[CARE AND USE MANUAL]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

ATLANTIS T3, DC_{18} AND HILIC SILICA COLUMNS

I. INTRODUCTION

Thank you for choosing an Atlantis® Column. The manufacture of Atlantis columns begins with ultrapure reagents to control the chemical composition and purity of the final product. Atlantis columns are manufactured in a cGMP, ISO 9001:2000 certified plant with each step being conducted within narrow tolerances. Every column is individually tested and Certificates of Batch Analysis and a Performance Chromatogram are provided with each column.

Waters recommends the use of Sentry™ guard columns to maximize column lifetime and protect the column from contaminants.

CONTENTS

I. INTRODUCTION

II. CONNECTING THE COLUMN OR CARTRIDGE TO THE

III. SYSTEM MODIFICATION RECOMMENDATIONS HPLC SYSTEM

IV. WATERS SMALL PARTICLE SIZE (3 μM) COLUMNS – FAST CHROMATOGRAPHY

V. COLUMN EQUILIBRATION

VI. COLUMN INSTALLATION PROCEDURE

VII. COLUMN PERFORMANCE VALIDATION

VIII. INITIAL COLUMN EFFICIENCY DETERMINATION

IX. COLUMN USAGE

XII. HILIC GETTING STARTED

XIII. COLUMN CLEANING, REGENERATING AND STORAGE

XIV. TROUBLESHOOTING

Atlantis® Columns

II. CONNECTING THE COLUMN OR CARTRIDGE TO THE HPLC SYSTEM

a. Column Connection

Handle the column with care. Do not drop or hit the column on a hard surface as this may disturb the bed and affect its performance.

1. Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results.

2. An arrow on the column identification label indicates correct direction of solvent flow.

3. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16 inch outer diameter stainless steel tubing. When tightening or loosening the compression screw, place a 5/16 inch wrench on the compression screw and a 3/8 inch wrench on the hex head of the column endfitting.

   Caution: If one of the wrenches is placed on the column flat during this process, the endfitting will be loosened and leak. Under-tightening compression screws or using worn ferrules can lead to solvent leaking. Care should be taken to check all column connections for leaks to avoid exposure to solvents and the hazards associated with such exposure including risks to health and electrical connections.

4. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing and ferrule must be assembled.

b. Cartridge Connection

Handle the cartridge with care. Do not drop or hit the cartridge on a hard surface as it may disturb the bed and affect its performance. Refer to Figure 1 for an exploded view of an Atlantis cartridge column with a Sentry guard column.

1. Unscrew end connectors from the old cartridge. Leave them connected to the inlet and outlet lines of the instrument.

2. Attach new cartridge column between connectors so that the direction of the flow arrow on the label is pointing in the direction of mobile phase flow (toward detector).

3. Fingertighten all fittings.

   Caution: Under-tightening the connectors can lead to solvent leaking. Care should be taken to check all column connections for leaks to avoid exposure to solvents and the hazards associated with such exposure including risks to health and electrical connections.

4. Check for leaks once flow has been initiated. If a leak occurs between the connector and the column endfitting, the column may be misaligned in the connector or the Kalrez O-ring must be replaced in the connector.

It is important to realize that extra column peak broadening can destroy a successful separation. The choice of appropriate column connectors and system tubing is discussed in detail below.

c. Column Connectors and System Tubing Considerations

Due to the absence of an industry standard, various column man facturers have employed different types of chromatographic column connectors. The chromatographic performance of the separation can be negatively affected if the style of the column endfittings does not match the existing tubing ferrule setting. This section explains the differences between Waters style and Parker style ferrules and endfittings (Figure 2). Each endfitting style varies in the required length of the tubing protruding from the ferrule. The Atlantis column is equipped with Waters style endfittings, which require a 0.130 inch ferrule. If a non-Waters style column is presently being used, it is critical that ferrule depth be reset for optimal performance prior to installing an Atlantis column. In a proper tubing/column connection (Figure 3), the tubing touches the bottom of the column endfitting, with no void between them.

