ACQUITY UPLC and ACQUITY PREMIER HSS Columns

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VI. CAUTIONARY NOTE

I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY™ UPLC™ and/or ACQUITY PREMIER HSS Column. The ACQUITY HSS packing materials were designed specifically for use with the Waters ACQUITY UPLC System and are manufactured in a cGMP, ISO 9001:2000 certified plant using ultra pure reagents. Each batch of ACQUITY HSS 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.

The ACQUITY PREMIER HSS Column is offered with or without a VanGuard™ Fully-Integrated Technology [FIT] Cartridge. To address the desire to extend the operating lifetimes of analytical columns, the VanGuard FIT Cartridge is designed to prevent the non-desired 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.

In addition, the ACQUITY PREMIER HSS Columns utilize MaxPeak™ High Performance Surfaces, an innovative technology designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

II. GETTING STARTED

A Certificate of Analysis and Performance Test Chromatogram are provided with each ACQUITY HSS Column, either in the column box or on the column's eCord Intelligent Chip. 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. These data should be stored for future reference. For those not able to access the information on the eCord Intelligent Chip, the Certificate of Analysis and Performance Test Chromatogram are available on request at www.waters.com/coa.

a. eCord Installation

(May not be available for all column configurations)

The eCord Intelligent Chip button is designed for use on ACQUITY UPLC and ACQUITY Arc™ Systems, and should be attached to the side of the instruments' column heater module. The eCord button is magnetized and does not require specific orientation. For more information on eCord Intelligent Chip functionality, see Section V.

b. Column Installation

(with or without a VanGuard FIT Cartridge)

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

ACQUITY HSS columns are shipped in 100% acetonitrile. The flow rates given in the procedure below are for 2.1 mm I.D. 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.

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

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).

d. 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. The analyte mixture as found on the "Performance Test Chromatogram" that 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 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.

e. Column QR Code

The quick reference (QR) code that is located on the column label provides column-specific information (i.e., 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 (i.e., 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 (i.e., certificate of analysis, application notes).

f. VanGuard Pre-columns for ACQUITY UPLC Columns

VanGuard Pre-columns are designed to work with ACQUITY UPLC Columns. They 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.

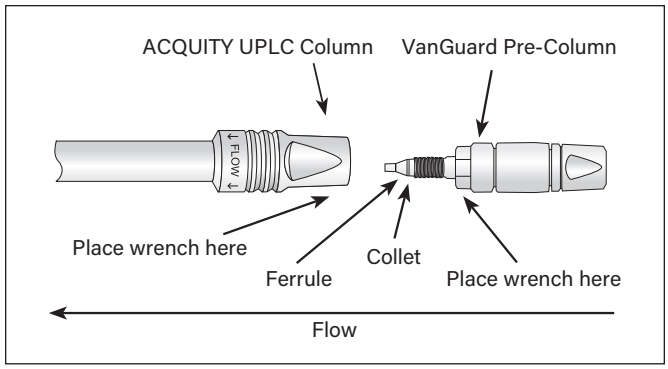


Figure 1. VanGuard Pre-Column installation diagram.

g. Installation Instructions

1. Remove the VanGuard Pre-column from its box and shipping tube and remove plastic plug.
2. Orient the 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 the ACQUITY column perpendicular to the work surface so that column inlet is on the bottom (column outlet on top).
4. From below, insert the VanGuard Pre-column into the 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 the ACQUITY UPLC Column flats and the VanGuard Pre-column hex nut (male end) as shown above.
7. Tighten 1/4 turn to set collet and ferrule.
8. Check that the ferrule is set by loosening the 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.

h. Replacing the VanGuard FIT Cartridge

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 3/8" wrenches. Simply apply the wrenches to the flats on the guard and column end nut and turn in a counter clockwise direction (Figure 1). This will allow the VanGuard FIT Cartridge to be removed and appropriately discarded when following good laboratory practices.

A new VanGuard FIT Cartridge can now be used to replace the discarded one. Extra cartridges can be obtained separately, as needed. Hand-tighten the new cartridge in a clockwise direction, then tighten using two 3/8" wrenches. Proper sealing should not require more than a 1/4 turn past the hand-tightened position.

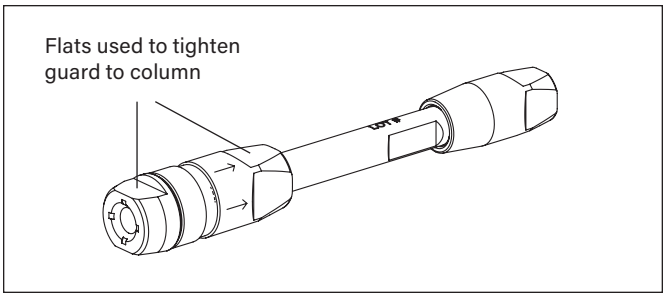


Figure 2. Recommended 3/8" wrench placement to remove the VanGuard FIT Cartridge from the ACQUITY PREMIER VanGuard FIT Column.

II. COLUMN USE

To ensure the continued high performance of ACQUITY HSS 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 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.
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.

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.

b. pH Range

The recommended operating pH range for ACQUITY HSS columns is 2–8 for the HSS T3, HSS C_{18} SB, HSS Cyano and HSS PFP chemistries, and 1–8 for the HSS C_{18} 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.

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

ACQUITY HSS 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.

Table 2. Buffer Recommendations for Using ACQUITY HSS 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 ($NH_4CH_3CO_2$)	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.

e. Temperature

Temperatures between 20–45 °C are recommended for operating ACQUITY HSS 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., >40 °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 (Table 4). 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.

