

ACQUITY™ UPLC™ and ACQUITY Premier Columns

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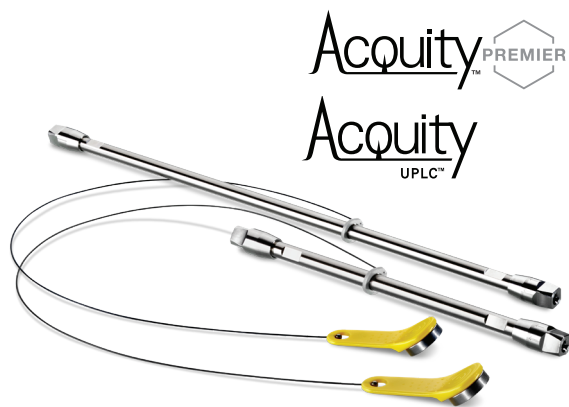
IX. CAUTIONARY NOTE

I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY UPLC and/or ACQUITY Premier Column. ACQUITY BEH™ packing materials are designed to provide excellent peak shape, high efficiency, and excellent stability for acidic and basic mobile phases. ACQUITY HSS™ Columns feature Waters High Strength Silica (HSS) Technology which provides excellent peak shape, high efficiency, and excellent reversed-phase retention for polar analytes. ACQUITY CSH™ Columns feature Waters Charged Surface Hybrid (CSH) Technology which provides excellent peak shape, high efficiency, and loading capacity for basic compounds when using low ionic strength, acidic mobile phases like formic acid. The same BEH particle technology is used in the XBridge™ BEH Column family and the same CSH and the HSS particle technologies are used in the XSelect™ CSH and XSelect HSS Column families, enabling seamless transferability between HPLC, UHPLC and UPLC System Platforms.

All ACQUITY UPLC 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 packing 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 Test Chromatogram is provided for each column along with the Certificate of Analysis. These documents are available on [waters.com/coa](https://www.waters.com/coa), or by scanning the QR code on the column label.

ACQUITY Premier Columns utilize MaxPeak™ High Performance Surfaces (HPS) Technology, an innovative technology designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses. The ACQUITY Premier Column is offered with or without a VanGuard™ Fully Integrated Technology [FIT] Cartridge. The VanGuard FIT Cartridge is designed to prevent the unwanted introduction of sample matrix or particulates onto the column without degrading the separation. It can be easily replaced to restore separation performance and extend the analytical column's lifetime.



II. GETTING STARTED

With each ACQUITY UPLC Column or ACQUITY Premier Column, a Certificate of Analysis and Performance Test Chromatogram is available on waters.com/coa or by scanning the QR code on the column label. 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, and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains the batch number, column serial number, USP tangent efficiency, USP tailing factor, retention factor, and chromatographic conditions. This data should be stored for future reference.

a. LC Considerations

We recommend using the correct system for your column. Our 1mm I.D. columns will work best on ACQUITY Premier UPLC Systems at flow rates between 50-200 $\mu\text{L}/\text{min}$, and ACQUITY UPLC M-Class Systems at flow rates between 50-100 $\mu\text{L}/\text{min}$.

If using an ACQUITY Premier UPLC System or similar analytical scale LC, we recommend the following modifications to reduce peak dispersion when using a 1 mm ID column. If possible, position the column outlet as close to the detector inlet as possible. The standard outlet tubing i.d. (0.004 in.) needs to be reduced to 0.0025 in. (e.g., P/N: 430001749). For best results with mass spectrometry applications, bypass any valving, such as the divert valve, in order to reduce dispersion, and the ESI stainless steel capillary (125 μm i.d.) should be replaced with a narrow-bore variant (50 μm i.d., P/N: 186007529, Lit code: 715004382).

b. eCord Installation

(May not be available for all column configurations.)

Depending on the column configuration, your ACQUITY UPLC or ACQUITY Premier Column may be equipped with an eCord Intelligent Chip Button. The eCord Button is designed for use on the ACQUITY UPLC and ACQUITY Arc™ Systems and it should be attached to the side of the instrument's column heater module. The eCord button is magnetized and does not require a specific orientation.

