ACQUITY UPLC CSH COLUMNS

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Thank you for choosing a Waters ACQUITY UPLC® CSH™ column. ACQUITY UPLC CSH columns feature Waters Charged Surface Hybrid (CSH) Technology which provides excellent peak shape, high efficiency and loading capacity for basic compounds when using acidic, low ionic strength mobile phases. This same particle technology is used in the XSelect™ CSH family of HPLC columns, thus enabling seamless transferability between HPLC and UPLC® system platforms. The ACQUITY UPLC CSH packing materials were designed specifically for use with the ACQUITY UPLC systems and are manufactured in a cGMP, ISO 9001 certified manufacturing facility using ultra pure reagents. Each batch of ACQUITY UPLC CSH 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 CSH columns were designed and tested specifically for use on ACQUITY UPLC systems. ACQUITY UPLC CSH columns will exhibit maximum chromatographic performance and benefits ONLY when used on holistically-designed ACQUITY UPLC systems since these systems and columns 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®

I. GETTING STARTED

Each ACQUITY UPLC CSH 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 CSH 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

ACQUITY UPLC systems utilize tubing and connectors which have been designed to meet stringent tolerance levels and to minimize extra column volumes.For information on system tubing and connectors, please refer to the ACQUITY UPLC System Operator's Guide (Part Number 71500082502).

b. Column Installation

Note: The flow rates given in the procedure below are for typical 2.1 mm i.d. by 50 mm length 1.7 µm columns. 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 mobile-phase additives are present in low concentrations (e.g., ion-pairing reagents), 100 to 200 column volumes may be required for complete equilibration. In addition, mobile phases that contain formate (e.g., ammonium formate, formic acid, etc.) may also require longer initial column equilibration times.

c. Column Equilibration

ACQUITY UPLC CSH 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)

Cloumn 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. 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.

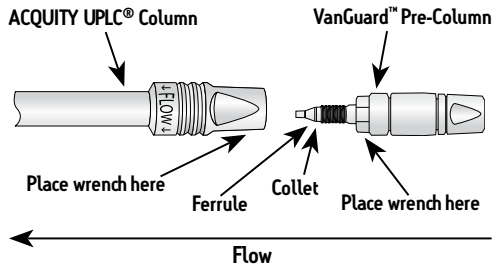
Note: If b. is performed, the isocratic efficiencies measured in your laboratory may be less than those given on the Waters "Performance Test Chromatogram". This is normal. The Waters isocratic column testing systems have been modified in order to achieve extremely low system volumes. This presents a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

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 systems. VanGuard Pre-columns are packed with the same chemistries and frits as our 2.1 mm i.d. ACQUITY UPLC CSH columns. VanGuard Pre-columns are designed to be attached directly to the inlet side of an ACQUITY UPLC CSH 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 CSH 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 CSH 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 CSH 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.

II. COLUMN USE

To ensure the continued high performance of ACQUITY UPLC CSH 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.
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.

b. pH Range

Please see Table 2 below for information on pH range and suggested operating temperatures.

A listing of commonly used buffers and additives is given in Table 3. 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.

Table 2: Recommended pH and Temperature Limits for ACQUITY UPLC CSH Columns

Column Name	Particle Size (µm)	Pore Diameter (Å)	Surface Area (m^2)	pH Limits	Temperature Limits		Ligand Density (µmol/m^2)	% Carbon
					Low pH	High pH		
ACQUITY UPLC CSH C_{18}	1.7	135	185	1-11	80	45	2.3	15
ACQUITY UPLC CSH Phenyl-Hexyl	1.7	135	185	1-11	80	45	2.3	14
ACQUITY UPLC CSH Fluoro-Phenyl	1.7	135	185	1-8	60	45	2.3	10

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

Table 3. Buffer Recommendations for Using ACQUITY UPLC CSH Columns up to pH 11

Additive/Buffer	pKa	Buffer Range	Volatility	Used for Mass Spec	Comments
TFA	0.3		Volatile	Yes	Ion pair additive, can suppress MS signal, used in the 0.02-0.1% range.
Acetic Acid	4.76		Volatile	Yes	Maximum buffering obtained when used with ammonium acetate salt. Used in 0.1-1.0% range.
Formic Acid	3.75		Volatile	Yes	Maximum buffering obtained when used with ammonium formate salt. Used in 0.1-1.0% range.
Acetate (NH_4CH_2COOH)	4.76	3.76 – 5.76	Volatile	Yes	Used in the 1-10 mM range. Note that sodium or potassium salts are not volatile.
Formate (NH_4COOH)	3.75	2.75 – 4.75	Volatile	Yes	Used in the 1-10 mM range. Note that sodium or potassium salts are not volatile.
Phosphate 1	2.15	1.15 – 3.15	Non-volatile	No	Traditional low pH buffer, good UV transparency.
Phosphate 2	7.2	6.20 – 8.20	Non-volatile	No	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime.
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.

