
	ards holders												
	rd cartridge ho			069580	069580	069580	069580	069580	069580	069580	069580	069580	069580
	rd cartridge-co	· · · ·		074188	074188	074188	074188	074188	074188	074188	074188	074188	074188
Acclaim gua	rd kit (Holder a	and coupler)		069707	069707	069707	069707	069707	069707	069707	069707	069707	069707

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