For more information on the eCord Intelligent Chip functionality, see [Section VI](#) in this care and use manual.

c. Connecting the Column to the LC System (With or Without a VanGuard FIT Cartridge)

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

i. General Recommendations for Tubing Connections

1. An arrow on the column identification label indicates the correct direction of solvent flow.
2. Correct connection of 1/16-inch outer diameter tubing leading to and/or from the column is essential for high quality chromatographic results.
3. To obtain a void-free connection, the tubing must touch the bottom of the column endfitting. Extra-column peak broadening due to voids can destroy an otherwise successful separation.
2. In a proper tubing/column connection (Figure 1a), the tubing touches the bottom of the column endfitting, with no void between them.

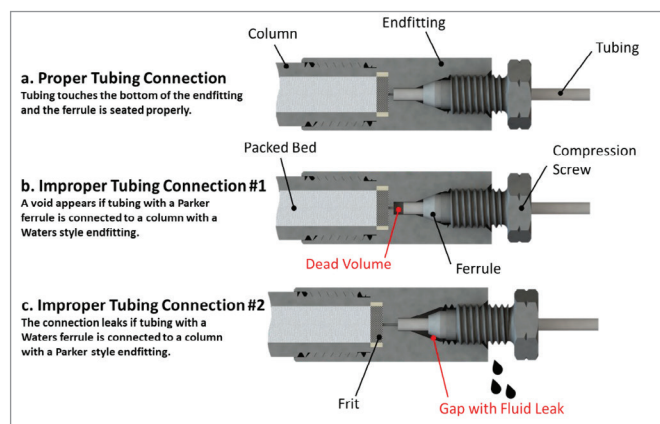


Figure 1. Proper tubing connections require the tubing to touch the bottom of the endfitting and the ferrule to be seated properly.

d. Column Startup Guides

Note: Prior to handling ACQUITY UPLC or ACQUITY Premier Columns and any chemical, consult with your safety department and/or local regulations on the use of proper protective equipment.

All ACQUITY UPLC Columns are shipped in 100% acetonitrile. The flow rates given in the procedure below are for 2.1 mm ID columns.

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 five 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.
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.

e. Column Equilibration

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 (see Table 1 for a list of column volumes). The column may be considered thermally equilibrated once a constant backpressure is achieved.

Column Length (mm)	Internal Diameter		
	1.0 mm	2.1 mm	3.0 mm
10	–	0.03	–
20	–	0.07	0.14
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

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

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.)

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.

For columns packed with BEH HILIC material, flush with 50-column volumes of 50:50 acetonitrile:water with 10 mM final buffer concentration. For columns packed with BEH Amide material, 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 (see Table 1 for a list of column volumes). See “Special Considerations for HILIC and Amide Columns” for additional information.

f. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. This test may consist of:
 - i. An analyte test mixture that is commonly used in your laboratory, and/or
 - ii. The analyte mixture as found on the “Performance Test Chromatogram” that accompanied your column.

Note: If (ii) 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 to achieve extremely low system volumes. This presents a more challenging test of how well the column was packed and 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 observed due to differences in the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

g. Column QR Code

The quick reference (QR) code that is located on the column label provides column-specific information (e.g., the part and serial numbers that are unique identifiers for the column), and its encoding follows a widely adopted industry-standard.

1. Scan QR code using any device that is capable of scanning QR codes (e.g., for smart phones and tablets, use the built-in camera app).
2. Be directed to the column's information hub on waters.com.
3. Access technical and scientific information for the column (e.g., certificate of analysis, application notes).

h. VanGuard/VanGuard FIT Column Protection

VanGuard Pre-Columns are designed specifically for ACQUITY UPLC Columns and for use on ACQUITY UPLC Systems. These 2.1 mm I.D. x 5 mm length guard column devices are packed with the same UPLC chemistries and frits as the Waters 2.1 mm I.D. UPLC columns. The VanGuard Pre-Columns attach directly to the inlet side of an ACQUITY UPLC Column. For more information, visit the VanGuard Column Protection page on waters.com.

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.

