Waters

ACQUITY UPLC™ BEH Column
Care and Use Instructions

Thank you for choosing a Waters ACQUITY UPLC™ BEH column. The ACQUITY UPLC™ BEH packing materials were designed specifically for use with the Waters ACQUITY UPLC™ system and are manufactured in a cGMP, ISO 9001 certified plant using ultra pure reagents. Each batch of ACQUITY UPLC™ BEH material is tested chromatographically with acidic, basic and neutral analytes and the results are held to narrow specification ranges to assure excellent, reproducible performance. Every column is individually tested and a Performance Chromatogram and Certificate of Batch Analysis are provided on the eCord™ intelligent chip.

Please note that ACQUITY UPLC™ BEH columns are designed for use with the ACQUITY UPLC™ system ONLY. Use of these columns on conventional HPLC systems is NOT recommended.

Acquity
Ultra Performance LC™

Contents

I. GETTING STARTED

Each ACQUITY UPLC™ BEH column comes with a Certificate of Analysis and a Performance Test Chromatogram embedded within the eCord™ intelligent chip. The Certificate of Analysis is specific to each batch of packing material contained in the ACQUITY UPLC™ BEH column and includes the gel batch number, analysis of unbonded particles, analysis of bonded particles, and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains such information as: gel batch number, column serial number, USP plate count, USP tailing factor, capacity factor, and chromatographic conditions. These data should be stored for future reference.

a. Column Connectors

The ACQUITY UPLC™ system utilizes tubing and gold plated compression screws which have been designed to meet stringent tolerance levels and to minimize extra column volumes.

Optimized column inlet tubing (part number 430001084) is supplied with the ACQUITY UPLC™ system. The inject valve end of the tubing is clearly marked with a blue shrink tube marker. Insert the opposite end of the tubing into the ACQUITY UPLC™ column and tighten the compression fitting using two 5/16-inch wrenches.

For information on the correct column outlet tubing, please refer to the relevant detector section in the ACQUITY UPLC™ System Operator's Guide (part number 71500082502).

b. Column Installation

Note: The flow rates given in the procedure below are for a typical 2.1 mm i.d. by 50 mm length 1.7 µm column. Scale the flow rate up or down accordingly based upon the flow rate and pressure guide provided in Section V (Additional Information).

1. Purge the pumping system of any buffer-containing mobile phases and connect the inlet end of the column to the injector outlet.
2. Flush column with 100% organic mobile phase (methanol or acetonitrile) by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.5 mL/min over 5 minutes.
3. When the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the detector. This prevents entry of air into the detection system and gives more rapid baseline equilibration.
4. Gradually increase the flow rate as described in step 2.
5. Once a steady backpressure and baseline have been achieved, proceed to the next section.

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 longer initial column equilibration times.

c. Column Equilibration

ACQUITY UPLC™ BEH columns are shipped in 100% acetonitrile. It is important to ensure mobile phase compatibility before changing to a different mobile phase system. Equilibrate the column with a minimum of 10 column volumes of the mobile phase to be used (refer to Table 1 for a list of column volumes). The column may be considered thermally equilibrated once a constant backpressure is achieved.

Table 1. Empty Column Volumes in mL (multiply by 10 for flush solvent volumes)

Column Length	Internal Diameter	
(mm)	1.0 mm	2.1 mm
20	0.016	0.07
30	0.024	0.1
50	0.04	0.2
100	0.08	0.4
150	0.12	0.5

To avoid precipitating mobile phase buffers on your column or in your system, flush the column with five column volumes of a water/organic solvent mixture, using the same or lower solvent content as in the desired buffered mobile phase. (For example, flush the column and system with 60% methanol in water prior to introducing 60% methanol/40% buffer mobile phase).

For ACQUITY UPLC™ BEH HILIC columns, equilibrate with 50 column volumes of 50:50 acetonitrile:water with 10 mm final buffer concentration. Prior to the first injection, equilibrate with 20 column volumes of initial mobile phase conditions (see Table 1 for a list of column volumes). See Getting Started with ACQUITY UPLC™ BEH HILIC Columns for additional information.

d. eCord™ Installation

The eCord™ button should be attached to the side of the column heater module. The eCord™ button is magnetized and does not require specific orientation.

e. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. This test may consist of:

   a) An analyte test mixture that is commonly used in your laboratory, and/or

   b) An analyte mixture as found on the "Performance Test Chromatogram" which accompanied your column.

