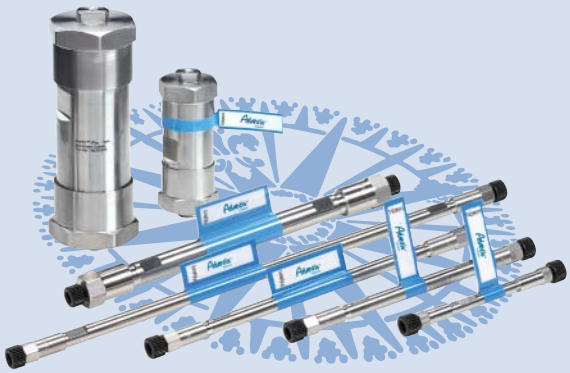
Atlantis™ Columns

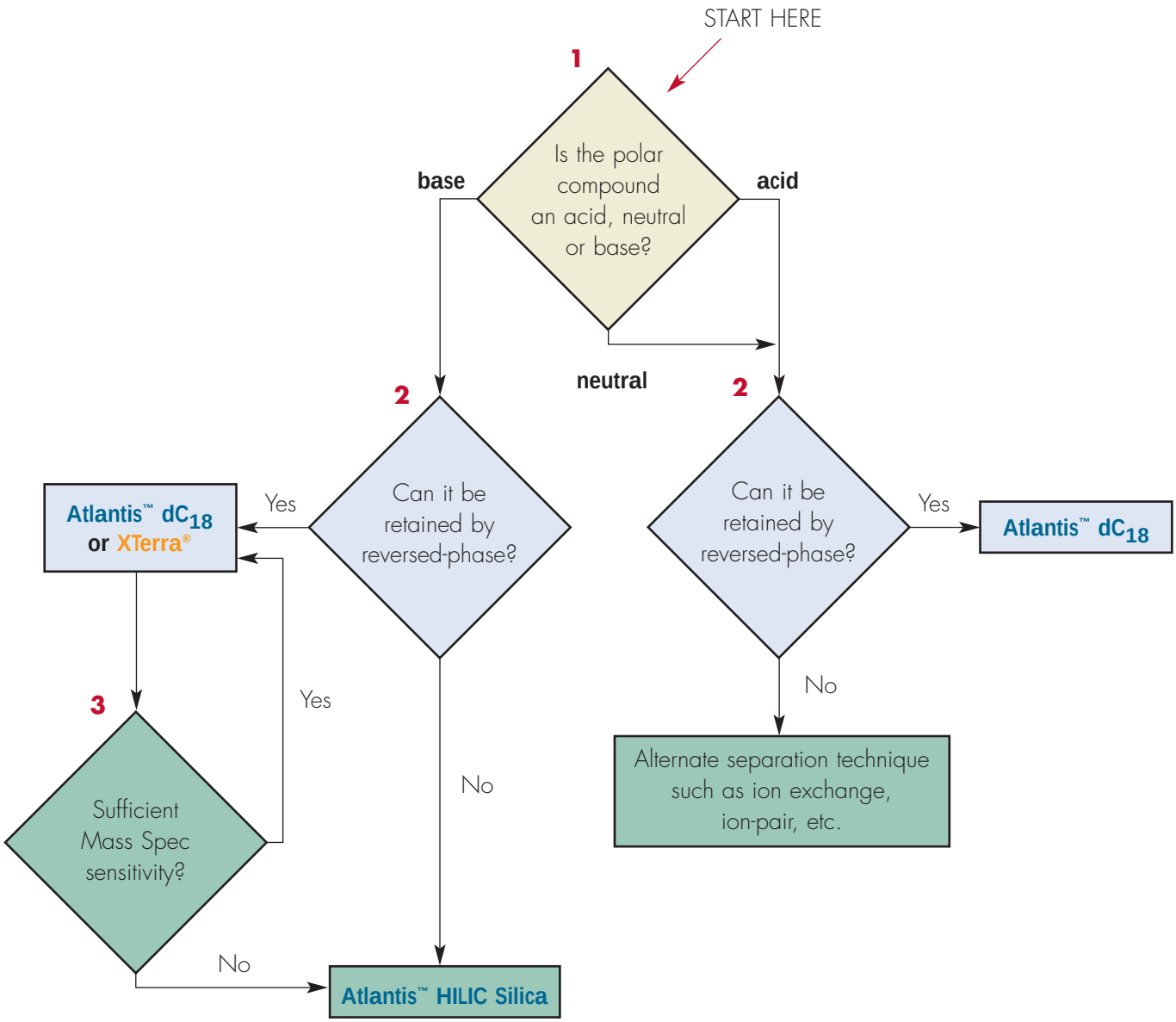
The search for polar retention leads to Atlantis™



Atlantis™ Columns

Waters

Retaining Polar Compounds



To learn more about polar compound retention, contact your local Waters representative or visit us online at **www.waters.com/atlantis**

NOTE:
Atlantis™ dC18 = High aqueous, Low pH
Atlantis™ HILIC Silica = High organic, Low pH
XTerra® = High aqueous, High pH

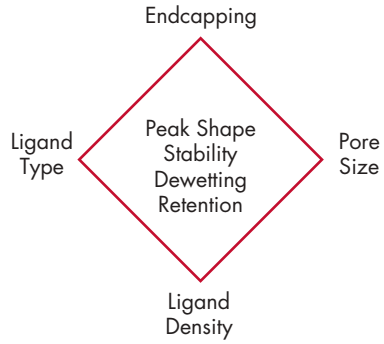
WATERS ATLANTIS™ COLUMNS

Most chromatographers have experienced problems retaining and separating polar compounds using conventional reversed-phase chromatography. These difficult-to-analyze compounds either pass through the column unretained or, if retained at all, co-elute at the beginning of the chromatogram. Although today's sensitive and selective mass detectors may help identify these early co-eluting compounds, MS ion suppression often occurs if these analytes are not sufficiently separated from the solvent front. Waters Atlantis™ columns are designed for these types of challenging separations. Atlantis™ columns are available in two chemistries: **dC_{18}** and **HILIC Silica**.

Atlantis™ dC_{18} columns are a fully LC/MS compatible line of universal C_{18} columns that offer the perfect balance of retention for both polar and non-polar compounds. Atlantis™ dC_{18} columns exhibit superior retention of polar compounds as compared to conventional reversed-phase HPLC columns without exhibiting excessive retention of hydrophobic compounds. Atlantis™ dC_{18} columns are compatible with aqueous mobile phases, provide enhanced low pH stability and are available in a wide variety of column configurations ranging from nanoscale to preparative.

Atlantis™ HILIC Silica columns retain and separate very polar, water-soluble basic organic compounds such as actives, metabolites and peptides using Hydrophilic Interaction Chromatography (HILIC). These compounds are often too polar to retain by reversed-phase HPLC and require an alternate separation technique such as HILIC. Why HILIC? Besides very polar compound retention, HILIC affords improved LC/ESI-MS response, direct compatibility with SPE solvents and complementary selectivity as compared to reversed-phase HPLC. Atlantis™ HILIC Silica columns provide long column lifetime, universal compatibility with all LC detectors and excellent column-to-column reproducibility.

ATLANTIS™ dC_{18} COLUMNS—AN INTELLIGENT DESIGN



Optimizing key stationary phase attributes results in the optimal combination of peak shape, low pH stability, resistance to dewetting and polar compound retention.