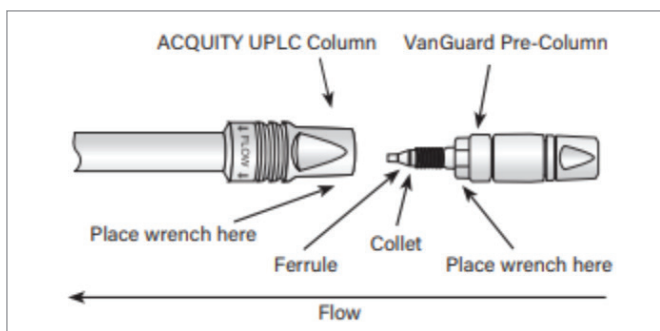


Figure 2. VanGuard Pre-Column installation diagram.

- i. Remove VanGuard Pre-column from box and shipping tube and remove plastic plug.
- ii. 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).
- iii. Orient ACQUITY UPLC Column perpendicular to work surface so that column inlet is on the bottom (column outlet on top).

- iv. From below, insert VanGuard Pre-column into ACQUITY UPLC Column inlet and hand-tighten (collet and ferrule are not yet permanently attached).
- v. 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.
- vi. Tighten with two $\frac{5}{16}$ " wrenches placed onto ACQUITY UPLC Column flats and VanGuard Pre-column hex nut (male end) as shown above.
- vii. Tighten $\frac{1}{4}$ turn to set collet and ferrule.
- viii. 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.
- ix. Reattach pre-column, apply mobile-phase flow, and inspect for leaks.

2. The VanGuard FIT Cartridge is designed specifically for ACQUITY Premier Columns. For the ACQUITY Premier Columns that have a VanGuard FIT Cartridge, the VanGuard FIT Cartridge may be replaced using two $\frac{3}{8}$ -inch wrenches. Simply apply the wrenches to the flats on the guard and column end nut and turn in a counterclockwise direction (see Figure 3) to remove the guard cartridge.

A new VanGuard FIT Cartridge can now be used to replace the discarded one. Hand-tighten the new cartridge in a clockwise direction, then tighten using two $\frac{3}{8}$ -inch wrenches. Proper sealing should not require more than a $\frac{1}{4}$ turn past the hand-tightened position.

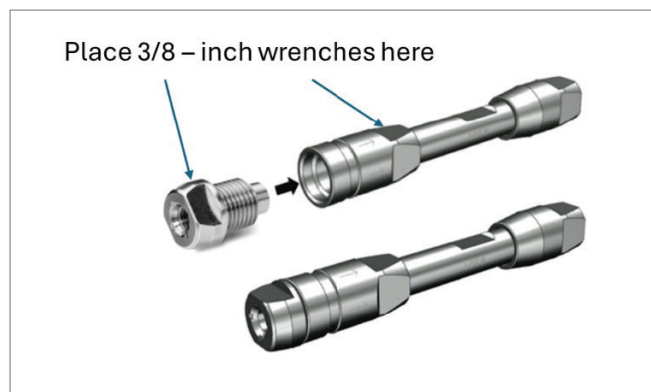


Figure 3. Recommended $\frac{3}{8}$ " wrench placement to remove the VanGuard FIT Cartridge from the ACQUITY Premier VanGuard FIT Column.

III. COLUMN USE

To ensure the continued high performance of ACQUITY and ACQUITY Premier Columns, follow these guidelines:

a. Guard Columns

Use a Waters guard column of matching chemistry and particle size between the injector and main column. It is important to use a high-performance matching guard column to protect the main column while not compromising or changing the analytical resolution.

Guard columns need to be replaced at regular intervals as determined by sample contamination. When system backpressure steadily 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. 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 clean up the sample before analysis. For more information, visit the Sample Preparation Page on waters.com.
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 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 a high percentage of organic solvents (e.g., 95% acetonitrile). See "Special Considerations for HILIC and Amide" section for additional information.

c. pH Range

The recommended operating pH limits for ACQUITY BEH and ACQUITY CSH Columns are listed in Table 2. A listing of commonly used buffers and additives for using ACQUITY BEH and ACQUITY CSH columns up to pH 12 is given in Table 3. The recommended operating pH limits for ACQUITY HSS Columns are listed in table 4. A listing of commonly used buffers and additives for using ACQUITY HSS columns up to pH 8 is given in Table 5.

The column lifetime will vary depending upon the operating temperature, the type, and concentration of buffer used.

d. 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 (Tips for Maximizing ACQUITY Column Lifetime) for more information.

e. Pressure

ACQUITY and ACQUITY Premier Columns can tolerate operating pressures up to 18,000 psi (1241 bar or 124 MPa).