Figure 1: Installation of Atlantis Cartridge Column with Sentry Guard Column

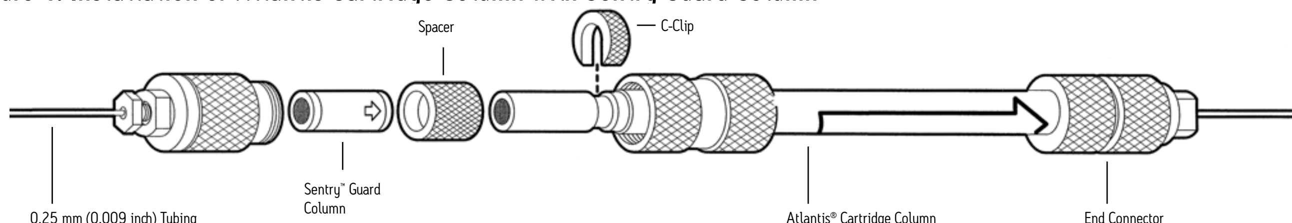


Figure 2: Waters and Parker Ferrule Types

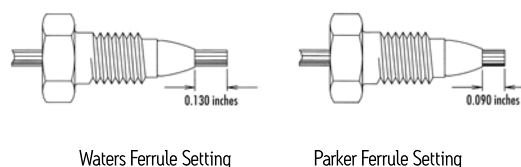
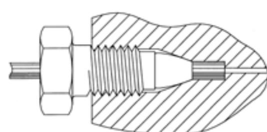


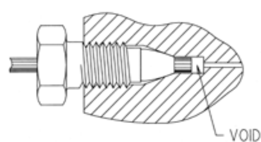
Figure 3: Proper Tubing/Column Connection

Tubing touches the bottom of the column endfitting, with no void between them.



Attention: A void will occur if tubing with a Parker ferrule is connected to a Waters style endfitting (Figure 4). This will dramatically reduce the efficiency of the column and cause peak shape distortion.

Figure 4: Parker Ferrule in a Waters Style Endfitting

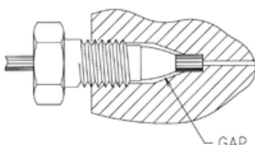


To fix this problem: Cut the end of the tubing with the ferrule, place a new ferrule on the tubing and make a new connection. Before tightening the screw, make sure that the tubing bottoms out in the endfitting of the column.

Conversely, if tubing with a Waters ferrule is connected to a column with Parker style endfitting, the end of the tubing will bottom out before the ferrule reaches its proper sealing position. This will leave a gap and create a leak (Figure 5).

Caution: The connection will leak if a Waters ferrule is connected to a column with a Parker style endfitting.

Figure 5: Waters Ferrule in a Parker Style Endfitting



There are two ways to fix the problem:

1. Tighten the screw a bit more. The ferrule moves forward, and reaches the sealing surface. Do not overtighten since this may break the screw.

2. Cut the tubing, replace the ferrule and make a new connection.

Alternatively, replace the conventional compression screw fitting with an all-in-one PEEK™ fitting (Waters Part Number PSL613315) that allows resetting of the ferrule depth. Another approach is to use a Thermo Hypersil™ Keystone, Inc. SLIPFREE® connector to ensure the correct fit. The fingertight SLIPFREE connectors automatically adjust to fit all compression screw type fittings without the use of tools (Figure 6).

Figure 6: Single and Double SLIPFREE Connectors

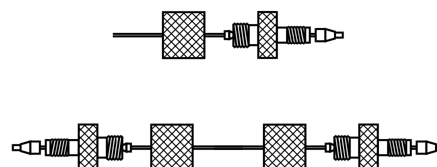


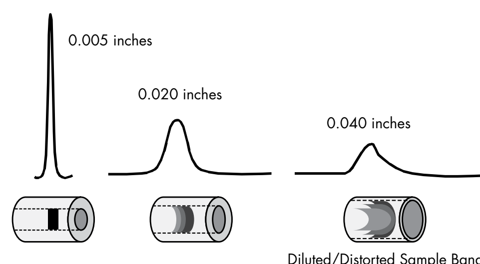
Table 1. Waters Part Numbers for SLIPFREE Connectors

SLIPFREE Type and Tubing Length	Tubing Internal Diameter 0.005"	0.010"	0.020"
Single 6 cm	PSL 618000	PSL 618006	PSL 618012
Single 10 cm	PSL 618002	PSL 618008	PSL 618014
Single 20 cm	PSL 618004	PSL 618010	PSL 618016
Double 6 cm	PSL 618001	PSL 618007	PSL 618013
Double 10 cm	PSL 618003	PSL 618009	PSL 618015
Double 20 cm	PSL 618005	PSL 618001	PSL 618017

d. Band Spreading Minimization

Internal tubing diameter influences system band spreading and peak shape. Larger tubing diameters cause excessive peak broadening and lower sensitivity (Figure 7).