ATLANTIS™ dC_{18}: THE IDEAL COLUMN FOR REVERSED-PHASE HPLC

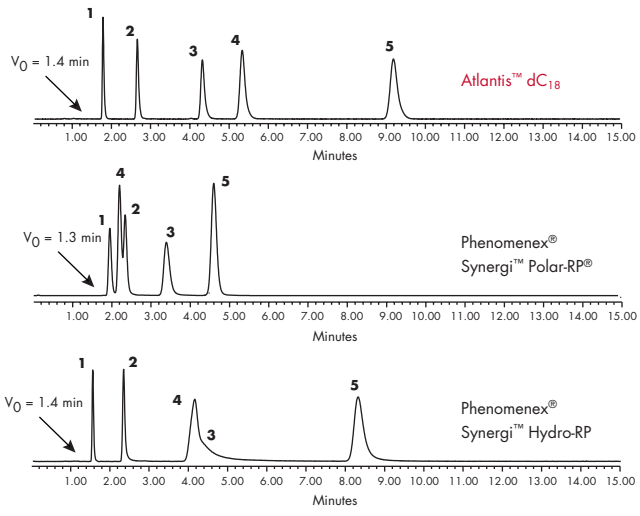
In order to create a reversed-phase HPLC column for the retention and separation of polar, water-soluble compounds, a new and unique stationary phase packing material had to be created. The result of this two year stationary phase creation project was the silica-based, difunctionally bonded C_{18} material of Atlantis™ dC_{18} columns. Stationary phase physical attributes such as endcapping, silica pore size, bonded phase ligand density and ligand type were all optimized in order to create a column that exhibits superior peak shape, low pH stability, resistance to dewetting (hydrophobic collapse) and enhanced polar compound retention.

Waters studied the effects of the stationary phase on polar compound retention and created a column that not only retains polar compounds, but provides excellent peak shape for all compounds and is fully LC/MS compatible. Atlantis™ dC_{18} columns combine all the desirable characteristics of an ideal reversed-phase HPLC column, making it suitable for separating polar compounds as well as standard reversed-phase applications.

ENHANCED POLAR COMPOUND RETENTION USING ATLANTIS™ dC_{18} COLUMNS

CONDITIONS	
Columns:	4.6 x 150 mm, 5 µm
Isocratic Mobile Phase:	10 mM NH_4COOH, pH 3.0
Flow Rate:	1.2 mL/min
Injection Volume:	7 µL
Temperature:	Ambient
Detection:	UV @ 254 nm
Instrument:	Alliance® 2695, 2996 PDA

COMPOUNDS
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Guanosine-5-monophosphate
5. Thymine



Atlantis™ dC_{18} columns provide enhanced polar compound retention when compared to other "polar retention" columns.

ENHANCED POLAR COMPOUND RETENTION

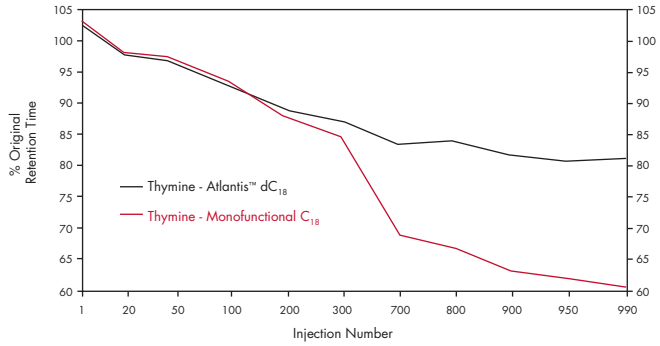
Atlantis™ dC_{18} columns exhibit enhanced retention of polar compounds due to the intelligent design of its stationary phase. Carefully designing and understanding the role of the stationary phase in polar compound retention results in a column that is compatible with the aqueous mobile phases necessary for retaining these hydrophilic compounds. All of this is possible while still achieving superior peak shapes for bases since Atlantis™ dC_{18} columns are fully endcapped.

EXTENDED COLUMN LIFETIME AND LOW pH STABILITY

In order to provide superior peak shapes for amine-containing bases, HPLC separations are often run under acidic conditions (pH ≤ 3.0). Unfortunately, a gradual loss in retention and shorter column lifetimes are observed under these harsh conditions. This is due to the gradual loss of bonded phase due to cleavage of the siloxane bond that occurs as the column ages. The results are frequent column replacement, increased column costs and instrument downtime.

Atlantis™ dC_{18} columns address this problem by incorporating a difunctional silane bonding chemistry that provides excellent stability under acidic mobile phase conditions. More consistent retention times and longer column lifetimes are realized as a result of this highly stable stationary phase.

ACCELERATED LOW pH TFA DEGRADATION TEST



Waters designed this accelerated low pH degradation test to promote the loss in stationary phase that occurs in a column under acidic pH conditions using 0.1% TFA combined with a steep, sweeping gradient that elutes the cleaved ligand. As compared to a popular monofunctionally-bonded "AQ-type" column, Atlantis™ dC_{18} columns' proprietary difunctional bonding chemistry provides longer, more stable column lifetimes.

OPTIMIZED FOR AQUEOUS MOBILE PHASES

With conventional reversed-phase HPLC columns you may experience difficulties retaining and separating highly polar, water-soluble organic compounds. Retention of these types of analytes requires the use of mobile phases that contain little or no organic modifier. Under these aqueous conditions, conventional C_{18} stationary phases can exhibit a sudden loss of retention. In the past, this was attributed to a proposed phenomenon where the hydrophobic C_{18} chains "collapse."

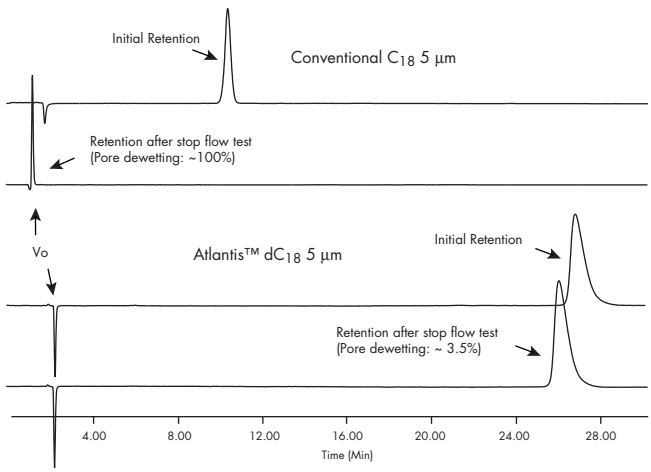
Tests at Waters have revealed that the silica pores (where the majority of the surface area lies) actually expel aqueous mobile phase in the absence of pressure. Under these conditions, analytes do not migrate into the pores and, therefore, pass through the column unretained. This phenomenon is termed "dewetting."

Waters Atlantis™ dC_{18} columns were developed specifically for operating in aqueous mobile phases without fear of dewetting.

ATLANTIS™ dC_{18} COLUMNS RESIST DEWETTING

Waters "stop flow" test determines the susceptibility of a stationary phase to pore dewetting using 100% aqueous mobile phases. Under these difficult testing conditions, Atlantis™ dC_{18} columns resist phase dewetting. Note also the increased retention of amoxicillin on the Atlantis™ column compared to the conventional C_{18} column.

Amoxicillin retention with 0.1% formic acid mobile phase



PORE DEWETTING MECHANISM

Flow stoppage relieves the pressure that forces aqueous mobile phase into the pores. When this pressure is decreased, the hydrophobic pore surface expels the polar mobile phase and the pore "dewets," resulting in retention loss.

Note: When the column is restricted, the mobile phase pressure is not sufficient to fully rewet all the pores throughout the column length. This results in the loss of retention.

