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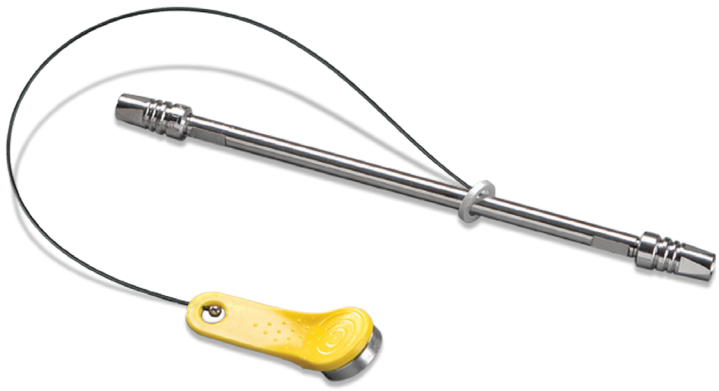
ACQUITY UPLC BEH Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing a Waters® ACQUITY UPLC® BEH Column. The ACQUITY UPLC BEH packing materials were designed specifically for use with the 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.

ACQUITY UPLC Columns were designed and tested specifically for use on the ACQUITY UPLC System. ACQUITY UPLC Columns will exhibit maximum chromatographic performance and benefits ONLY when used on the holistically-designed ACQUITY UPLC System since the ACQUITY UPLC System and column were created and designed to operate together. For these reasons, Waters cannot support the use of ACQUITY UPLC Columns on any system other than an ACQUITY UPLC System.



Acquity UPLC®

II. GETTING STARTED

Each ACQUITY UPLC BEH Column comes with a Certificate of Analysis and 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 Columns 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 (p/n 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.

For information on the correct column outlet tubing, please refer to the relevant detector section in the ACQUITY UPLC System Operator's Guide (p/n 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 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 you are using a BEH HILIC column with low concentrations of mobile-phase additives (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 (mm)	Internal diameter		
	1.0 mm	2.1 mm	3.0 mm
30	—	0.1	0.2
50	0.04	0.2	0.4
100	0.08	0.4	0.8
150	0.12	0.5	1.0

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, flush with 50 column volumes of 50:50 acetonitrile:water with 10 mM final buffer concentration. For ACQUITY UPLC BEH Amide Columns, flush with 50 column volumes of 60:40 acetonitrile:aqueous. Prior to the first injection, equilibrate with 20 column volumes of initial mobile phase conditions (refer to Table 1 for a list of column volumes). See "Getting Started with ACQUITY UPLC BEH HILIC Columns" or "Getting Started with ACQUITY UPLC Amide 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.

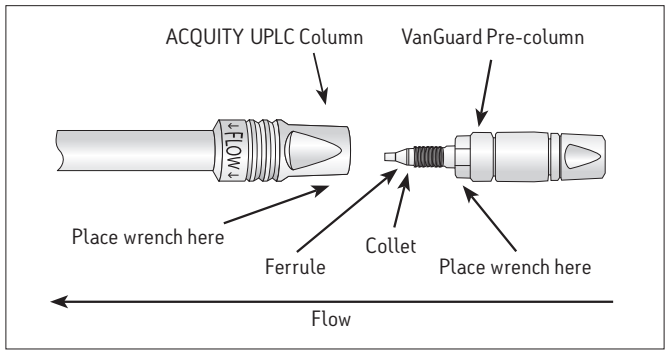
Note: If b) is performed, the isocratic efficiencies measured in your laboratory may be less than those given on the Waters "Performance Test Chromatogram." This is normal. The Waters isocratic column testing systems have been modified in order to achieve extremely low system volumes. This presents a 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.

f. VanGuard Pre-Columns

VanGuard Pre-columns are 2.1 mm I.D. x 5 mm length guard column devices designed specifically for use in the ACQUITY UPLC System. VanGuard Pre-columns are packed with the same UPLC chemistries and frits as our 2.1 mm I.D. UPLC columns. VanGuard Pre-columns are designed to be attached directly to the inlet side of an ACQUITY UPLC Column.

Note: In order to ensure void-free and leak-free connections, the VanGuard Pre-column is shipped with the collet and ferrule NOT permanently attached. Care must be taken when removing the O-ring that holds these two pieces on the pre-column tubing.