Figure 7: Effect of Connecting Tubing on System



e. Measuring System Bandspread Volume

This test should be performed on an HPLC system with a single wavelength UV detector (not a Photodiode Array (PDA)).

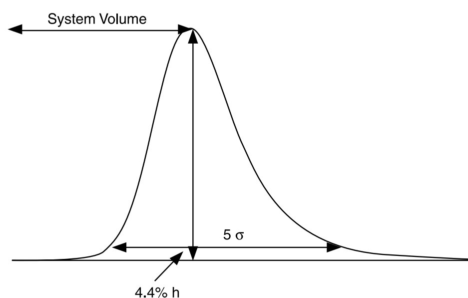
1. Disconnect column from system and replace with a zero dead volume union.
2. Set flow rate to 1 mL/min.
3. Dilute a test mix in mobile phase to give a detector sensitivity 0.5-1.0 AUFS (system start up test mix can be used which contains uracil, ethyl and propyl parabens; Waters Part Number WAT034544).
4. Inject 2 to 5 μL of this solution.
5. Using 5 sigma method measure the peak width at 4.4% of peak height:

   Band Spreading (μL) = Peak Width (min) x Flow Rate (μL/min)

   = 0.1 min x 1000 μL/min

   = 100 μL

Figure 8: Determination of System Bandspread Volume Using 5-Sigma Method



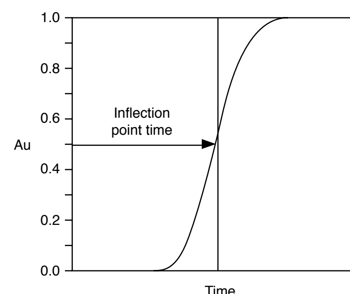
In a typical HPLC system, the Bandspread Volume should be 100 μL ± 30 μL.

In a microbore (for 2.1 mm i.d. columns) system, the Bandspread Volume should be no greater than 20 to 40 μL.

f. Measuring Gradient Delay Volume

1. Replace the column with a zero dead volume union.
2. Prepare eluent A (pure solvent, such as methanol) and eluent B (solvent plus sample, such as 5.6 mg/mL propylparaben in methanol).
3. Equilibrate the system with eluent A until a stable baseline is achieved.
4. Switch to 100% eluent B.
5. Record the half height of the step and determine dwell volume (Figure 9).

Figure 9: Determination of Dwell Volume



The dwell volume should be less than 1 mL. If the dwell volume is greater than 1 mL, see „System Modification Recommendations„ for how to reduce system volume.

g. Use of Smaller i.d. Columns

A 3.0 mm i.d. narrow-bore column usually requires no system modifications. A 2.1 mm i.d. microbore column, however, requires modifications to the HPLC system to eliminate excessive system bandspread volume. Without proper system modifications, excessive system bandspread volume causes peak broadening and has a large impact on peak width as peak volume decreases.

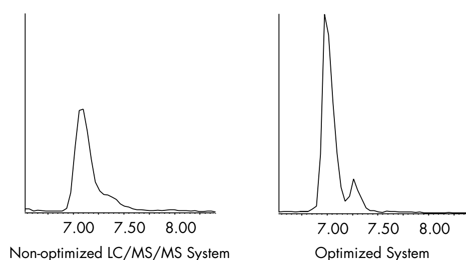
h. Impact of Bandspread Volume on 2.1 mm i.d. Column Performance

System with 70 μL bandspread:	10,000 plates
System with 130 μL bandspread:	8,000 plates (same column)

Attention: Flow splitters after the column will introduce additional band spreading which will reduce sensitivity and resolution. Loss of sensitivity or resolution may affect the accuracy and/or precision of results.

System optimization, especially in a system that contains a flow splitter, can have dramatic effects on sensitivity and resolution. Optimization includes using correct-depth ferrules and minimizing tubing diameter and lengths. System optimization results in a doubling of sensitivity and resolution of the metabolite in an LC/MS/MS system (Figure 10).

Figure 10: Chromatograms Obtained Using Non-Optimized vs. Optimized LC/MS/MS System



III. SYSTEM MODIFICATION RECOMMENDATIONS

1. Use a microbore detector flowcell with ≤ 2.1 mm i.d. columns.

   Attention: Detector sensitivity is reduced with the shorter flowcell pathlength in order to achieve lower bandspread volume.