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

f. Temperature

Temperatures between 20–90 °C are recommended for operating ACQUITY BEH and ACQUITY CSH Columns and temperatures between 20 to 60 °C are recommended for operating ACQUITY HSS columns to enhance selectivity, lower solvent viscosity, and increase mass transfer rates. When operating near the pH limits, lower operating temperatures are recommended for longer column lifetime. Reference Table 2 and Table 4 for temperature guidance when operating near the extremes of pH.

Table 2. Recommended pH and Temperature Limits for ACQUITY BEH and ACQUITY CSH Columns

Bonded Phase	Pore Diameter	Surface Area	pH Limit	Temp. Guidance* for pH Extremes		Ligand Density	Carbon %
				Low pH	High pH		
ACQUITY BEH C ₁₈	130 Å	185 m ² /g	1–12	80° C	60° C	3.1 µmol/m ²	18
ACQUITY BEH C ₈	130 Å	185 m ² /g	1–12	60° C	60° C	3.2 µmol/m ²	13
ACQUITY BEH Shield RP18	130 Å	185 m ² /g	2–11	50° C	45° C	3.3 µmol/m ²	17
ACQUITY BEH Phenyl	130 Å	185 m ² /g	1–12	80° C	60° C	3.0 µmol/m ²	15
ACQUITY BEH Biphenyl	130 Å	185 m ² /g	1.5–10**	60° C	45° C	3.3 µmol/m ²	16
ACQUITY BEH HILIC	130 Å	185 m ² /g	1–9	45° C	45° C	N/A	Unbonded
ACQUITY BEH Amide	130 Å	185 m ² /g	2–11	90° C	90° C	7.5 µmol/m ²	12
ACQUITY CSH C ₁₈	130 Å	185 m ² /g	1–11	80° C	45° C	2.3 µmol/m ²	15
ACQUITY CSH Phenyl-Hexyl	130 Å	185 m ² /g	1–11	80° C	45° C	2.3 µmol/m ²	14
ACQUITY CSH Fluoro-Phenyl	130 Å	185 m ² /g	1–8	60° C	45° C	2.3 µmol/m ²	10

*Note: Higher operating temperatures are acceptable when working in the mid-range of the recommended pH limit. When operating near the pH limits, lower operating temperatures are recommended for longer column lifetime.

**Note: To minimize UV-detected column bleed from BEH Biphenyl columns, the pH should be less than 7. See section VIII for additional information.

Table 3. Buffer Recommendation for Using ACQUITY BEH and ACQUITY CSH Columns

Additive/Buffer	pK _a	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 ₄ CH ₃ CO ₂)	4.76	3.76–5.76	Volatile	Yes	Used in the 1–10 mM range. Note: Sodium or potassium salts are not volatile.
Formate (NH ₄ HCO ₂)	3.75	2.75–4.75	Volatile	Yes	Used in the 1–10 mM 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	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 ₄ OH)	9.2	8.2–10.2	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 ₄ HCO ₃), not ammonium carbonate ((NH ₄) ₂ CO ₃).
Ammonium bicarbonate	10.3 (HCO ₃ ⁻) 9.2 (NH ₄ ⁺)	8.2–11.3	Volatile	Yes	
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.

Table 4. Recommended pH and Temperature Limits for ACQUITY HSS Columns

Bonded Phase	Pore Diameter	Surface Area	pH Limit	Temp. Guidance* for pH Extremes		Ligand Density	Carbon %
				Low pH	High pH		
ACQUITY HSS C ₁₈	100 Å	230 m ² /g	1–8	60° C	45° C	3.2 µmol/m ²	15
ACQUITY HSS T3	100 Å	230 m ² /g	2–8	60° C	45° C	1.6 µmol/m ²	11
ACQUITY HSS C ₁₈ SB	100 Å	230 m ² /g	2–8	60° C	45° C	1.6 µmol/m ²	8
ACQUITY HSS PFP	100 Å	230 m ² /g	2–8	60° C	45° C	3.2 µmol/m ²	7
ACQUITY HSS Cyano	100 Å	230 m ² /g	2–8	60° C	45° C	2.0 µmol/m ²	5

*Note: Higher operating temperatures are acceptable when working in the mid-range of the recommended pH limit. When operating near the pH limits, lower operating temperatures are recommended for longer column lifetime.