Installation Instructions

1. Remove VanGuard Pre-column from box and shipping tube and remove plastic plug.
2. Orient pre-column so that male end is facing up and carefully remove rubber O-ring that holds collet and ferrule in place during shipping (collet and ferrule are not yet permanently attached).
3. Orient ACQUITY UPLC Column perpendicular to work surface so that column inlet is on the bottom (column outlet on top).
4. From below, insert VanGuard Pre-column into ACQUITY UPLC Column inlet and hand-tighten (collet and ferrule are not yet permanently attached).
5. While pushing the VanGuard Pre-column into the column inlet, turn assembled column and pre-column 180° so that pre-column is now on top.
6. Tighten with two 5/16" wrenches placed onto ACQUITY UPLC Column flats and VanGuard Pre-column hex nut (male end) as shown above.
7. Tighten 1/4 turn to set collet and ferrule.
8. Check that ferrule is set by loosening connection and inspecting the ferrule depth. A properly set ferrule depth will resemble other connections in the ACQUITY UPLC System.
9. Reattach pre-column, apply mobile-phase flow, and inspect for leaks.

III. 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 Oasis® Solid-phase Extraction Cartridges/columns or Sep-Pak® Cartridges of the appropriate chemistry to clean up the sample before analysis. For more information, visit **www.waters.com/sampleprep**
2. It is preferable to prepare the sample in the operating mobile phase or a mobile phase that is weaker than the mobile phase for the best peak shape and sensitivity. Acetone should not be used as a sample solvent/diluent unless a Hexane Tetrahydrofuran Compatibility Kit has been installed.
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 8000 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 a high percentage of organic solvents (e.g., 95% acetonitrile). See "Getting Started with ACQUITY UPLC BEH HILIC Columns" or "Getting Started with ACQUITY UPLC Amide 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 RP18 and BEH Amide chemistries; and 1 to 9 for the BEH 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.

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 VI for more information.

d. Pressure

ACQUITY UPLC BEH Columns can tolerate operating pressures up to 18000 psi (1241 bar or 124 MPa). *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. Under HILIC conditions, ACQUITY UPLC BEH Amide Columns can be used at high pH and at high temperatures without issue (see recommended conditions in Getting Started with BEH Amide section).

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 ($NH_4CH_3CO_2$)	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_4HCO_2)	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.
Phosphate 3	12.3	11.3 – 13.3	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) Ammonium Bicarbonate	9.2 10.3 (HCO_3^-) 9.2 (NH_4^+)	8.2 – 10.2 8.2 – 11.3	Volatile Volatile	Yes 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.
Borate	9.2	8.2 – 10.2	Non-volatile	No	Reduce temperature/concentration and use a guard column to maximize lifetime.
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.

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

IV. 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 solvent.

Increasing column temperature 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.

To clean polar contaminants from ACQUITY UPLC BEH Amide Columns, run a 10 minute gradient from 0–100% water. Please note that as aqueous concentration increases, backpressure will rapidly increase as well. Reduce flow rate when operating at greater than 60% aqueous. Repeat if necessary.

Table 3. Recommended pH and temperature limits for ACQUITY UPLC BEH Columns

Name of column	Particle size	Pore diameter	Surface area	pH limits	Temperature limits		Surface	Carbon %
					Low pH	High pH		
BEH C_{18}	1.7 µm	130Å	185 m^2/g	1–12	80 °C	60 °C	3.1 µmol/m^2	17.7
BEH C_8	1.7 µm	130Å	185 m^2/g	1–12	60 °C	60 °C	3.1 µmol/m^2	12.8
BEH Phenyl	1.7 µm	130Å	185 m^2/g	1–12	80 °C	60 °C	3.0 µmol/m^2	14.5
BEH Shield RP_{18}	1.7 µm	130Å	185 m^2/g	2–11	50 °C	45 °C	3.2 µmol/m^2	16.6
BEH HILIC	1.7 µm	130Å	185 m^2/g	1–9	60 °C	45 °C	—	—
BEH Amide	1.7 µm	130Å	185 m^2/g	2–11	90 °C	90 °C	7.5 µmol/m^2	12

Table 4. Reversed-Phase 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 dimethylsulfoxide (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 (CH_3CN)
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.

** Unless a Hexane Tetrahydrofuran Compatibility Kit has been installed, running solvents such as THF or hexane should only be considered when the column cannot be cleaning by running neat, reversed-phase organic solvents such as acetonitrile. Reduce flow rate, lower operating temperatures, and limit system exposure to THF and/or hexane.

b. Storage

For periods longer than four days at room temperature, store reversed-phase 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 reversed-phase ACQUITY UPLC BEH Columns 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. Run a gradient to 100% ACN in order to flush all aqueous solvent from an ACQUITY UPLC BEH Amide Column prior to storage in 100% ACN. 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).