2. Minimize injector sample loop volume.
3. Use 0.009 inch (0.25 mm) i.d. tubing between pump and injector.
4. Use 0.009 inch (0.25 mm) i.d. tubing for rest of connections with > 3.0 mm i.d. columns. Use 0.005 inch (0.12 mm) i.d. tubing for narrow-bore (≤ 2.1 mm i.d.) systems.
5. Use perfect (pre-cut) connections (with a variable depth inlet if using columns from different suppliers).
6. Detector time constants should be shortened to less than 0.2 seconds and the sampling rate adjusted to obtain at least 20 data points across peaks of interest.

IV. WATERS SMALL PARTICLE SIZE (3 μM) COLUMNS – FAST CHROMATOGRAPHY

Waters columns with 3 μm particles provide faster and more efficient separations without sacrificing column lifetime. This section describes five parameters to consider when performing separations with columns containing 3 μm particles.

Note: Columns that contain 3 μm particles have smaller pore-size outlet frits to retain packing material than are used at the inlet. These columns should not be backflushed.

1. *Flow Rate* — Compared to columns with 5 μm particles, columns with 3 μm particles have higher optimum flow rates and are used when high efficiency and short analysis times are required. These higher flow rates, however, lead to increased backpressure.

   Note: Use a flow rate that is practical for your system.

2. *Backpressure* — Backpressures for columns with 3 μm particles are higher than for 5 μm columns with the same dimensions. Waters suggests using a shorter column to compensate for increased back pressure and to obtain a shorter analysis time.
3. *Temperature* — Use a higher temperature to reduce backpressure caused by smaller particle sizes. The recommended temperature range for Atlantis columns is 20 °C to 45 °C. See "Column Usage" for a discussion of elevated temperature use with Atlantis columns.
4. *Sampling Rate* — Use a sampling rate of about 10 points per second.
5. *Detector Time Constant* — Use a time constant of 0.1 seconds for fast analyses.

V. COLUMN EQUILIBRATION

Atlantis columns are packed and shipped in 100% acetonitrile. It is important to ensure solvent compatibility before changing to a new solvent.

Atlantis T3 and dC_{18} - Equilibrate with a minimum of 10 column volumes of the mobile phase to be used (refer to Table 2 for a listing of standard column volumes).

Atlantis HILIC Silica – Upon receipt, equilibrate with 50 column volumes of 50:50 acetonitrile:water with 10 mM final buffer concentration (refer to Table 2 for a listing of standard column volumes). Prior to the first injection, equilibrate with 20 column volumes of initial mobile phase conditions. See "HILIC Getting Started" for additional information.

Table 2. Standard Column Volumes in mL (Multiply by 10 for Equilibration Mobile Phase Volumes)

	Column Volume (mL)									
Column Length	Column internal diameter (mm) 1.0	2.1	3.0	3.9	4.6	7.8	10	19	30	50
15 mm	–	0.1	–	–	–	–	–	–	–	–
20 mm	–	0.1	0.1	–	0.3	–	–	–	–	–
30 mm	–	0.1	0.2	–	0.5	–	2.4	8	–	–
50 mm	0.1	0.2	0.3	–	0.8	2.4	4	14	35	98
100 mm	0.1	0.4	0.7	1.2	1.7	5	8	28	70	–
150 mm	0.1	0.5	1.0	1.8	2.5	7	12	42	106	294
250 mm	–	0.9	1.8	–	4	–	20	70	176	490
300 mm	–	–	–	–	–	14	24	85	212	589

VI. COLUMN INSTALLATION PROCEDURE

Note: The flow rates given in the procedure below are for a typical 4.6 mm i.d. column. Scale the flow rate up or down accordingly based upon the column i.d., length, particle size and backpressure of the Atlantis column being installed. See "Scaling Up/Down" *for calculating flow rates when changing column i.d. and/or length.*

1. Purge the pumping system and connect the inlet end of the column to the injector outlet.
2. Set the pump flow to 0.1 mL/min and increase to 1 mL/min over 5 minutes.
3. When the mobile phase is flowing freely from the column outlet, stop the flow, then attach the column to the detector. This prevents entry of air into the detector and provides more rapid baseline equilibration.