   Note: If b) 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.

2. Determine the number of theoretical plates (N) and use this value for periodic comparisons.

3. Repeat the test at predetermined intervals to track column performance over time. Slight variations may be obtained on two different UPLC systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition and operator technique.

II. COLUMN USE

To ensure the continued high performance of ACQUITY UPLC™ BEH columns, follow these guidelines:

a. Sample Preparation

1. Sample impurities often contribute to column contamination. One option to avoid this is to use Waters Oasis® solid-phase extraction cartridges/columns or Sep-Pak® cartridges of the appropriate chemistry to cleanup the sample before analysis. For more information, vistit www.waters.com/sampleprep

2. It is preferable to prepare the sample in the operating mobile phase or a mobile phase that is weaker (less organic modifier) than the mobile phase for the best peak shape and sensitivity.

3. If the sample is not dissolved in the mobile phase, ensure that the sample, solvent and mobile phases are miscible in order to avoid sample and/or buffer precipitation.

4. Filter sample with 0.2 µm membranes 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. Alternatively, centrifugation for 20 minutes at 8,000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial, could be considered.

5. For Hydrophilic Interaction Chromatography (HILIC) separations, the samples must be prepared in 100% organic solvents (e.g., acetonitrile). See Getting Started with ACQUITY UPLC™ BEH Columns for additional information.

b. pH Range

The recommended operating pH range for ACQUITY UPLC™ BEH columns is 1 to 12 for the C_{18}, C_8 and Phenyl chemistries; 2 to 11 for the Shield RP_{18} chemistry and 1 to 8 for the HILIC chemistry. A listing of commonly used buffers and additives is given in Table 2. Additionally, the column lifetime will vary depending upon the operating temperature, the type and concentration of buffer used. For example, the use of phosphate buffer at pH 8 in combination with elevated temperatures will lead to shorter column lifetimes.

Note: Working at the extremes of pH, temperature and/or pressure will result in shorter column lifetimes.

Table 2: Buffer Recommendations for Using ACQUITY UPLC™ BEH Columns from pH 2 to 12

Additive/Buffer	pKa	Buffer range	Volatility (±1 pH unit)	Used for Mass Spec	Comments
TFA	0.3		Volatile	Yes	Ion pair additive, can suppress MS signal, used in the 0.02-0.1% range.
Acetic Acid	4.76		Volatile	Yes	Maximum buffering obtained when used with ammonium acetate salt. Used in 0.1-1.0% range.
Formic Acid	3.75		Volatile	Yes	Maximum buffering obtained when used with ammonium formate salt. Used in 0.1-1.0% range.
Acetate (NH_4CH_2COOH)	4.76	3.76 – 5.76	Volatile	Yes	Used in the 1-10 mM range. Note that sodium or potassium salts are **not volatile**.
Formate (NH_4COOH)	3.75	2.75 – 4.75	Volatile	Yes	Used in the 1-10 mM range. Note that 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	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime.
4-Methylmorpholine	~8.4	7.4 – 9.4	Volatile	Yes	Generally used at 10 mM or less.
Ammonia (NH_4OH)	9.2	8.2 – 10.2	Volatile	Yes	Keep concentration below 10 mM and temperatures below 30 °C.
Ammonium Bicarbonate	10.3 (HCO_3) 9.2 (NH_4) 6.3 (H_2CO_3)	6.8 – 11.3	Volatile	Yes	Used in the 5-10 mM range (for MS work keep source >150 °C). Adjust pH with ammonium hydroxide or acetic acid. Good buffering capacity at pH 10. Note: use ammonium bicarbonate (NH_4HCO_3), not ammonium carbonate ($(NH_4)_2CO_3$).
Ammonium (Acetate)	9.2	8.2 – 10.2	Volatile	Yes	Used in the 1-10 mM range.
Ammonium (Formate)	9.2	8.2 – 10.2	Volatile	Yes	Used in the 1-10 mM range.
CAPSO	9.7	8.7 – 10.7	Non-Volatile	No	Zwitterionic buffer, compatible with acetonitrile, used in the 1-10 mM range. Low odor.
Glycine	2.4, 9.8	8.8 – 10.8	Non-Volatile	No	Zwitterionic buffer, can give longer lifetimes than borate buffer.
1-Methylpiperidine	10.2	9.3 – 11.3	Volatile	Yes	Used in the 1-10 mM range.
CAPS	10.4	9.5 – 11.5	Non-Volatile	No	Zwitterionic buffer, compatible with acetonitrile, used in the 1-10 mM range. Low odor.
Triethylamine (as acetate salt)	10.7	9.7 – 11.7	Volatile	Yes	Used in the 0.1-1.0% range. Volatile only when titrated with acetic acid (not hydrochloric or phosphoric). Used as ion-pair for DNA analysis at pH 7-9.
Pyrrolidine	11.3	10.3 – 12.3	Volatile	Yes	Mild buffer, gives long lifetime.

c. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use through a 0.2 µm filter. Pall Gelman Laboratory Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poorer performance. See **Section V** for more information.

d. Pressure

Although ACQUITY UPLC™ BEH columns can tolerate pressures of up to 15,000 psi (1034 bar or 103 MPa), for longer column lifetimes it is recommended that you operate at pressures of less than 10,000 psi (689 bar or 69 MPa). This is an optimal balance between speed (linear velocity or flow rate), resolution and column lifetime. In addition, ballistic gradients which produce sharp increases in backpressure can adversely affect column lifetime.

Note: Working at the extremes of pressure, pH and/or temperature will result in shorter column lifetimes.

e. Temperature

Temperatures between 20°C – 90°C are recommended for operating ACQUITY UPLC™ BEH columns in order to enhance selectivity, lower solvent viscosity and increase mass transfer rates. When operating at high pH, lower operating temperatures are recommended for longer column lifetime. Working at high temperatures (e.g. > 70°C) may also result in shorter column lifetimes.

Note: Working at the extremes of temperature, pressure and/or pH will result in shorter column lifetimes.

III. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

Changes in peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution or increasing backpressure may indicate contamination of the column. Flushing with a neat organic solvent, taking care not to precipitate buffers, is usually sufficient to remove the contaminant. If the flushing procedure does not solve the problem, purge the column using the following cleaning and regeneration procedures.

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 3 below). Flush columns with 20 column volumes of HPLC-grade solvents. Increasing mobile phase temperature to 35-55 °C increases cleaning efficiency. If the column performance is poor after regenerating and cleaning, call your local Waters office for additional support.

Flush ACQUITY UPLC™ BEH HILIC columns with 50:50 acetonitrile: water to remove polar contaminants. If this flushing procedure does not solve the problem, purge the column with 5:95 acetonitrile/water.

Table 3. Column Cleaning Sequence

Polar Samples	Non-polar Samples	Proteinaceous Samples
1. water	1. isopropanol (or an appropriate isopropanol/ water mixture*)	Option 1: Inject repeated aliquots of dimethyl sulfoxide (DMSO)
2. methanol	2. tetrahydrofuran (THF)	Option 2: gradient of 10% to 90% B where: A = 0.1% trifluoroacetic acid (TFA) in water B = 0.1% trifluoroacetic acid (TFA) in acetonitrile (CH3CN)
3. tetrahydrofuran (THF)	3. dichloromethane	
4. methanol	4. hexane	
5. water	5. isopropanol (followed by an appropriate isopropanol/ water mixture*)	Option 3: Flush column with 7M guanidine hydrochloride, or 7M urea
6. mobile phase	6. mobile phase	

* Use low organic solvent content to avoid precipitating buffers.

b. Storage

For periods longer than four days at room temperature, store reversed-phase ACQUITY UPLC™ BEH columns in 100% acetonitrile. For elevated temperature applications, store immediately after use in 100% acetonitrile for the best column lifetime. 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 1 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. Completely seal column to avoid evaporation and drying out of the bed.

For periods longer than four days, store ACQUITY UPLC™ BEH HILIC columns in 95:5 acetonitrile:water. Do not store in buffered solvent. If the mobile phase contained a buffered salt, flush the column with 10 column volumes of 95:5 acetonitrile:water (see Table 1 for common column volumes).