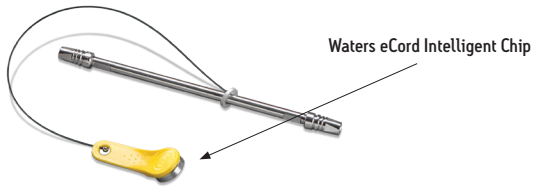
Note: If a column has been run with a mobile phase that contains formate (e.g., ammonium formate, formic acid, etc.) and is then flushed with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and run again with a formate-containing mobile phase.

V. INTRODUCING eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

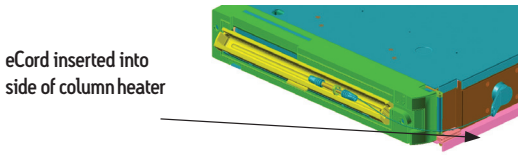
The eCord Intelligent Chip is a new technology that will provide the history of a column's performance throughout its lifetime. The eCord will be permanently attached to the column to assure that the column's performance history is maintained in the event that the column is moved from one instrument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

Figure 1. Waters eCord Intelligent Chip



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.

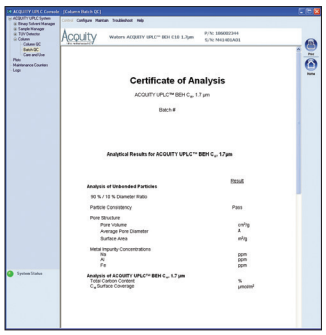
Figure 2. eCord Inserted into Side of Column Heater



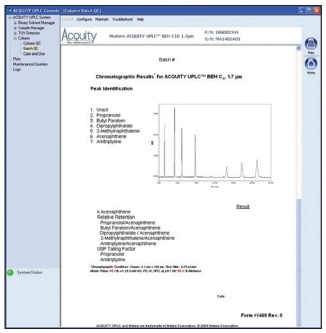
b. Installation

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.

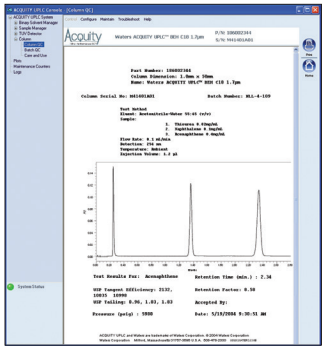
c. Manufacturing Information



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

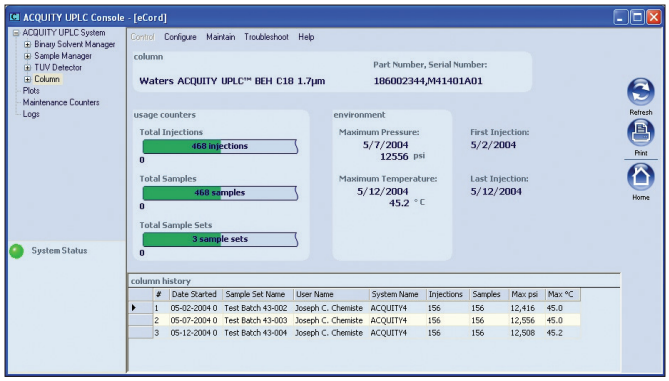


The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition the QC results and acceptance is placed onto the column.



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. Up to 50 sample sets can be stored on the eCord chip.



VI. ADDITIONAL INFORMATION

a. Tips for Maximizing ACQUITY UPLC BEH 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. It is recommended to monitor your systems health by incorporating a quality control (QC) reference material into your work flow. (Reference 720004535en)
3. When problems arise, often only one improper practice must be changed.
4. Always remember to:
   - Use in-line filter unit or, preferably, a VanGuard Pre-column.
   - 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).
   - Discard 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) water and highest quality solvents possible. HPLC grade water is not UPLC grade water.
- Consider sample preparation (e.g., solid-phase extraction, filtration, etc.).