Table 5. Buffer Recommendation for Using ACQUITY HSS Columns up to pH 8

Additive/Buffer	pK _a	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 ₄ CH ₃ CO ₂)	4.76	3.76–5.76	Volatile	Yes	Used in the 1–10 mM range. Note: Sodium or potassium salts are not volatile.
Formate (NH ₄ HCO ₂)	3.75	2.75–4.75	Volatile	Yes	Used in the 1–10 mM 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	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime.

IV. COLUMN CLEANING, REGENERATION 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 6). 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 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 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.

i. For Normal-Phase Conditions:

The ACQUITY HSS Cyano Column can be used for both reversed-phase separations as well as normal phase separations. The column is originally shipped in acetonitrile and is ready to use for reversed-phase conditions. If you intend to use the column for normal phase applications you will need to condition the column with the following procedure:

1. Flush the column with a minimum of 20 column volumes of 100% methanol using a low flow rate to avoid overpressuring the LC system. Refer to table 1 for the minimum solvent volume.
2. Flush the column with a minimum of 20 column volumes of 100% isopropanol using a low flow rate to avoid overpressuring the LC system. Refer to table 1 for the minimum solvent volume.
3. Flush the column with a minimum of 20 column volumes of 100% dichloromethane using a low flow rate to avoid overpressuring the LC system. Refer to table 1 for the minimum solvent volume.
4. Flush the column with the intended mobile-phase conditions until a stable baseline is achieved.

b. Storage

For periods longer than four days at room temperature, store ACQUITY Columns in 100% acetonitrile. Immediately after use with elevated temperatures and/or at pH extremes, store in 100% acetonitrile for the best column lifetime. Do not store columns in highly aqueous (<20% organic) mobile phases, as this may promote bacterial growth. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of ultra-pure (18 megohm-cm) 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 or system when 100% acetonitrile is introduced. Completely seal column to avoid evaporation and drying out of the packed 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.

For Normal-Phase Use:

For rapid equilibration upon start-up, it is recommended that you store the ACQUITY HSS Cyano Column in the mobile phase that is commonly used for your normal-phase separation. Completely seal the column to avoid evaporation and drying out of the bed.

Table 6. 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 ₃ CN)
3. Tetrahydrofuran (THF)	3. Dichloromethane	
4. Methanol	4. Hexane	Option 3: Flush column with 7 M guanidine hydrochloride or 7 M urea
5. Water	5. Isopropanol (or an appropriate isopropanol/water mixture**)	
6. Mobile phase	6. Mobile phase	

* Prior to using THF or hexane, ensure your system is compatible with these solvents. THF or hexane should only be considered when the column cannot be cleaned 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.

**Use low organic solvent content to avoid precipitating buffers.

V. TIPS FOR MAXIMIZING ACQUITY COLUMN LIFETIMES

1. To maximize ACQUITY 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 system's health by incorporating a quality control (QC) reference material into your workflow. (Reference 720004535en)

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

4. Always remember to:

- Use an in-line filter unit or, preferably, a VanGuard Pre-Column or VanGuard FIT 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 (18 megohm-cm) water and the 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, remembering 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
- Sample quality
- Injection solvent strength.

7. Remember: the diameter of ACQUITY 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 the 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?

VI. ADDITIONAL INFORMATION

a. eCord Electronic Column Tags

"Smart columns" are those enabled with electronic devices (tags) permanently affixed to enable identification and/or tracking of column history and use information. These tags contain unique product identifying information, such as part numbers, serial numbers, and product descriptions. Data residing on these devices is automatically imported by complementary Waters' systems, whether LC instrumentation or the Empower™ Chromatographic Data System. This type of product data is used to aid in tracking and decision-making and troubleshooting, especially valuable for GLP/GMP compliant laboratories. The eCord is a standard feature on ACQUITY UPLC™ Columns for use with Waters ACQUITY and Arc UHPLC Systems. The eCord chip is permanently attached to the column to assure that the column performance history is maintained if the column is moved from one instrument to another. It is designed to be robust and durable. When in operation, the eCord enabled column is connected via a cable tether to the system, allowing the electronic tag to be physically located outside the column manager. This placement reduces exposure to variable operating temperatures and solvents.