ATLANTIS™ dC_{18} COLUMN VS. EMBEDDED POLAR GROUP COLUMNS FOR THE SEPARATION OF CATECHOLAMINES

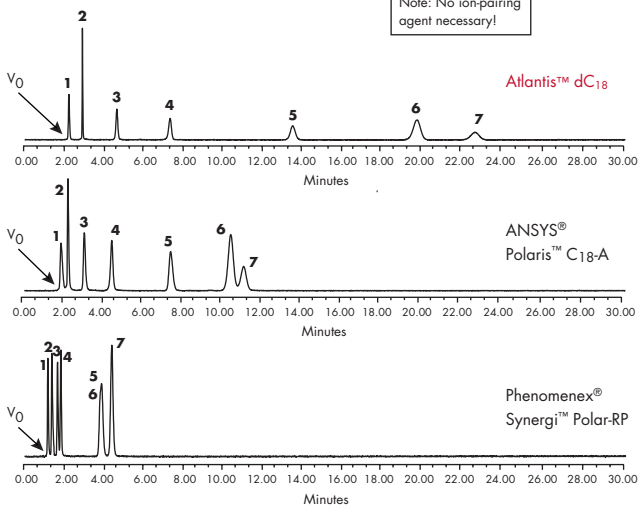
CONDITIONS

Columns:	4.6 x 150 mm, 5 µm
Mobile Phase A:	H_2O
Mobile Phase B:	ACN
Mobile Phase C:	100 mM CH_3COONH_4, pH 5.0
Flow Rate:	1.0 mL/min
Isocratic Mobile Phase Composition:	88% A; 2% B; 10% C
Injection Volume:	10 µL
Temperature:	30° C
Detection:	UV @ 280 nm
Instrument:	Alliance® 2695, 2996 PDA

COMPOUNDS

1. Norepinephrine (NE)
2. Epinephrine (E)
3. Dopamine (DA)
4. 3,4-Dihydroxyphenylacetic acid (DOPAC)
5. Serotonin (5-HT)
6. 5-Hydroxy-3-indoleacetic acid (5-HIAA)
7. Homovanillic acid (HVA)

Note: No ion-pairing agent necessary!



Embedded polar group stationary phases were designed for improved peak shape for bases, not for enhanced polar compound retention. Atlantis™ dC_{18} columns provide superior peak shape and aqueous compatibility while also imparting enhanced polar compound retention.

EXCELLENT PEAK SHAPES AND AQUEOUS COMPATIBILITY

CONDITIONS

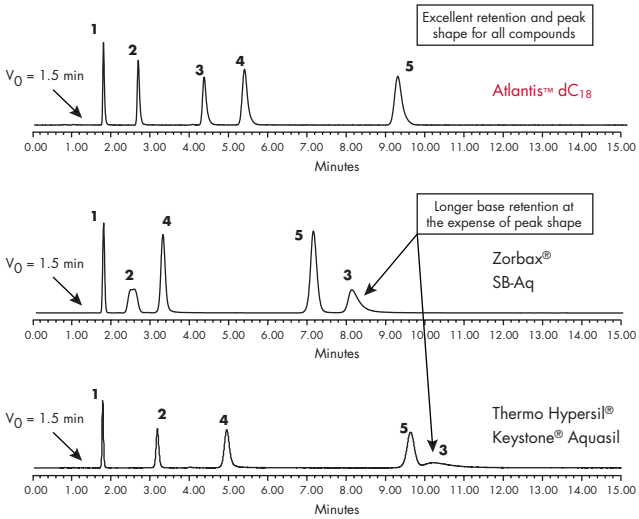
Columns:	4.6 x 150 mm, 5 µm
Isocratic Mobile Phase:	10 mM NH_4COOH, pH 3.0
Flow Rate:	1.2 mL/min
Injection Volume:	7 µL
Detection:	UV @ 254 nm
Instrument:	Alliance® 2695, 2996 PDA

COMPOUNDS

1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Guanosine-5-monophosphate
5. Thymine

Excellent retention and peak shape for all compounds

Longer base retention at the expense of peak shape



Atlantis™ dC_{18} columns are fully endcapped and avoid the tailing and extreme retention for amine-containing bases observed with unendcapped stationary phases.

AQUEOUS COMPATIBILITY WITHOUT AN EMBEDDED POLAR GROUP

One way to produce a stationary phase that is compatible with aqueous mobile phases is to incorporate an embedded polar group into the bonded phase ligand. Examples of embedded polar groups include carbamate, urea, amide, ether, etc. Chromatographers, however, confuse this aqueous compatibility with enhanced polar compound retention since these same highly aqueous mobile phases are necessary to promote retention.

Waters has found that embedded polar group stationary phases actually provide less retention for polar compounds. Atlantis™ dC_{18} columns neither contain nor require an embedded polar group and have succeeded where embedded polar groups have failed: aqueous compatibility and enhanced retention of polar compounds.

BENEFIT OF A FULLY ENDCAPPED COLUMN

Another way that a conventional high coverage C_{18} column can be made compatible with aqueous mobile phases is to omit the endcapping step during the stationary phase synthesis. An unendcapped stationary phase can produce severe peak tailing for amine-containing bases, however. Acetonitrile or methanol must then be added to the mobile phase(s) to improve the peak shapes, resulting in shortened retention times for acidic and neutral polar compounds.

Atlantis™ dC_{18} columns are fully endcapped and avoid the tailing and extreme retention for amine-containing bases observed with unendcapped stationary phases.

OPTIMAL COMBINATION OF POLAR AND NON-POLAR COMPOUND RETENTION

Using a conventional C_{18} column for polar compound retention involves either using a mobile phase that contains less organic modifier (isocratically) or using a shallower gradient. Besides the risk of dewetting, increased or extreme non-polar compound retention can occur. The chromatographer must then change the mobile phase and/or gradient to elute these strongly retained hydrophobic compounds. This can result in co-eluting peaks and makes method transfer difficult.

The optimal ligand density of Atlantis™ dC_{18} columns exhibits strong retention of polar compounds without excessive retention of non-polar compounds. This approaches the concept of the ideal reversed-phase HPLC column since polar and non-polar compounds can both be easily separated on one column.

SIMULTANEOUS SEPARATION OF WATER AND FAT SOLUBLE VITAMINS ON AN ATLANTIS™ dC_{18} COLUMN

CONDITIONS

Column:	4.6 x 150 mm, 5 µm
Part Number:	186001344
Mobile Phase A:	0.1% TFA in H_2O
Mobile Phase B:	0.1% TFA in ACN
Flow Rate:	1.4 mL/min
Gradient:	Time Profile

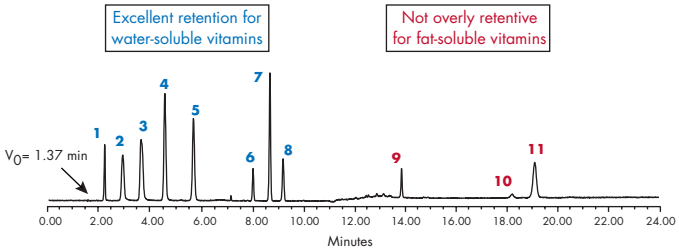
(min)	%A	%B
0.0	100	0
5.0	97	3
6.0	85	15
10.0	80	20
12.0	0	100
25.0	0	100

Injection Volume:	10 µL
Temperature:	30° C
Detection:	UV @ 280 nm
Instrument:	Alliance® 2695, 2996 PDA

COMPOUNDS / **CONCENTRATION (g/mL)**

1.	L-ascorbic acid (vitamin C)	19.6
2.	Nicotinic acid (niacin)	9.8
3.	Thiamine (vitamin B1)	19.6
4.	Pyridoxal	39.2
5.	Pyridoxine	39.2
6.	Folic acid (vitamin B11)	23.5
7.	Caffeine	9.8
8.	Riboflavin (vitamin B2)	3.9
9.	Retinol (vitamin A)	19.6
10.	Tocopherol (vitamin E)	39.2
11.	Ergocalciferol (vitamin D2)	9.8

Note: No ion-pairing agent necessary!