Table 3. Recommended pH and Temperature Limits for ACQUITY HSS Columns

Column	Particle Size	Pore Diameter	Surface Area	pH Limits	Temperature Limits		Surface	Carbon %
					Low pH	High pH		
HSS C_{18}	1.8 µm	100 Å	230 m^2/g	1–8	45 °C	45 °C	230 µmol/m^2	15
HSS T3	1.8 µm	100 Å	230 m^2/g	2–8	45 °C	45 °C	230 µmol/m^2	11
HSS C_{18} SB	1.8 µm	100 Å	230 m^2/g	2–8	45 °C	45 °C	230 µmol/m^2	8
HSS PFP	1.8 µm	100 Å	230 m^2/g	2–8	45 °C	45 °C	230 µmol/m^2	7
HSS CN	1.8 µm	100 Å	230 m^2/g	2–8	45 °C	45 °C	230 µmol/m^2	5

Table 4. Reversed-Phase Column Cleaning Sequence

Polar Samples	Non-polar Samples*	Proteinaceous Samples
1. Water	1. Isoproanol (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 7 M guanidine hydrochloride or 7 M urea
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 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.*

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

b. Normal-phase Conditions

The 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 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.

c. Storage

For periods longer than four days at room temperature, store reversed-phase ACQUITY HSS 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.

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.

d. Post Normal-phase Use

For rapid equilibration upon startup, it is recommended that you store the 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.

IV. eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip provides 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.

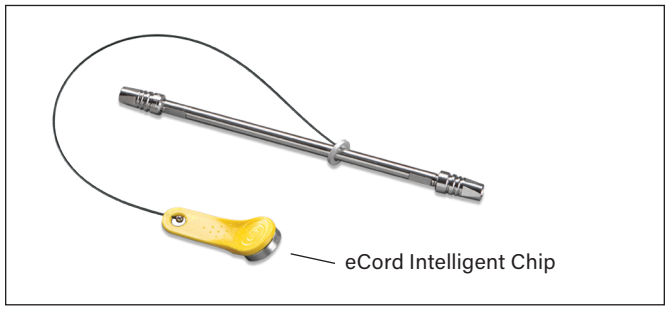


Figure 3. eCord Intelligent Chip.

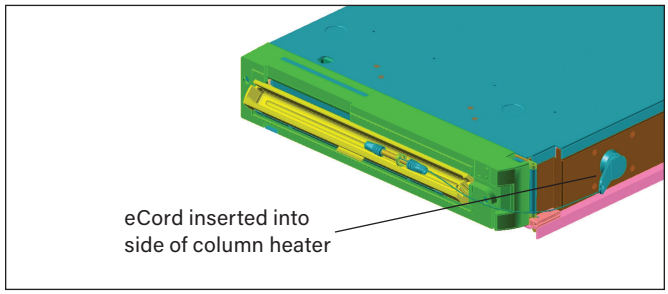


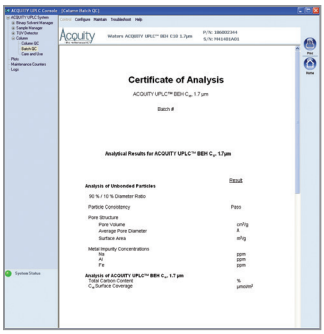
Figure 4. eCord inserted into side of column heater.

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 allowing the user to access column information on their desktop.

c. Manufacturing Information



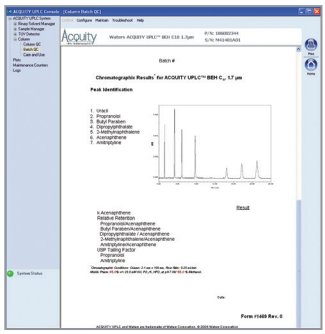


Figure 5. The eCord chip provides the user with an overview of the bulk material QC test results.

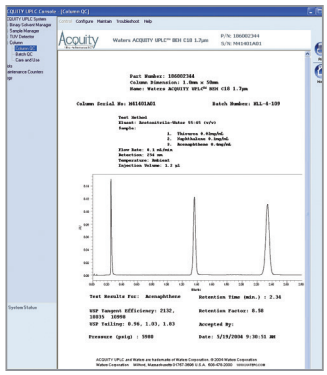


Figure 6. The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition, the QC results and acceptance is placed onto the column.

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.

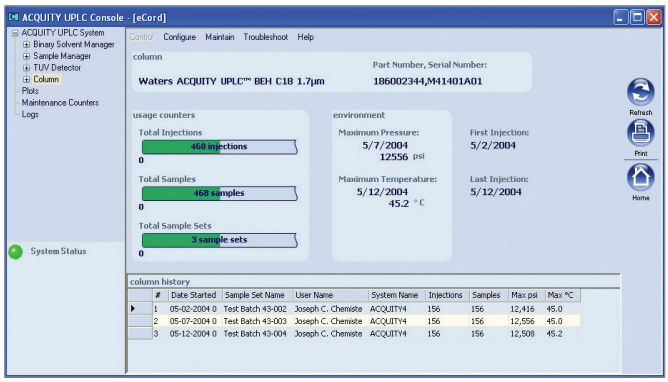


Figure 7. An example of column use information provided by the eCord chip.

V. ADDITIONAL INFORMATION

a. Tips for Maximizing ACQUITY HSS 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. When problems arise, often only one improper practice must be changed.
3. 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.

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 hrs
   - 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 backpressure 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: 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.
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?

VI. 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 www.waters.com/sds.

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