- i. eCord Intelligent Chip Technology (may not be available for all column configurations):

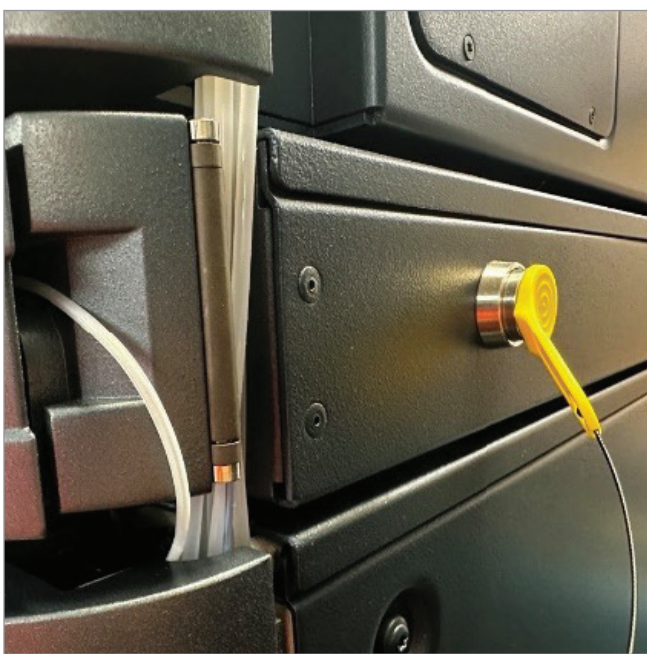


Figure 4. eCord Intelligent Chip attached to the side of column heater.

The eCord chip provides the customer with a history of column use data and performance throughout its lifetime. Once the column is installed into the column heater, attach the eCord to the side of the column heater (Figure 4) and the Empower or MassLynx™ Software will automatically download key parameters into a column history file stored on the chip, allowing access to the column information. The information will include the column 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, username, 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.

b. MaxPeak Premier Column Hardware

Most LC column hardware is stainless steel, which has excellent pressure tolerances and a highly reproducible machining process for a wide range of dimensions/formats. Non-Specific Adsorption (NSA) can be a problem with stainless steel column hardware, as certain analytes (such as acidic, phosphorylated or carboxylated compounds) can bind to the metal oxide layer, resulting in poor peak shape, poor analyte recovery and poor reproducibility. This problem is often noticed as a loss in signal intensity or inconsistent results injection to injection and/or column to column.

MaxPeak Premier Columns feature the MaxPeak High Performance Surfaces (HPS) Technology, which is a hybrid organic/inorganic silica surface comprised of a resilient, highly crosslinked layer containing ethylene bridged siloxane groups that is covalently bonded to the metal surface. MaxPeak Premier Columns are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

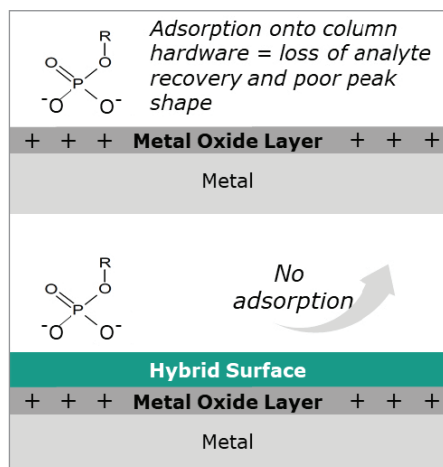


Figure 5: MaxPeak High Performance Surfaces Technology prevents adsorption of analytes to metal surfaces.