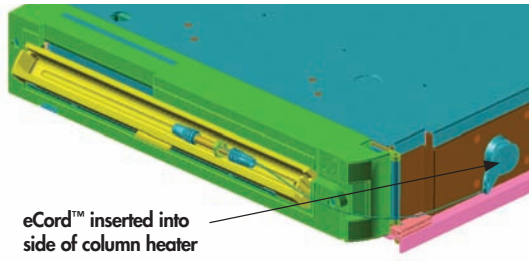
Note: If a column has been run with a mobile phase that contains formate (e.g., ammonium formate, formic acid, etc.) and is then flushed with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and run again with a formate-containing mobile phase.

IV. eCORD™

a. Introduction

The eCord™ intelligent chip is a new technology that will provide the history of a column's performance throughout its lifetime. The eCord™ will be permanently attached to the column to assure that the column's performance history is maintained in the event that the column is moved from one instrument to another.

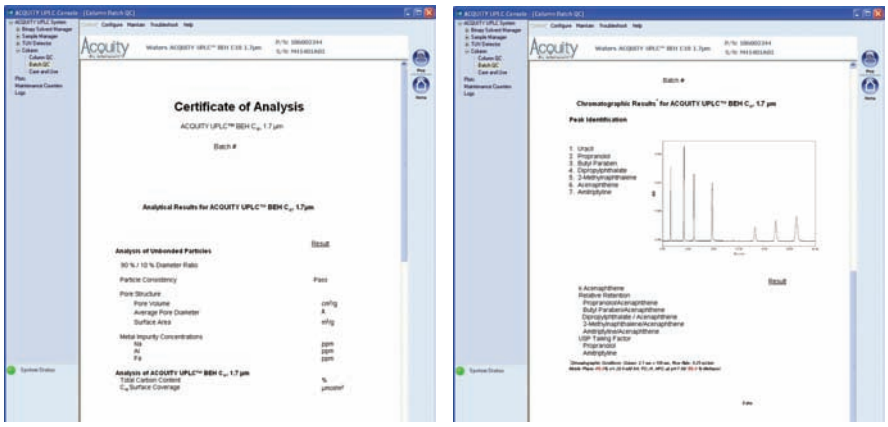
At the time of manufacture, tracking and quality control information will be downloaded to the eCord™. Storing this information on the chip will eliminate the need for a paper Certificate of Analysis. Once the user installs the column, the software will automatically download key parameters into a column history file stored on the chip. In this manual, we explain how the eCord™ will provide a solution for easily tracking the history of the columns, reduce the frustration of paperwork trails, and give customers the reassurance that a well-performing column is installed onto their instruments.



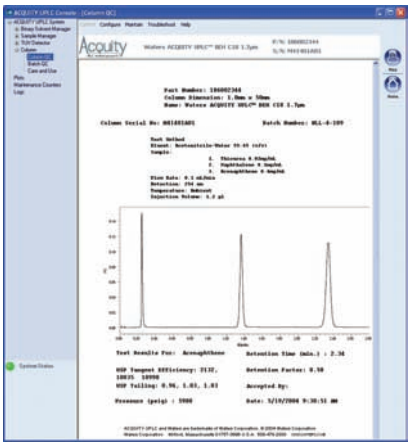
b. Installation

Install the column into the column heater. Plug the eCord™ into the side of the column heater. Once the eCord™ is inserted into the column heater the identification and overall column usage information will be available in Empower™ and MassLynx™ software allowing the user to access column information on their desktop.