5. Avoid (where possible):
   - 100% aqueous mobile phases (if possible).
   - HPLC-grade bottled water.
   - "Topping off" or adding "new" mobile phase to "old" mobile phase.
   - 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.
6. Don't assume a "bad" column is the culprit when high backpressure or split peaks are observed.
   Investigate cause of column failure by checking:
   - Backpressure
   - Mobile phase(s), bacteria, precipitation and/or samples
   - Peak splitting
   - Sample quality
   - Injection solvent strength.
7. Remember, the diameter of UPLC columns (1.0, 2.1, and 3.0 mm I.D.) are often lower than that of a conventional HPLC column and therefore, mobile phases last much longer. To reduce the chances of mobile-phase contamination or degradation, only prepare what you need for analysis or store excess bulk quantities in a refrigerated environment.
8. 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 hours?
   - 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)?
9. 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 I.D. 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 50 mm	0.1	4300	0.13	5600	0.17	7400	0.2	8700
1.0 x 100 mm	0.1	8600	0.13	11200	0.17	14600	0.2	17200
1.0 x 150 mm	0.1	12800	0.13	16700	0.17	21800	0.2	25600

2.1 mm I.D. 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	0.9	6100
2.1 x 50 mm	0.45	4800	0.60	6400	0.75	8000	0.9	9500
2.1 x 100 mm	0.45	9100	0.60	12100	0.75	15200	0.9	18200
2.1 x 150 mm	0.45	13400	0.60	17900	0.75	22400	0.9	26900

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

3.0 mm I.D. 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)
3.0 x 30 mm	0.9	3400	1.17	4400	1.53	5800	1.8	6800
3.0 x 50 mm	0.9	5100	1.17	6600	1.53	8700	1.8	10200
3.0 x 100 mm	0.9	9300	1.17	12100	1.53	15900	1.8	18700
3.0 x 150 mm	0.9	13600	1.17	17600	1.53	23100	1.8	27100

c. Getting Started With ACQUITY UPLC BEH HILIC Columns

1. Because ACQUITY UPLC BEH HILIC Columns do not possess a bonded phase, the pH operating range is 1 to 9, and they can be operated at temperatures up to 45 °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, flush 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 3% polar solvent in the mobile phase or gradient (e.g., 3% aqueous/3% methanol or 2% aqueous/ 1% 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 salt buffers to avoid precipitation in HILIC mobile phases. Phosphoric acid is okay.
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 95% acetonitrile. The polar solvent (i.e., water, methanol, isopropanol) should be minimized to 25% of the total volume.
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. Acidic, neutral, and/or non-polar compounds will have limited retention.
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 HPLC 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. If using an ACQUITY UPLC System, the weak needle wash should closely match the % organic present in the initial mobile-phase conditions, otherwise, analyte peak shape or retention may suffer.
8. It is recommended to monitor your systems health by incorporating the HILIC Quality Control (QC) Reference Material into your work flow. (Reference 720004535en).

d. Getting Started with ACQUITY UPLC BEH Amide Columns

Operating Ranges

1. ACQUITY UPLC BEH Amide Columns can be used routinely under HILIC conditions between pH 2 to 11, 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, flush in 60% acetonitrile: 40% aqueous (or initial starting conditions) 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 3% polar solvent in the mobile phase or gradient (e.g., 3% aqueous, 3% methanol, or 2% aqueous/1% methanol, etc.).
2. Buffered mobile phases are recommended over additives. For best results maintain at least a 10 mM buffer concentration on column at all times.
3. If additives are to be used, it is recommended to maintain at least a 0.2% additive concentration on column at all times.
4. Maintain at least 40% organic solvent (e.g., acetonitrile) in your mobile phase or gradient.
5. At aqueous concentrations greater than 60%, lower flow rates should be used due to high backpressure. This includes all aqueous wash procedures.
6. Avoid phosphate salt buffers to avoid precipitation in HILIC mobile phases. Phosphoric acid is OK.

Injection Solvents

1. If possible, injection solvents should be as close to the mobile phase composition as possible (if isocratic) or the starting gradient conditions. Acetone should not be used as a sample solvent/diluent unless a Hexane Tetrahydrofuran Compatibility Kit has been installed.
2. A generic injection solvent is 75:25 acetonitrile:methanol. This is a good compromise between analyte solubility and peak shape. When separating saccharides with limited solubility in organic solvents, higher concentrations of aqueous solvent in the sample are acceptable. 50:50 acetonitrile:water can provide satisfactory results.
3. The injection solvent's influence on peak shape should be determined experimentally. In some cases, injections of water (or highly aqueous solutions) may not adversely affect peak shape.

Miscellaneous Tips

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 to increase retention.
3. Ensure that your weak needle wash solvent/purge solvent is your starting mobile phase (i.e., high organic), or your peak shapes will suffer. Typical needle wash conditions: 800 µL strong wash in 20:80 ACN/H_2O, 500 µL weak wash in 75:25 ACN/H_2O.
4. Acetone should not be used as a sample solvent/diluent unless a Hexane Tetrahydrofuran Compatibility Kit has been installed.