ATLANTIS™ dC_{18} COLUMNS FOR PEPTIDE MAPPING

TFA is commonly used as a mobile phase additive in peptide mapping in order to improve retention of hydrophilic peptides and enhance peak shape. However, TFA promotes ion suppression and lowers signal response when using MS. Since Atlantis™ dC_{18} columns retain peptides much longer than conventional C_{18} columns, TFA is no longer necessary and more LC/MS-compatible additives such as formic acid can be used without sacrificing retention or peak shape. This results in higher MS response and increased sensitivity.

Peak capacity is a measure of the number of peaks that can be separated in a given period of time. The more peaks that can be separated, the more powerful the separation technique. A column with a high peak capacity is an important tool in peptide mapping due to the complexity and number of peaks present in tryptic digests. In peptide mapping studies, Atlantis™ dC_{18} columns exhibit the highest peak capacity of any commercially available reversed-phase column.

Column	Peak Capacity*
Waters® Atlantis™ dC_{18} (100Å)	**115.9**
Vydac® 238MS™ LC/MS C_{18} (300Å)	105.8
Agilent Zorbax® 300 SB-C_{18} (300Å)	95.1
Phenomenex® Jupiter™ Proteo C_{12} (4 µm, 90Å)	87.0
Vydac® 218TP™ C_{18} (300Å)	60.8

(*) – Measured at 4.4% peak height. All columns – 4.6 x 50 mm, 5 µm unless noted.

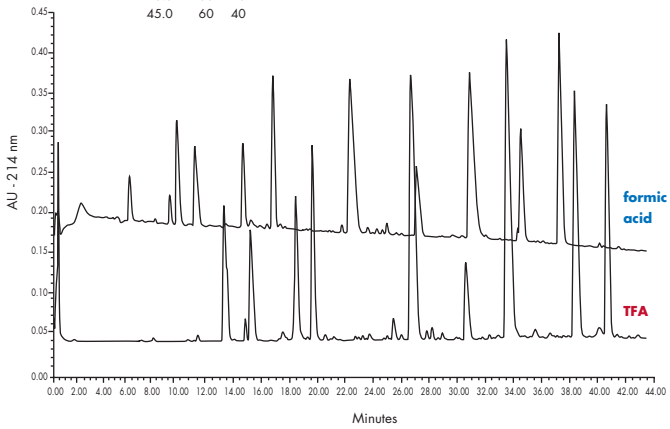
ELIMINATE TFA FOR PEPTIDE ANALYSES

CONDITIONS

Column:	Atlantis™ dC_{18}, 4.6 x 50 mm, 5 µm
Part Number:	186001331
Mobile Phase A:	0.1% HCOOH or 0.2% TFA
Mobile Phase B:	0.065% HCOOH in ACN or 0.016% TFA in ACN
Flow Rate:	0.75 mL/min, 0.2 mL/min split to MS
Gradient:	Time Profile

(min)	%A	%B
0.0	100	0
45.0	60	40

Injection Volume:	20 µL
Injection Mass:	9 µg
Temperature:	Ambient
Detection:	UV @ 214 nM
Instrument:	Alliance® 2695, 2996 PDA



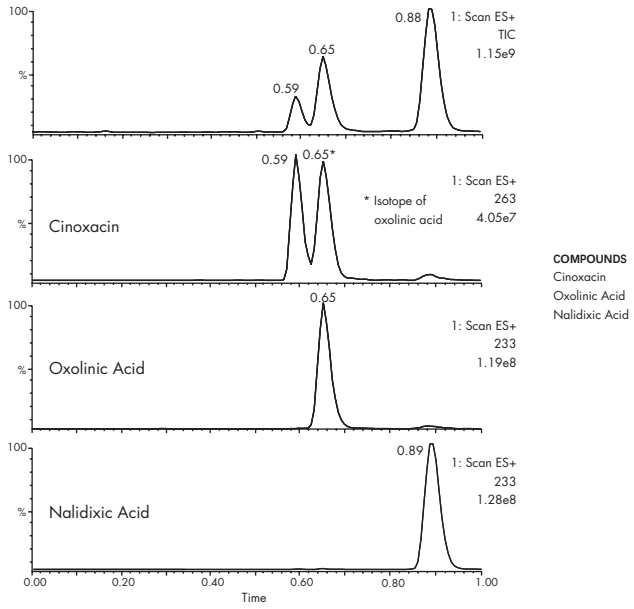
Separations of 9.0 µg of tryptic cytochrome C peptides using formic acid (top) instead of TFA (bottom) in the mobile phases.

MS-READY FLOW RATES FOR HIGH-THROUGHPUT LC/MS SEPARATIONS

CONDITIONS

Column:	2.1 x 20 mm *IS*™, 5 µm	Gradient:	
Part Number:	186002058		
Mobile Phase A:	Water		
Mobile Phase B:	MeOH		
Mobile Phase C:	1% HCOOH		
Flow Rate:	0.4 mL/min		
		Injection Volume:	2 µL
		Concentration:	10 µg/mL
		Temperature:	30° C
		Instrument:	Alliance® 2795 HT, Waters ZQ™

Time (min)	Profile %A	%B
0.0	50	10
1.0	30	10



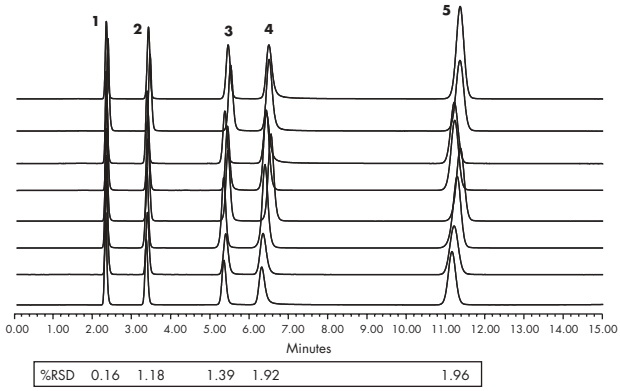
COMPOUNDS
Cinoxacin
Oxolinic Acid
Nalidixic Acid

EXCELLENT BATCH-TO-BATCH REPRODUCIBILITY

CONDITIONS

Columns:	4.6 x 150 mm, 5 µm
Part Number:	186001344
Mobile Phase:	10 mM NH_4COOH, pH 3.0
Flow Rate:	1.0 mL/min
Injection Volume:	7 µL
Detection:	UV @ 254 nm
Instrument:	Alliance® 2695, 2996 PDA

COMPOUNDS
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Guanosine-5-monophosphate
5. Thymine



%RSD	0.16	1.18	1.39	1.92	1.96

Overlay of actual QC chromatograms from eight separate batches of Atlantis™ dC_{18} 5 µm packing material. Note that these columns were not specially packed for this test.

ATLANTIS™ dC_{18} INTELLIGENT SPEED (*IS*™) COLUMNS

Chromatographers are being asked to produce more results in less time. Atlantis™ dC_{18} *IS*™ 20 mm length columns help make this possible by combining speed, resolution and retention. For high-throughput LC/MS applications, the MS-ready flow from the 2.1 mm ID *IS*™ columns do not require splitting and can flow directly into the MS source. Flow splitting is common in LC/MS after the column in order to reduce the flow and/or concentration of analyte delivered to the MS source. Flow splitting, however, can be difficult to do accurately and reproducibly due to the constant change in viscosity which occurs throughout gradient cycles. In addition, flow splitting can cause band spreading and sensitivity loss. By eliminating or reducing the need for flow splitting, Atlantis™ dC_{18} *IS*™ columns make scaling down existing separations faster and easier.