   Caution: Care should be taken to check column connections for leaks to avoid exposure to solvents and the hazards associated with such exposure including risks to health and electrical connections.

4. When the mobile phase is changed, gradually increase the flow rate of the new mobile phase from 0.0 mL/min to 1.0 mL/min in 0.1 mL/min increments.
5. Once a steady backpressure and baseline have been achieved, the column is ready to be used (or equilibrated).

   Note: If mobile phase additives are present in low concentrations (e.g., ion-pairing reagents), 100 to 200 column volumes may be required for complete equilibration. In addition, mobile phases that contain formate (e.g., ammonium formate, formic acid, etc.) may also require slightly longer initial column equilibration times. Please see additional equilibration information for Atlantis HILIC Silica columns in "HILIC Getting Started."

VII. COLUMN PERFORMANCE VALIDATION

Each Atlantis column comes with a Certificate of Batch Analysis and a Performance Test Chromatogram. The Certificate of Analysis is specific to each batch of packing material and includes the batch number, analysis of unbonded particles, analysis of bonded particles (Atlantis T3 and Atlantis dC_{18}), and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains information such as batch number, column serial number, USP plate count, USP tailing factor, capacity factor and chromatographic results and conditions. These data should be stored for future reference.

VIII. INITIAL COLUMN EFFICIENCY DETERMINATION

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture, as found in the "Performance Test Chromatogram", to verify the performance of the column upon receipt.
2. Determine the number of theoretical plates (N) and use for periodic comparison.
3. Repeat the test periodically to track column performance over time. Slight variations may be obtained on two different HPLC systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition and operator technique.

   Note: If 1) is performed, the isocratic efficiencies measured in your laboratory may be less than those given on the Waters "Performance Test Chromatogram." This is normal. The Waters isocratic column testing systems have been modified in order to achieve extremely low system volumes. This presents a more challenging test of how well the column was packed. This guarantees the highest quality packed column. These special testing systems have been modified to such an extent that they are not commercially viable and have limited method flexibility other than isocratic column testing.

IX. COLUMN USAGE

Caution: Accumulation of particulates from solvents, samples, or pump seals may cause the column backpressure to increase over time. This may lead to a system shutdown or leaking of column connections. Accumulation of contaminants from "dirty" samples at the column inlet may lead to a loss of resolution or ion suppression in a mass spectrometer, resulting in erroneous results.

To ensure the continued high performance of Atlantis columns and cartridges, follow these guidelines:

a. Guard Columns

Use a Waters Sentry guard cartridge of matching i.d., chemistry and particle size between the injector and main column. For best results, the guard column should be replaced prior to the observation of a substantial loss in resolution or increase in system backpressure. It is important to use a high-performance matching guard column to protect the main column while not compromising or changing analytical resolution.

b. Sample Preparation

1. Sample impurities often contribute to column contamination. Use Waters Oasis® or Sep-Pak® solid-phase extraction cartridges/columns of the appropriate chemistry to cleanup the sample before analysis.
2. For reversed-phase separations (Atlantis T3 and dC_{18}) prepare the sample in mobile phase or a solvent that is weaker (less organic modifier) than the mobile phase. For Hydrophilic Interaction Chromatography (HILIC) separations (Atlantis HILIC Silica), the samples must be prepared in 100% organic solvents (e.g., acetonitrile). See "HILIC Getting Started" for additional information.
3. If the sample is not dissolved in the mobile phase, ensure that the sample and diluent are miscible in the mobile phase(s) in order to avoid sample and/or diluent precipitation.
4. Filter sample through a 0.2 µm membrane to remove particulates. If the sample is dissolved in a solvent that contains an organic modifier (e.g., acetonitrile, methanol, etc.) ensure that the membrane material does not dissolve in the solvent. Contact the membrane manufacturer with solvent compatibility questions.

c. Recommended pH Range

Atlantis HILIC Silica: 1-5 Atlantis T3: 2-8 Atlantis dC_{18}: 3-7

Column lifetime will vary depending upon the temperature, type and concentration of buffer used. A listing of recommended and non-recommended buffers is given in Table 3. Please use this as a guideline when developing methods.

Attention: Operating at the upper or lower end of the pH range in combination with elevated temperatures will lead to shorter column lifetime and/or may result in the column generating high backpressure.