Tips for Separating Sugars/Saccharides/Carbohydrates

1. If separating sugars or sugar-containing compounds that do not include reducing sugars (see below) follow generic 'Getting Started with ACQUITY UPLC BEH Amide Columns' recommendations described above.
2. If separating reducing sugars, please review the following information.
3. Reducing sugars can undergo mutarotation which produces the undesired separation of the α and β ring forms (anomers).
4. Collapsing anomers into one peak is accomplished through the use of a combination of elevated temperature and high pH:
   a. Use of 35 °C with high pH (0.2% triethylamine (TEA) or 0.1% ammonium hydroxide (NH_4OH)) and/or
   b. Use of >80 °C with 0.05% TEA high temperature (>80 °C)
5. When separating reducing sugars (e.g., fructose, glucose, maltose, lactose, arabinose, glyceraldehyde, etc.) please pay attention to the following suggestions. Failure to do so will result in the appearance of split peaks (anomer separation) for these analytes:
   a. Operate at a slow flow rate (e.g., 0.10–0.13 mL/min on 2.1 x 50 mm column) to facilitate anomer collapse.
   b. With longer columns, increased flow rates (e.g., up to 0.3 mL/min) can be used. As with all LC separations, optimal flow rates should be determined experimentally.
   c. Add triethylamine (TEA) or ammonium hydroxide (NH_4OH) modifiers to both mobile phase (e.g., A2, B2, etc.) reservoirs.
   d. For UPLC/ELSD separations of mono- and/or disaccharides, typical isocratic UPLC conditions include:
      i. 75% acetonitrile (ACN) with 0.2% TEA, 35 °C, 0.13 mL/min, 2.1 x 50 mm BEH Amide Column;
      ii. 77% acetone with 0.05% TEA, 85 °C, 0.15 mL/min, 2.1 x 50 mm BEH Amide Column;
      iii. 75% ACN with 0.2% TEA, 35 °C, 0.2mL/min, 2.1 x 100 mm BEH Amide Column.
   e. For UPLC-ELSD separations of more complex sugar mixtures (e.g., polysaccharides), typical gradient UPLC conditions include (add TEA modifier to both mobile phases A and B):
      i. Gradient going from 80% to 50% ACN with 0.2% TEA in 10 min, 35 °C, 0.13 mL/min, 2.1 x 100 mm BEH Amide Column or up to 0.3 mL/min flow rate with 2.1 x 150 mm BEH Amide column;
      ii. 80%–55% acetone with 0.05% TEA in 10 min, 85 °C, 0.15 mL/min, 2.1 x 100 mm BEH Amide Column.

f. For UPLC-MS separations of mono- and disaccharides, typical isocratic UPLC conditions include:

   i. 75% ACN with 0.1% NH_4OH, 35 °C, 0.13 mL/min, 2.1 x 50 mm BEH Amide Column.

g. For UPLC-MS separations of more complex sugar mixtures (e.g., polysaccharides), typical gradient UPLC conditions include (add NH_4OH modifier to both mobile phases A and B):

   i. Gradient going from 75% to 45% ACN with 0.1% NH_4OH in 10 min, 35 °C, 0.2 mL/min, 2.1 x 100 mm BEH Amide Column.

6. More complex sample mixtures may require the use of gradient conditions and/or longer UPLC column lengths.
7. If acetone is used in one or more mobile phases, do not use acetone as a sample diluent or needle wash solvent. Refer to injection solvents section for sample diluent recommendations and miscellaneous tip (#3) for needle wash solvent/purge solvent recommendations.
8. Typical sample preparation suggestions for samples that contain sugars/saccharides/carbohydrates:

   a. Liquid Samples

      i. Dilute with 50:50 ACN/H_2O.

      ii. Filter using 0.45 µm or 0.22 µm syringe filter (if necessary).

   b. Solid Samples

      i. Weigh out sample (~3 g) into 50 mL centrifuge tube.

      ii. Add 25 mL of 50:50 ACN/H_2O and homogenize (mechanically).

      iii. Centrifuge at 3200 rpm for 30 minutes.

      iv. Collect supernatant and filter using 0.45 µm or 0.22 µm syringe filter (if necessary).

   c. Depending on sample and/or analyte concentrations, additional sample dilutions may be necessary.

   d. More complex samples and/or lower analyte concentrations may require additional sample preparation steps and/or procedures such as solid phase extraction (SPE).

   e. Consider VanGuard BEH Amide Pre-columns for UPLC column protection.

   f. It is recommended to monitor your systems health by incorporating the HILIC Quality Control (QC) Reference Material into your work flow. (Reference 720004535en).

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