EXCELLENT REPRODUCIBILITY

As with other modern Waters packing materials such as Symmetry® and XTerra®, Waters paid close attention to batch-to-batch reproducibility in the development of the Atlantis™ dC_{18} stationary phase material.

When highly reproducible stationary phases are packed in cGMP, ISO 9002 certified facilities by a company that has over 30 years of HPLC column manufacturing experience, the result is a rugged and robust reversed-phase HPLC column product that produces consistent results year after year.

EASE OF SCALE-UP AND LONG, PREDICTABLE COLUMN LIFETIMES

One of the most frustrating and time-consuming aspects of the isolation and purification process is when an analytical separation does not scale-up linearly. In these situations, the preparative separation method must then be "redeveloped" in order to obtain and mimic the required resolution of the previously developed analytical separation. This delays moving potential new drugs from lead generation to lead optimization.

Separations that do not scale-up occur when the preparative column contains a different and/or lower cost material than the analytical column. Successful scale-up can only occur when the same stationary phase material in the analytical column is present in the preparative column. All Atlantis™ dC_{18} columns contain the same high-quality; fully tested stationary phase material in analytical and preparative column formats, thus ensuring trouble-free, linear scale-up.

Atlantis™ dC_{18} preparative columns also benefit from Waters' new patent-pending optimum packed bed density design which provides maximum bed column stability even when injecting high concentration samples dissolved in DMSO. And for the first time, analytical column performance in preparative column formats has been realized. More importantly, Atlantis™ dC_{18} preparative columns can be used in UV or mass directed, multiple preparative column systems (i.e., MUX™) with greater confidence during long, unattended preparative runs.

OPTIMAL RETENTION AND HIGH MASS LOADING

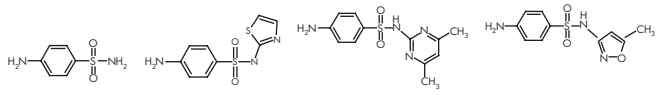
Polar compounds present a unique and difficult challenge since these unretained and/or poorly separated analytes must be re-analyzed separately, thus becoming a bottleneck in the high-throughout laboratory. If some analyte separation is realized, the peak fraction is a highly aqueous, non-volatile solvent (i.e., very weak mobile phase) that requires long evaporation times. Since Atlantis™ dC_{18} preparative columns retain compounds longer, stronger mobile phases and/or steeper gradient profiles can now be used. This optimal retention results in more volatile peak fractions, faster fraction evaporation, less sample handling and higher recoveries.

Atlantis™ dC_{18} preparative columns also provide high mass loading for polar compounds. The isolation and purification scientist can choose to use either a larger preparative column which provides a capacity safety margin for unknown sample sets or a smaller preparative column to decrease solvent consumption, operating backpressures and peak volumes. This ability to inject high mass loads translates into less preparative runs and faster library screening and purification.

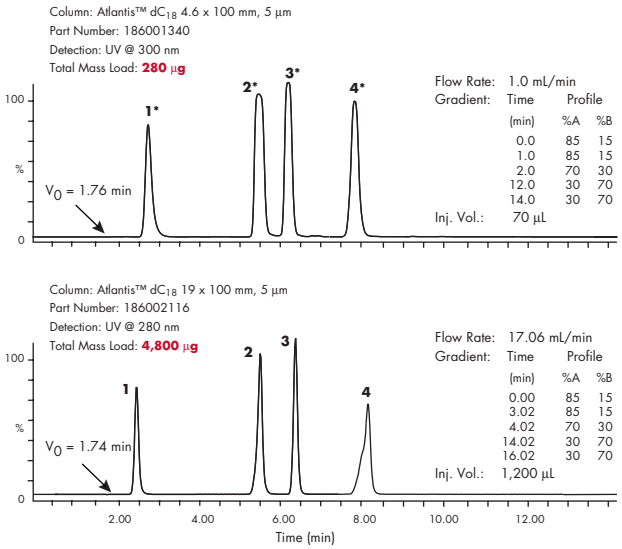
LINEAR SCALE-UP OF SULFA MEDICINES (SULFONAMIDES)

CONDITIONS

Mobile Phase A:	0.1% HCOOH
Mobile Phase B:	ACN/0.1% HCOOH (90:10)
Temperature:	Ambient
Instrument:	AutoPurification™ System
Sample Conc:	1 mg/mL each in DMSO



1. Sulfanilamide 2. Sulfathiazole 3. Sulfamethazine 4. Sulfamethoxazole

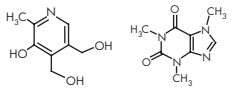


The same high efficiency separations are realized when using Atlantis™ dC_{18} columns in analytical and preparative formats. (*) – The flattened peak profiles on the analytical column separation reflect the saturation of the PDA detector, not column overload.

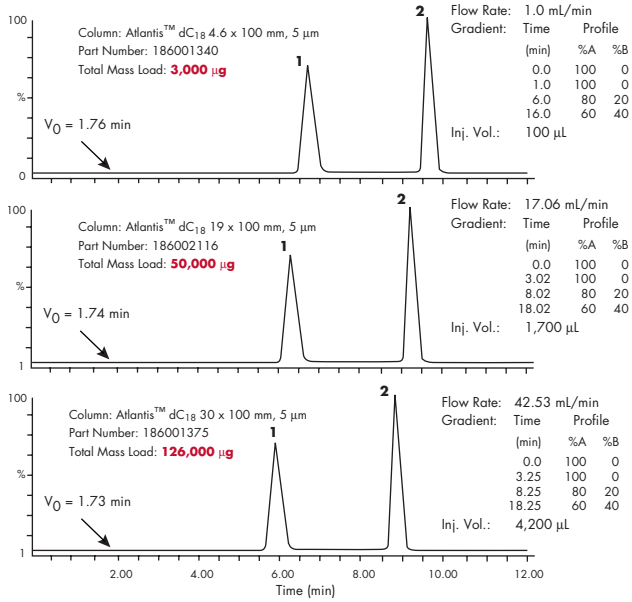
HIGH MASS LOADING OF WATER-SOLUBLE VITAMINS

CONDITIONS

Mobile Phase A:	0.1% TFA
Mobile Phase B:	ACN/0.1% TFA (90:10)
Temperature:	Ambient
Detection:	UV @ 290 nm
Instrument:	AutoPurification™ System

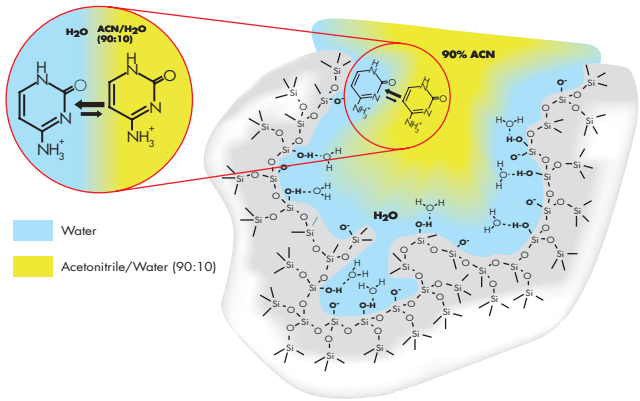


1. Pyridoxine (5 mg/mL in H_2O) 2. Caffeine (25 mg/mL in H_2O)



Linear scale-up, enhanced retention and high mass loading are achieved using Atlantis™ dC_{18} preparative columns.