Table 3: Buffer recommendations for using Atlantis columns from pH 1 to 7

Additive or Buffer	pKa	Buffer range (±1 pH unit)	Volatility	Used for Mass Spec?	Comments
TFA	0.3		Volatile	Yes	Ion pair additive, can suppress MS signal. Used in the 0.01-0.1% range.
Formic Acid	3.75		Volatile	Yes	Maximum buffering obtained when used with Ammonium Formate salt. Used in 0.1-1.0% range.
Acetic Acid	4.76		Volatile	Yes	Maximum buffering obtained when used with Ammonium Acetate salt. Used in 0.1-1.0% range.
Formate (NH_4COOH)	3.75	2.75 – 4.75	Volatile	Yes	Used in the 1-10mM range. Note: sodium or potassium salts are not volatile.
Acetate (NH_4CH_2COOH)	4.76	3.76 – 5.76	Volatile	Yes	Used in the 1-10mM range. Note: sodium or potassium salts are not volatile.
Phosphate 1*	2.15	1.15 – 3.15	Non-volatile	No	Traditional low pH buffer, good UV transparency
Phosphate 2*	7.2	6.20 – 8.20	Non-volatile	No	Much shorter colum lifetimes will be realized using phosphate at pH 7

* Phosphate salt buffers are not recommended for HILIC (phosphoric acid is OK) due to phosphate buffer salt insolubility at high acetonitrile concentrations.

d. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use. The addition of at least 5% organic to neutral pH buffers is recommended to prevent bacterial growth. Pall Corporation Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet frit of the column. This will result in higher operating pressure and poorer performance.

Degas all solvents thoroughly before use to prevent bubble formation in the pump and detector. The use of an on-line degassing unit is also recommended. This is especially important when running low pressure gradients since bubble formation can occur as a result of aqueous and organic solvent mixing during the gradient.

e. Pressure

Atlantis columns can tolerate pressures of up to 6,000 psi (400 bar or 40 Mpa) although pressures greater than 4,000 - 5,000 psi should be avoided in order to maximize column and system lifetimes, and the risk of system shutdowns and leaking.

f. Temperature

Temperatures between 20 °C - 45 °C are recommended for operating Atlantis columns in order to enhance selectivity, lower solvent viscosity and increase mass transfer rates. However, any temperature rise above ambient will have a negative effect on lifetime which will vary depending on the pH and buffer conditions used. The combination of operating at elevated temperatures and at pH extremes should be avoided.

g. Scaling Up/Down Isocratic Methods

The following formulas will allow scale up or scale down, while maintaining the same linear velocity (retention time), and provide new sample loading values:

If column i.d. and length are altered: $F_2 = F_1(r_2/r_1)^2$

or: $Load_2 = Load_1(r_2/r_1)^2(L_2/L_1)$

or: $Inj\ vol_1 = Inj\ vol_2\ (r_2/r_1)^2\ (L_2/L_1)$

Where: r = Radius of the column, in mm

F = Flow rate, in mL/min

L = Length of column, in mm

1 = Original, or reference column

2 = New column

XII. HILIC GETTING STARTED

a. Equilibration of Atlantis HILIC Silica Columns

1. Upon receipt, equilibrate in 50% acetonitrile/50% aqueous buffer (10 mM final buffer concentration) for 50 column volumes.
2. Prior to first injection, equilibrate with 20 column volumes of initial mobile phase conditions.
3. When running gradients, equilibrate with 10 column volumes between injections.

Failure to appropriately equilibrate the column could result in drifting retention times.

b. HILIC Mobile Phase Considerations

1. Always maintain at least 5% polar solvent in the mobile phase or gradient (e.g., 5% water, 5% methanol or 3% methanol/2% aqueous buffer, etc.). This ensures that the Atlantis HILIC Silica particle is always hydrated.
2. Maintain at least 40% organic solvent (e.g., acetonitrile) in your mobile phase or gradient.
3. Avoid phosphate salt buffers to avoid precipitation in HILIC mobile phases (phosphoric acid is OK).
4. Buffers such as ammonium formate or ammonium acetate, will produce more reproducible results than additives such as formic acid or acetic acid. If an additive (e.g., formic acid) must be used instead of a buffer, use 0.2% (v:v) instead of 0.1%.
5. For best peak shape, maintain a buffer concentration of 10 mM in your mobile phase/gradient at all times.