c. Manufacturing Information



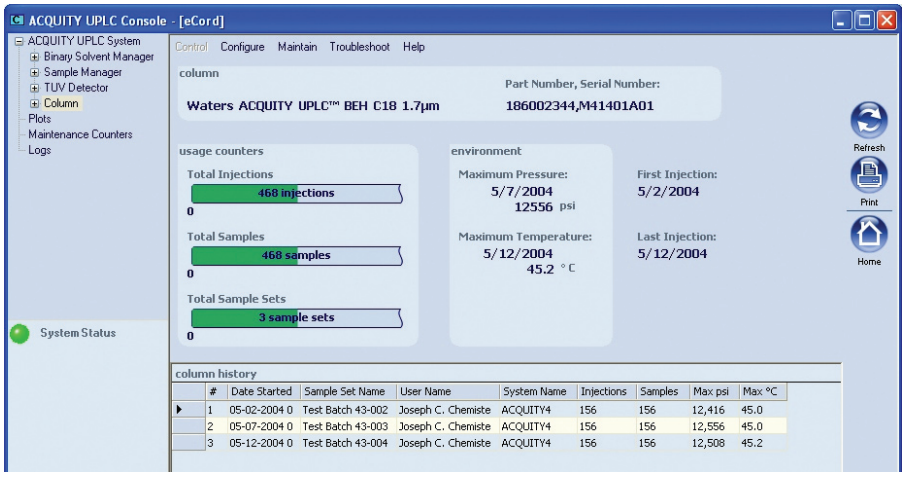
The eCord™ chip provides the user with an overview of the bulk material QC test results.



The eCord™ chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions and analytes used to test the columns. In addition the QC results and acceptance is placed onto the column.

d. Column Use Information

The eCord™ chip provides the customer with column use data. The top of the screen identifies the column including chemistry type, column dimensions and serial number. The overall column usage information includes the total number of samples, total number of injections, total sample sets, date of first injection, date of last injection, maximum pressure and temperature. The information also details the column history by sample set including date started, sample set name, user name, system name, number of injections in the sample set, number of samples in the sample set, maximum pressure and temperature in the sample set and if the column met basic system suitability requirements.



V. ADDITIONAL INFORMATION

a. Tips for Maximizing ACQUITY UPLC™ Column Lifetimes

1. To maximize ACQUITY UPLC™ column lifetime, pay close attention to:

   - Water quality (including water purification system)
   - Solvent quality
   - Mobile phase preparation, storage and age
   - Sample, buffer and mobile phase solubilities
   - Sample quality and preparation

2. When problems arise, often only one improper practice must be changed.

3. Always remember to:

   - Use in-line filter unit.
   - Discourage bacterial growth by minimizing the use of 100% aqueous mobile phases where possible.
   - Change aqueous mobile phase every 24 – 48 hours (if 100% aqueous mobile phase use is required).
   - FSiscard old 100% aqueous mobile phases every 24-48 hours to discourage bacterial growth.
   - Add 5% - 10% organic modifier to mobile phase A and adjust gradient profile.
   - Filter aqueous portions of mobile phase through 0.2 µm filter.
   - Maintain your water purification system so that it is in good working order.
   - Only use ultra pure water (18 megohm-cm) and highest quality solvents possible. HPLC grade water is not UPLC™ grade water.
   - Consider sample preparation (e.g., solid-phase extraction, filtration, etc.).

4. Avoid (where possible):

   - 100% aqueous mobile phases (if possible).
   - HPLC-grade bottled water.
   - "Topping off" your mobile phases.
   - Old aqueous mobile phases. Remember to rinse bottles thoroughly and prepare fresh every 24 to 48 hours.
   - Using phosphate salt buffer in combination with high ACN concentrations (e.g., >70%) due to precipitation.

5. Don't: assume a "bad" column is the culprit when high back-pressure or split peaks are observed:

   - Investigate cause of column failure
     - Backpressure
     - Mobile phase(s), bacteria, precipitation and/or samples
     - Peak splitting
     - Sample quality
     - Injection solvent strength

6. Remember: UPLC™ flow rates are often much lower and, therefore, mobile phases last much longer (only prepare what you need).

7. Mobile phase-related questions to ask:

   - Am I using 100% aqueous mobile phases? Am I able to add a small amount of organic modifier to my Mobile Phase A?
   - Do I filter my aqueous mobile phases through 0.2 µm filters?
   - How old is my mobile phase? Do I label the bottle with preparation date?
   - Do I "top off" or do I prepare fresh mobile phases every 24 – 48 hrs?
   - What is the quality of my water? Has the quality recently changed? How is my water purification system working? When was it last serviced?
   - Am I working with pH 7 phosphate buffer (which is VERY susceptible to bacterial growth)?