RETENTION MECHANISMS IN HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC)



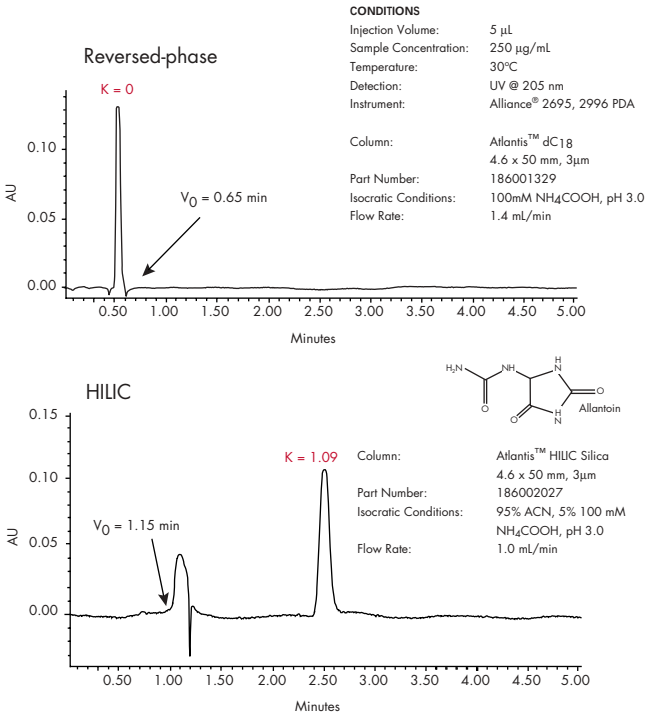
The combination of partitioning and weak cation-exchange results in retention of polar bases with Atlantis™ HILIC Silica columns.

HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC)

HILIC is a variation of normal phase chromatography where a polar stationary phase is used with a mobile phase which contains a high concentration of organic (non-polar) solvent and a low concentration of aqueous (polar) solvent. In HILIC, the organic portion of the mobile phase is the weak solvent, the aqueous portion is strong solvent and the compound elution is in the order of increasing hydrophilicity. HILIC is also referred to as "aqueous normal phase" or "reverse reversed-phase" since the elution order is similar to that of normal phase and the solvents used are similar to those of reversed-phase chromatography.

The retention mechanism of HILIC is the partitioning of the polar analyte between the water-rich stationary phase and the water-poor mobile phase[1]. On silica stationary phases (e.g., Atlantis™ HILIC Silica columns) polar bases can also undergo weak cation exchange with the negatively charged silanols. This combination of partitioning and cation-exchange results in retention for these difficult to analyze compounds.

HILIC OFFERS RETENTION WHERE NONE IS POSSIBLE WITH REVERSED-PHASE HPLC



Allantoin does not retain using reversed-phase chromatography (even with 100% aqueous mobile phases). With Atlantis™ HILIC columns, however, retention of allantoin is achieved.

ATLANTIS™ HILIC SILICA COLUMNS FOR COMPOUNDS UNRETAINED BY REVERSED-PHASE CHROMATOGRAPHY

The hydrophilic nature of very polar basic analytes, when combined with their net positive charge at acidic pH, makes retention and separation of these compounds extremely difficult using reversed-phase chromatography. This is why drug metabolism, drug discovery and combinatorial chemistry scientists have turned to HILIC to solve these challenging separations problems.

One of the many advantages of this "reverse reversed-phase" separation technique is retention of compounds that simply will not retain using reversed-phase HPLC.

(1) Neue, Uwe D., HPLC Columns: Theory, Technology and Practice, Wiley-VCH, New York, 1997, pp. 217-223.

HILIC AFFORDS ENHANCED ESI-MS SENSITIVITY

In order to promote retention of highly polar analytes using reversed-phase chromatography, very weak mobile phases must be used. These highly aqueous mobile phases maximize the hydrophobic attraction between the analyte and the stationary phase. However, these non-volatile mobile phases are not ideal for compound ionization by ESI-MS, resulting in poor sensitivity.

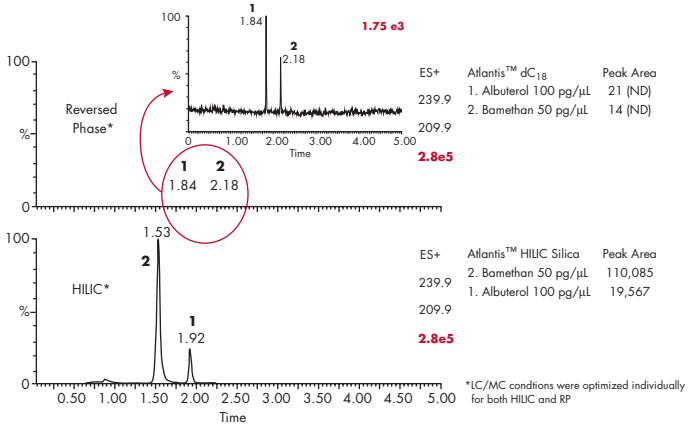
Atlantis™ HILIC Silica columns retain highly polar basic analytes with volatile mobile phases that are ideal for compound ionization by ESI-MS. This results in much higher sensitivity and lower limits of detection. This is important in drug metabolism studies since metabolites are often more polar than the parent compound and are present at much lower concentrations.

ENHANCED LC/MS SENSITIVITY USING HILIC

Column: Atlantis™ dC_{18}, 2.1 x 50 mm, 3µm
Part Number: 186001291
Flow Rate: 0.2 mL/min
Gradient:

Time (min)	%A	%B	%C
0.0	95	0	5
5.0	45	50	5

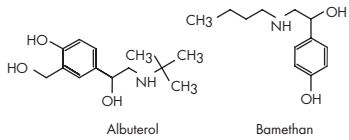
CONDITIONS
Moblie Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 200 mM NH_4COOH, pH 3.0
Injection Volume: 10 µL
Temperature: Ambient
Instrument: Alliance® 2795, Waters ZQ™



*LC/MC condtions were optimized individually for both HILIC and RP

Column: Atlantis™ HILIC Silica, 2.1 x 50 mm, 3µm
Part Number: 186002011
Flow Rate: 0.2 mL/min
Gradient:

Time (min)	%A	%B	%C
0.0	0	95	5
5.0	45	50	5



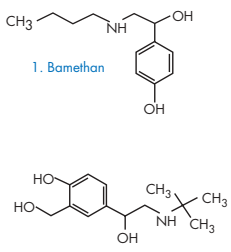
A reversal in elution order and an increase in ES+ response of 900X and 7,800X were realized for albuterol and bamethan, respectively, on the Atlantis™ HILIC Silica column as compared to the reversed-phase separation on the Atlantis™ dC_{18} column.

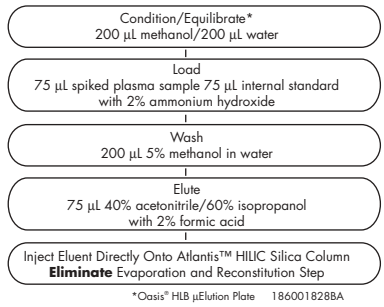
FACILITATE SAMPLE PREPARATION

Sample preparation techniques such as solid-phase extraction (SPE), protein precipitation or liquid/liquid extraction often have a final step that consists of a strong organic solvent (e.g., acetonitrile, isopropanol, etc.). These solvents are too strong to be directly injected onto a reversed-phase column and must be evaporated to dryness and reconstituted into a weak solvent that is compatible with the reversed-phase conditions. This laborious and time consuming step can account for 50% of the total time spent processing samples and can be the bottleneck of the high-through-put analytical laboratory.