c. Injection Solvents for HILIC

1. If possible, injection solvents should be 100% organic solvent. Water must be eliminated or minimized. Choose weak HILIC solvents such as acetonitrile, isopropanol, methanol, etc.
2. A generic injection solvent is 75:25 acetonitrile methanol. This is a good compromise between analyte solubility and peak shape.
3. Avoid water and dimethylsulfoxide (DMSO) as injection solvents. These solvents will produce very poor peak shapes.
4. Exchange water or DMSO with acetonitrile by using reversed-phase solid-phase extraction. If this is not possible, dilute the water or DMSO with organic solvent.

d. Additional HILIC Recommendations

1. For initial scouting conditions, run a gradient from 95% acetonitrile to 50% acetonitrile. If no retention occurs, run isocratically with 95:3:2 acetonitrile:methanol:aqueous buffer.
2. Alternate polar solvents such as methanol, acetone or isopropanol can also be used in place of water in the mobile phase to increase retention.
3. Be sure that your needle wash solvent/purge solvent contains the same high organic solvent concentration as your mobile phase, else peak shapes will suffer.

XIII. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

A sudden increase in pressure or shift in retention or resolution may indicate contamination of the column.

Atlantis T3 and dC$_{18}$ – Flush with a neat organic solvent to remove the non-polar contaminant(s). If this flushing procedure does not solve the problem, purge the column with a sequence of progressively more non-polar solvents. For example, switch from water to tetrahydrofuran to methylene chloride. Return to the standard mobile phase conditions by reversing the sequence.

Atlantis HILIC Silica – Flush with 50:50 acetonitrile:water to remove the polar contaminant(s). If this flushing procedure does not solve the problem, purge the column with 5:95 acetonitrile:water.

Guard columns require replacement at regular intervals as determined by sample contamination. When system backpressure increases above a set pressure limit, it is usually an indication that the guard column should be replaced. A sudden appearance of split peaks is also indicative of a need to replace the guard column.

b. Storage

Atlantis T3 and dC$_{18}$ – For periods longer than four days, store the column in 100% acetonitrile. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of HPLC grade water (see Table 2 for common column volumes) and replace with 100% acetonitrile for storage. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced.

Atlantis HILIC Silica – For periods longer than four days, store the column in 95:5 acetonitrile:water. Do not store in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of 95:5 acetonitrile:water (refer to Table 2 for a listing of standard column volumes) prior to storage.

Completely seal column to avoid evaporation and drying out of the bed.

Note: If a column has been run with a formate-containing mobile phase (e.g., ammonium formate, formic acid, etc.) and is flushed to remove the buffer, slightly longer equilibration times may be required after the column is re-installed and run again with a formate-containing mobile phase.

XIV. Troubleshooting

Changes in retention time, resolution, or backpressure are often due to column contamination (refer to "Column Cleaning, Regenerating and Storage"). Information on column troubleshooting problems may be found in HPLC Columns Theory, Technology and Practice, U.D. Neue, (Wiley-VCH, 1997) or the Waters HPLC Troubleshooting Guide (Literature Code 720000181EN).

Sales Offices:

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 431 877 18 07

Australia 2 9933 1777

Belgium 32 2 726 1000

Brazil 55 11 5094 3788

Canada 800 252 4752

China 8621 6495 6999

CIS/Russia +7 495 3367000

Czech Republic 42 02 617 11384

Denmark 45 46 59 8080

Finland +358 9 5659 6288

France (33) 1 30 48 72 00

Germany 49 6196 400600

Hong Kong 852 29 64 1800

Hungary 36 1 350 5086

India and India Subcontinent 91 80 2 837 1900

Ireland 353 1 448 1500

Italy 39 02 274 211

Japan (81) 3 3471 7191

Korea (82) 2 820 2700

Mexico 5255 5200 1860

The Netherlands +31 (0)76-50 87 200

Norway 47 63 84 60 50

Poland (48) 22 833 4400

Puerto Rico 787 747 8445

Singapore 65 6273 1221

Spain 34 93 600 93 00

Sweden 46 8 555 11500

Switzerland 41 62 889 2030

Taiwan 886 2 2543 1898

United Kingdom 44 208 238 6100

购买联系方式 ：
Tel：010-67113925/67136152/67100708
Fax： 010-67114016
E-mail：china.hplc@163.com
北京金欧亚科技发展有限公司
地址：北京崇文区左安门内大街8号伟图大厦301室
邮编：100061
网址：www.chromatogr.com