VII. SPECIAL CONSIDERATIONS FOR BEH HILIC AND BEH AMIDE COLUMNS

a. Recommendations for BEH HILIC Column Use

Because BEH HILIC columns do not possess a bonded phase, the pH operating range is 1–9, and they can operate at temperatures up to 45 °C when working near the upper and lower pH limits. As with any LC column, operating at the extremes of pH, pressures, and temperatures will result in decreased column lifetime.

i. Column Equilibration

1. When the 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.

ii. 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 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 the best peak shape, always maintain a buffer concentration of 10 mM in your mobile phase/gradient.

iii. 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.

iv. Miscellaneous Tips

1. 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.
3. As compared to Atlantis™ HILIC Silica HPLC Columns, the BEH HILIC columns are approximately 20% less retentive for gradient analysis and 35–65% 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 system's health by incorporating the HILIC Quality Control (QC) Reference Material into your workflow. (Reference 720004535EN).

b. Recommendations for BEH Amide Column Use

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. As with any LC column, operating at the extremes of pH, pressures and temperatures will result in decreased column lifetime.

i. 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.

ii. 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, always maintain at least a 10 mM buffer concentration on column.
3. If additives must be used instead of a buffer, it is recommended to always maintain at least a 0.2% additive concentration on column.
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 okay.

iii. 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.

iv. 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 μ L strong wash in 20:80 ACN H₂O, 500 μ L weak wash in 75:25 ACN/H₂O.
4. Acetone should not be used as a sample solvent diluent unless a Hexane Tetrahydrofuran Compatibility Kit has been installed.

v. Tips for Separating Sugars/Saccharides/Carbohydrates

If separating sugars or sugar-containing compounds that do not include reducing sugars, follow the generic recommendations described above. If separating reducing sugars, please review the following information:

1. Reducing sugars can undergo mutarotation which produces the undesired separation of the α and β ring forms (anomers).
2. Collapsing anomers into one peak is accomplished with 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 (NH₄OH)) and/or

- b. Use of high temperature >80 °C with 0.05% TEA.

3. 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 (NH₄OH) modifiers to aqueous and organic mobile phase reservoirs at equal concentrations.
 - d. For UPLC/ELSD separations of mono- and/or disaccharides typical isocratic conditions include:
 - i. 75% acetonitrile (ACN) with 0.2% TEA, 35 °C
 - ii. 77% acetone with 0.05% TEA, 85 °C.
 - e. For UPLC-ESLD separations of more complex sugar mixtures (*e.g.*, polysaccharides) using XBridge Amide Columns 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, 35 °C,
 - ii. 80%–55% acetone with 0.05% TEA, 85 °C.
 - f. For UPLC-MS separations of mono- and disaccharides using BEH Amide columns typical isocratic UPLC conditions include:
 - i. 75% ACN with 0.1% NH₄OH, 35 °C.
 - g. For UPLC-MS separations of more complex sugar mixtures (*tt.*, polysaccharides), using BEH Amide Columns typical gradient UPLC conditions include (add NH₄OH modifier to aqueous and organic mobile phase reservoirs):
 - i. Gradient going from 75% to 45% ACN with 0.1% NH₄OH, 35 °C.
4. More complex sample mixtures may require the use of gradient conditions and/or longer column lengths.
5. 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.

6. Typical sample preparation suggestions for samples that contain sugars/saccharides/carbohydrates:

a. Liquid Samples

- i. Dilute with 50:50 ACN/H₂O.
- 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/H₂O and homogenize (mechanically).
- iii. Centrifuge at 3200 rpm for 30 min.
- 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 column protection.

f. It is recommended to monitor your system's health by incorporating the HILIC Quality Control (QC) Reference Material into your workflow. (Reference 720004535en).

VIII. SPECIAL CONSIDERATIONS FOR MINIMIZING UV-DETECTED BIPHENYL COLUMN BLEED

Due to the high molar absorptivity of the biphenyl group at 250 nm, the elution of bonded phase hydrolysis products ("column bleed") from BEH Biphenyl columns may cause a rising baseline when using gradient elution with high pH mobile phases and UV detection at wavelengths near 250 nm. The severity of the baseline rise depends on the gradient span, the mobile phase pH, the column temperature and the use history of the column. To minimize this issue, it is recommended that the mobile phase pH be no greater than 7. Higher pH mobile phases (up to 10) may be used with mass spectrometry detection. As discussed in section IVb, BEH Biphenyl columns should be stored in 100% acetonitrile to prevent hydrolysis, ensuring the lowest column bleed.

IX. CAUTIONARY NOTE

Depending on the user's application, these products may be classified as hazardous following their use, and as such are intended to be used by professional laboratory personnel trained in the competent handling of such materials.

Responsibility for the safe use and disposal of products rests entirely with the purchaser and user. The Safety Data Sheet (SDS) for this product is available at waters.com/sds.

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