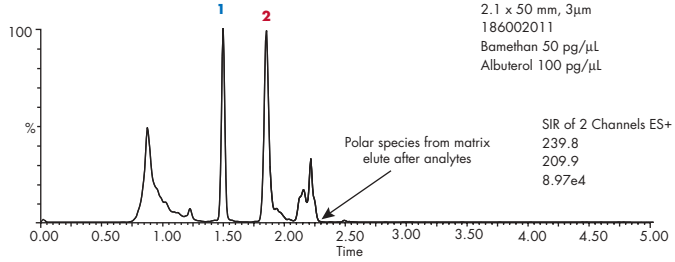
Eliminating this laborious and time consuming step can result in a dramatic increase in sample throughput. In addition, if the analytes are unstable and/or are not amenable to evaporation and reconstitution, lower limits of detection and higher recoveries can be achieved by directly injecting samples from the final step of a solid phase extraction, protein precipitation or liquid/liquid extraction step. In HILIC, organic solvents such as acetonitrile and isopropanol are considered weak solvents and can be injected directly onto the Atlantis™ HILIC Silica column. The result: increased sample throughput, improved analyte recoveries and lower limits of detection.

ELIMINATE EVAPORATION AND RECONSTITUTION STEP WITH ATLANTIS™ HILIC SILICA COLUMNS





*Oasis® HLB µElution Plate 186001828BA



Increase sample throughput by eliminating the time consuming and laborious evaporation and reconstitution steps involved in solid phase extraction (SPE).

Problem Solving and Troubleshooting Using Atlantis™ dC_{18} Columns

Problem	Impact	Solution and Benefit
Little or no retention of polar compounds	• Re-run samples using separate methods for polar compounds • Increased method development time and labor	• Polar compounds are retained longer with Atlantis™ dC_{18} columns • One Atlantis™ dC_{18} column and method can be used for polar and non-polar compounds • Decreased labor costs
Method requires 100% aqueous mobile phase for desired separation	• Loss of retention is observed	• Atlantis™ dC_{18} packing material is tested with highly polar analytes in 100% aqueous conditions, thereby ensuring its utility in aqueous conditions
Sudden loss of analyte retention observed when using highly aqueous mobile phase	• Run organic modifier through column to rewet and regenerate column • Increased labor and solvent costs • Decreased throughput • Reproducibility issues	• Atlantis™ dC_{18} columns don't lose retention in 100% aqueous mobile phases • Less time spent rewetting columns resulting in lower labor costs • Increased throughput
Short column lifetime in acidic mobile phases	• High cost due to frequent column replacement • Increased instrument downtime • Retention time reproducibility issues	• The proprietary difunctional bonding chemistry of Atlantis™ dC_{18} columns results in low pH stability and longer column lifetime • Decreased costs associated with column replacement and instrument maintenance
Retaining polar compounds on a conventional C_{18} column results in increased or infinite retention of non-polar compounds	• Multiple columns are required to separate analytes with a wide range of polarities • Increased method development time, labor and column costs • Decreased throughput	• One Atlantis™ dC_{18} column and method can be used for polar and non-polar compounds • Easier and faster method development • Increased throughput
Severe peak tailing for polar bases is observed	• Method fails system suitability guidelines for peak tailing • Increased method development time	• Atlantis™ dC_{18} columns are optimally endcapped and provide excellent peak shapes using MS compatible mobile phases • Easier and faster method development
Column bleed is observed on MS	• Frequent cleaning of MS source • Incorrect or inconsistent results	• Atlantis™ dC_{18} columns do not exhibit MS detectable column bleed • Decreased instrument downtime and maintenance costs
Column to column reproducibility is inconsistent (e.g., selectivity, retention, etc.)	• Increased labor costs due to individual column QC testing • Revalidate/redevelop method with each new batch of columns	• The stringent Atlantis™ dC_{18} packing material QC batch test separates highly polar analytes in 100% aqueous mobile phase conditions • Decreased method revalidation and development time

Problem Solving and Troubleshooting Using Atlantis™ HILIC Silica Columns

Problem	Impact	Solution and Benefit
Polar metabolites or contaminants not retained by reversed-phase HPLC	• Re-run samples using alternate, non-MS compatible chromatographic techniques • Metabolites or contaminants are not detected • Increased method development time	• Atlantis™ HILIC Silica columns retain polar metabolites that cannot be retained by reversed-phase HPLC • Faster and easier method development
Severe peak tailing for polar bases is observed on reversed-phase column	• Method fails system suitability guidelines for peak tailing • Increased method development time	• Atlantis™ HILIC Silica columns provide superior peak shapes for polar bases • Faster and easier method development
Evaporation and reconstitution step in sample preparation is too time consuming	• Increased labor costs and higher cost per analysis • Decreased sample throughput	• Evaporation and reconstitution step is not necessary with Atlantis™ HILIC Silica columns since the mobile phases used are compatible with sample preparation organic solvents • Lower analysis costs • Increased throughput
Evaporation and reconstitution step in sample preparation results in poor analyte recoveries	• Unstable or mobile analytes are lost and/or not detected • Evaporated sample does not completely reconstitute • Method fails recovery and limits of detection requirements	• Evaporation and reconstitution step is not necessary with Atlantis™ HILIC Silica columns since the mobile phases used are compatible with sample preparation organic solvents • Greater analyte recoveries • Lower limits of detection
Insufficient MS sensitivity due to highly aqueous reversed-phase mobile phases	• Samples need to be concentrated and re-analyzed • Low concentration metabolites or contaminants not detected	• Highly volatile mobile phases used with Atlantis™ HILIC Silica columns provide increased ESI-MS sensitivity • Lower limits of detection
Poor polar stationary phase column lifetime (e.g., amino, diol, etc.)	• Frequent column replacement • Retention time reproducibility issues	• Atlantis™ HILIC Silica columns do not have an unstable polar bonded phase • Decreased costs associated with frequent column replacement
Polar bonded phase bleed is observed on MS, UV and/or ELSD	• Noisy baselines resulting in poor sensitivity • Incorrect false positive peaks • Frequent system cleaning	• Atlantis™ HILIC Silica columns do not exhibit detectable column bleed • Increased sensitivity • Decreased instrument downtime and maintenance costs
Column to column reproducibility is inconsistent (e.g., selectivity, retention, etc.)	• Increased labor costs due to individual column QC testing • Revalidate/redevelop method with each new batch of columns	• Atlantis™ HILIC Silica columns are tested under actual HILIC conditions • Decreased method revalidation and development time
Difficult separation requires complementary chromatographic selectivity	• Multiple dedicated LC systems required • Separation techniques must be developed independently	• Atlantis™ HILIC Silica columns use reversed-phase solvents • Single LC system running reversed-phase and HILIC separations can be easily automated • Greater flexibility, lower instrumentation costs, faster method development