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 UPLC CSH 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 up to 80 °C are recommended for operating ACQUITY UPLC CSH 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. See Table 2 above for more information on suggested operating temperatures and pH ranges.

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

III. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

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

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 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 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 dimethyl sulfoxide (DMSO)
2. tetrahydrofuran (THF)	2. methanol	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 (Part Number 205000464) has been installed, running solvents such as 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.*

b. Storage

For periods longer than four days at room temperature, store ACQUITY UPLC CSH 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 ACQUITY UPLC CSH 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. Completely seal column to avoid evaporation and drying out of the bed.

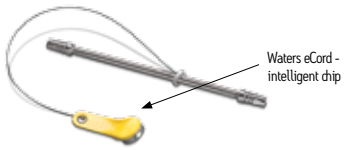
Note: If a column has been run with a mobile phase that contains formate (e.g., ammonium formate, formic acid, etc.) and is then flushed with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and run again with a formate-containing mobile phase.

IV. INTRODUCING eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

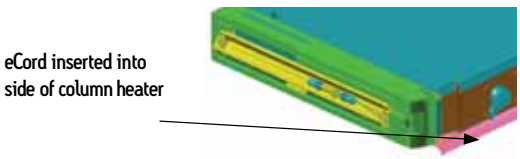
The eCord intelligent chip will provide the history of a column's performance throughout its lifetime. The eCord will be permanently attached to the column to assure that the column's performance history is maintained in the event that the column is moved from one instrument to another.

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.

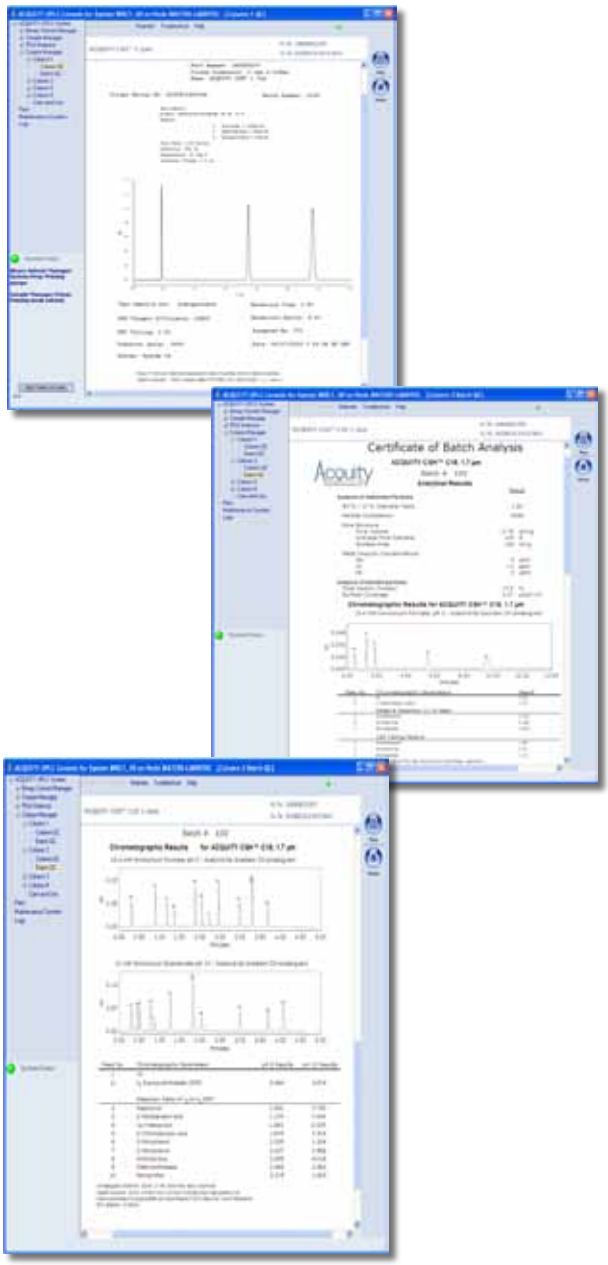
Figure 2. eCord Inserted into Side of Column Heater