8. Sample-related questions to ask:

   - If I inject neat standards prepared in mobile phase do I observe these problems?
   - If I prepare my standards in water and prepare them like samples (e.g., SPE, filtration, etc.) do I still observe these problems?
   - Has the quality of my samples changed over time?

b. Recommended Flow Rates and Backpressures for Reversed-Phase ACQUITY UPLC™ BEH Columns

1.0 mm ID Columns (40 °C)								
UPLC™ Linear Velocity (mm/sec)	3		4		5		6	
Column Dimensions	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)
1.0 x 30 mm	0.10	2600	0.13	3400	0.17	4500	0.20	5300
1.0 x 50 mm	0.10	4300	0.13	5600	0.17	7400	0.20	8700
1.0 x 100 mm	0.10	8600	0.13	11200	0.17	14600	0.20	17200
1.0 x 150 mm	0.1	12800	0.13	16700	0.17	21800	0.20	25600

2.1 mm ID Columns (40 °C)								
UPLC™ Linear Velocity (mm/sec)	3		4		5		6	
Column Dimensions	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)
2.1 x 30 mm	0.45	3000	0.60	4100	0.75	5100	0.90	6100
2.1 x 50 mm	0.45	4800	0.60	6400	0.75	8000	0.90	9500
2.1 x 100 mm	0.45	9100	0.60	12100	0.75	15200	0.90	18200
2.1 x 150 mm	0.45	13400	0.60	17900	0.75	22400	0.90	26900

Note: 1) ACQUITY UPLC™ 1.7 µm particle reversed-phase columns
2) ACN/Aqueous gradient, Pmax at ~30% ACN
3) Approximate maximum total system backpressure given

c. Getting Started with ACQUITY UPLC™ BEH HILIC Columns

Operating Ranges

1. Because ACQUITY UPLC™ BEH HILIC Columns do not posses a bonded phase, the pH operating range is 1 to 8, and they can be operated at temperatures up to 90 °C.
2. As with any LC column, operating at the extremes of pH, pressures and temperatures will result in decreased column lifetime.

Column Equilibration

1. When column is first received, equilibrate in 50% acetonitrile: 50% water with 10 mM final buffer concentration for 50 column volumes.
2. Equilibrate with 20 column volumes of initial mobile phase conditions before making first injection.
3. If gradient conditions are used, equilibrate with 8-10 column volumes between injections.
4. Failure to appropriately equilibrate the column could result in drifting retention times.

Mobile Phase Considerations

1. Always maintain at least 5% polar solvent in the mobile phase or gradient (e.g., 5% aqueous, 5% methanol or 2% aqueous/3% methanol, etc.). This ensures that the ACQUITY UPLC™ BEH particle is always hydrated.
2. Maintain at least 40% organic solvent (e.g., acetonitrile) in your mobile phase or gradient.
3. Avoid phosphate buffers to avoid precipitation in HILIC mobile phases.
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, etc.) 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.

Injection Solvents

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 (SPE). If this is not possible, dilute the water or DMSO with organic solvent.

Miscellaneous Tips

1. ACQUITY UPLC™ BEH HILIC Columns are designed to retain very polar bases ONLY. Acidic, neutral and/or non-polar compounds will not be retained.
2. Optimal flow rates for small (<200 daltons) very polar bases are in the 0.4 to 0.8 mL/min range with the ACQUITY UPLC™ BEH HILIC Columns.
3. As compared to Atlantis® HILIC Silica Columns, the ACQUITY UPLC™ BEH HILIC Columns are approximately 20% less retentive for gradient analysis and 35 to 65% less retentive for isocratic analysis. This is due to the lower residual surface silanol concentration of the BEH particle.
4. In HILIC, it is important to remember that water is the strongest solvent. Therefore, it must be eliminated or minimized in the injection solvent.
5. 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.
6. Alternate polar solvents such as methanol, acetone or isopropanol can also be used in place of water to increase retention.
7. Make sure that your weak needle wash solvent is your starting mobile phase (i.e., high organic), or your peak shapes will suffer.

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The quality management system of Waters' manufacturing facilities in Taunton, Massachusetts and Wexford, Ireland complies with the International Standard ISO 9001:2000 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.