ATLANTIS™ dC_{18} Analytical Columns

Part No.	Particle Size	Dimensions
186002194	3 µm	0.075 X 50 mm
186002195	3 µm	0.075 X 100 mm
186002197	3 µm	0.075 X 150 mm
186002207	3 µm	0.100 X 50 mm
186002208	3 µm	0.100 X 100 mm
186002209	3 µm	0.100 X 150 mm
186002304	3 µm	0.320 X 50 mm
186002305	3 µm	0.320 X 100 mm
186002306	3 µm	0.320 X 150 mm
186001279	3 µm	1.0 X 50 mm
186001281	5 µm	1.0 X 50 mm
186001283	3 µm	1.0 X 150 mm
186001285	5 µm	1.0 X 150 mm
186001377[1]	3 µm	2.1 X 10 mm Guard
186001379[1]	5 µm	2.1 X 10 mm Guard
186002064	3 µm	2.1 X 15 mm DC
186002065	5 µm	2.1 X 15 mm DC
186001381[2]	3 µm	2.1 X 20 mm Guard
186001383[2]	5 µm	2.1 X 20 mm Guard
186002058	3 µm	2.1 X 20 mm *IS*™
186002059	5 µm	2.1 X 20 mm *IS*™
186001287	3 µm	2.1 X 30 mm
186001289	5 µm	2.1 X 30 mm
186001291	3 µm	2.1 X 50 mm
186001293	5 µm	2.1 X 50 mm
186001295	3 µm	2.1 X 100 mm
186001297	5 µm	2.1 X 100 mm
186001299	3 µm	2.1 X 150 mm
186001301	5 µm	2.1 X 150 mm
186002060	3 µm	3.0 X 20 mm *IS*™
186002061	5 µm	3.0 X 20 mm *IS*™
186001389	3 µm	3.0 X 50 mm
186001391	5 µm	3.0 X 50 mm
186001303	3 µm	3.0 X 100 mm
186001305	5 µm	3.0 X 100 mm
186001307	3 µm	3.0 X 150 mm
186001309	5 µm	3.0 X 150 mm
186001311	5 µm	3.0 X 250 mm
186001313[3]	3 µm	3.9 X 20 mm Guard
186001315[3]	5 µm	3.9 X 20 mm Guard
186001385[4]	3 µm	3.9 X 50 mm
186001387[4]	5 µm	3.9 X 50 mm
186001393	3 µm	3.9 X 100 mm
186001395	5 µm	3.9 X 100 mm
186001317	3 µm	3.9 X 150 mm
186001319	5 µm	3.9 X 150 mm
186001321[3]	3 µm	4.6 X 20 mm Guard
186001323[3]	5 µm	4.6 X 20 mm Guard
186002062	3 µm	4.6 X 20 mm *IS*™
186002063	5 µm	4.6 X 20 mm *IS*™
186001325	3 µm	4.6 X 30 mm
186001327	5 µm	4.6 X 30 mm
186001329	3 µm	4.6 X 50 mm
186001331	5 µm	4.6 X 50 mm
186001333	3 µm	4.6 X 75 mm
186001335	5 µm	4.6 X 75 mm
186001337	3 µm	4.6 X 100 mm
186001340	5 µm	4.6 X 100 mm
186001342	3 µm	4.6 X 150 mm
186001344	5 µm	4.6 X 150 mm
186001346	5 µm	4.6 X 250 mm

ATLANTIS™ dC_{18} Method Validation Kits

Part No.	Particle Size	Dimensions
186002312	3 µm	4.6 X 150 mm
186002311	5 µm	4.6 X 150 mm
186002313	5 µm	4.6 X 250 mm

ATLANTIS™ dC_{18} Preparative Columns

Part No.	Particle Size	Dimensions
186002300[5]	5 µm	10 X 10 mm Guard
186002452[5]	10 µm	10 X 10 mm Guard
186002298	5 µm	10 X 50 mm
186002299	5 µm	10 X 100 mm
186002453	10 µm	10 X 150 mm
186002454	10 µm	10 X 250 mm
186001361[6]	5 µm	19 X 10 mm Guard
186001363[6]	10 µm	19 X 10 mm Guard
186001365	5 µm	19 X 50 mm
186001367	5 µm	19 X 100 mm
186001369	10 µm	19 X 150 mm
186001371	10 µm	19 X 250 mm
186001373	5 µm	30 X 50 mm
186001375	5 µm	30 X 100 mm
186002417	10 µm	30 X 150 mm
186002418	10 µm	30 X 250 mm

ATLANTIS™ HILIC Silica Analytical Columns

Part No.	Particle Size	Dimensions
186002003	3 µm	1.0 X 50 mm
186002004	5 µm	1.0 X 50 mm
186002005[1]	3 µm	2.1 X 10 mm Guard
186002006[1]	5 µm	2.1 X 10 mm Guard
186002007	3 µm	2.1 X 15 mm DC
186002008	5 µm	2.1 X 15 mm DC
186002009	3 µm	2.1 X 30 mm
186002010	5 µm	2.1 X 30 mm
186002011	3 µm	2.1 X 50 mm
186002012	5 µm	2.1 X 50 mm
186002013	3 µm	2.1 X 100 mm
186002014	5 µm	2.1 X 100 mm
186002015	3 µm	2.1 X 150 mm
186002016	5 µm	2.1 X 150 mm
186002017	3 µm	3.0 X 50 mm
186002018	5 µm	3.0 X 50 mm
186002019	3 µm	3.0 X 100 mm
186002020	5 µm	3.0 X 100 mm
186002021[3]	3 µm	3.9 X 20 mm Guard
186002022[3]	5 µm	3.9 X 20 mm Guard
186002023[3]	3 µm	4.6 X 20 mm Guard
186002024[3]	5 µm	4.6 X 20 mm Guard
186002025	3 µm	4.6 X 30 mm
186002026	5 µm	4.6 X 30 mm
186002027	3 µm	4.6 X 50 mm
186002028	5 µm	4.6 X 50 mm
186002029	3 µm	4.6 X 100 mm
186002030	5 µm	4.6 X 100 mm
186002031	3 µm	4.6 X 150 mm
186002032	5 µm	4.6 X 150 mm
186002033	5 µm	4.6 X 250 mm

ATLANTIS™ HILIC Silica Method Validation Kits

Part No.	Particle Size	Dimensions
186002315	3 µm	4.6 X 150 mm
186002314	5 µm	4.6 X 150 mm
186002316	5 µm	4.6 X 250 mm

NanoEase™ Columns
Guard Cartridges
Cartridge Column
Direct Connect Column
Intelligent Speed (*IS*™) Columns

[1] Requires Sentry Guard Holder	WAT097958
[2] Requires Sentry Guard Holder	186000262
[3] Requires Sentry Guard Holder	WAT046910
[4] Requires Cartridge End Fittings	WAT037525
[5] Requires Cartridge Guard Holder	289000779
[6] Requires Cartridge Guard Holder	186000709

visit us on the internet: *http://www.waters.com*

SALES OFFICES:

WATERS CORPORATION U.S.A.
508 478 2000/800 252 4752

AUSTRALIA
(61) 2 9933 1777

AUSTRIA AND EXPORT
(Central and Europe, CIS and Middle East, India and India Subcontinent)
(43) 1 8771807

BELGIUM AND LUXEMBOURG
(32) 0 27261000

BRAZIL
(55) 11 5543 7788

CANADA
800 252 4752

CZECH REPUBLIC
(42) 02 617 11384

DENMARK
(45) 46 598080

FINLAND
(358) 9 506 4140

FRANCE
(33) 1 30 48 72 00

GERMANY
(49) 6196 40 06 00

HONG KONG
(852) 2964 1800

HUNGARY
(36) 1 350 5086

INDIA
(91) 80 8371900

IRELAND
(353) 1 448 1500

ITALY
(39) 02 274211

JAPAN
(81) 3 3471 7191

MEXICO
(5255) 5524 76 36

THE NETHERLANDS
31 (0) 76 508 72 00

NORWAY
(47) 638 46 050

PEOPLE'S REPUBLIC OF CHINA
(86) 10 8451 8918

POLAND
(48) 22 833 4400

PUERTO RICO
(787) 747 8445

RUSSIA/CIS
(7) 095 931 9193

SINGAPORE
(65) 6278 7997

SPAIN
(34) 93 600 93 00

SWEDEN
468 555 11 500

SWITZERLAND
(41) 62 889 2030

TAIWAN
(886) 2 543 1898

UNITED KINGDOM
(44) 208 238 6100

WATERS CORPORATION
34 Maple Street
Milford MA 01757
508 478 2000/800 252 4752

Waters

The quality management system of Waters' manufacturing facilities in Taunton, Massachusetts and Wexford, Ireland complies with the International Standard ISO 9002 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.

11/03 **720000793EN**