b. Installation

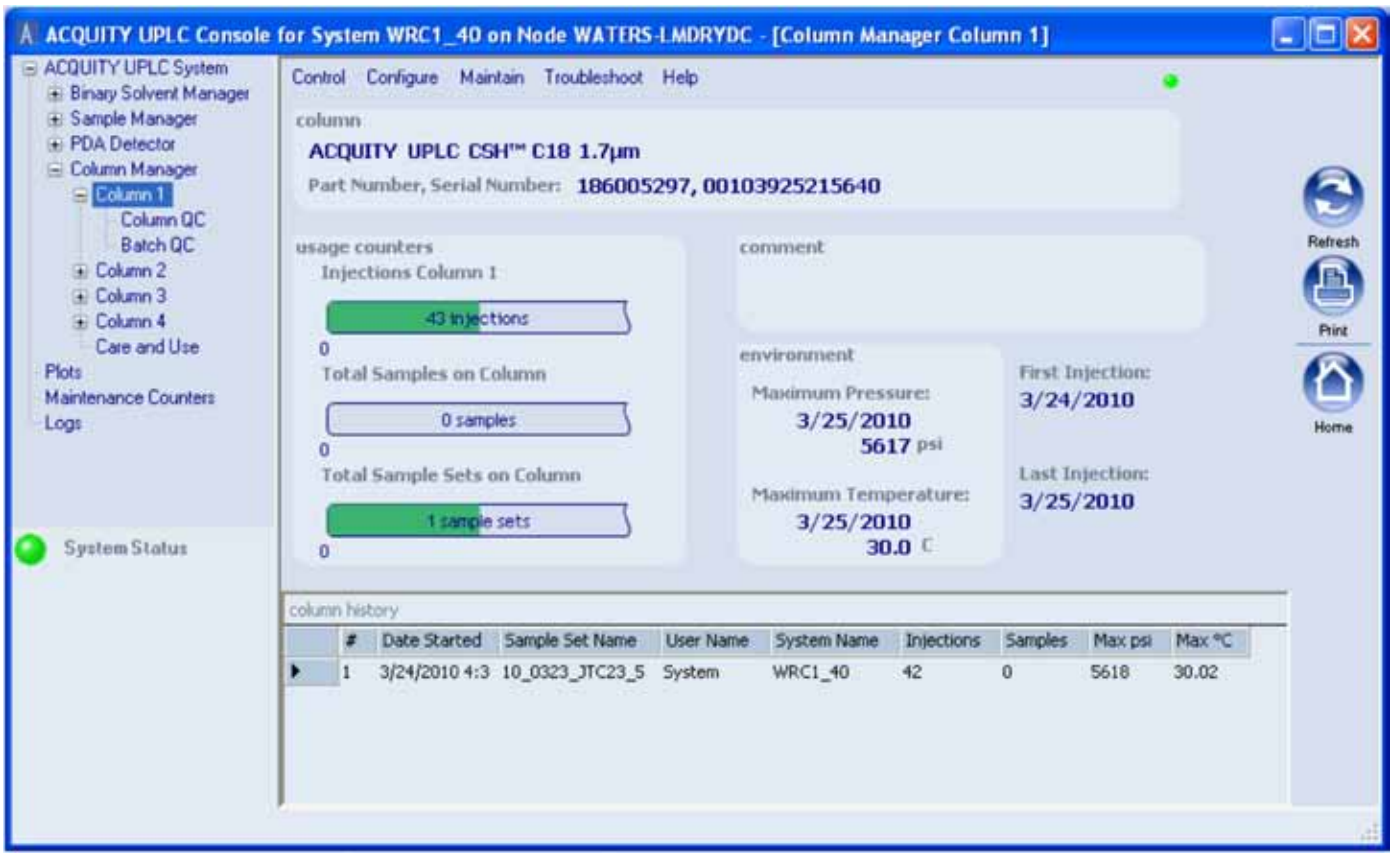
Install the column into the column heater. Plug the eCord into the side of the column heater. Once the eCord is inserted into the column heater the identification and overall column usage information will be available allowing the user to access column information on their desktop.

c. Manufacturing Information



d. Column Use Information

The eCord chip provides the customer with column use data. The 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.



V. ADDITIONAL INFORMATION

a. Tips for Maximizing ACQUITY UPLC CSH Column Lifetimes

1. To maximize ACQUITY UPLC CSH 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.
   - Consider sample preparation (e.g., solid-phase extraction, filtration, etc).
4. Avoid (where possible):
   - 100% aqueous mobile phases (if possible).
   - HPLC-grade bottled water.
   - "Topping off" or adding "new" mobile phase to "old" mobile phase.
   - Old aqueous mobile phases. Remember to rinse bottles thoroughly and prepare fresh every 24-48 hours.
   - Using phosphate salt buffer in combination with high ACN concentrations (e.g., > 70%) due to precipitation.
5. Don't: assume a "bad" column is the culprit when high 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: UPLC flow rates are often much lower and, therefore, mobile phases last much longer (only prepare what you need or store excess refrigerated).
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 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 a pH 7 phosphate buffer (which is VERY susceptible to bacterial growth)?
8. Sample-related questions to ask:
   - If I inject neat standards prepared in mobile phase do I observe these problems?
   - If I prepare my standards in water and prepare them like samples (e.g., SPE, filtration, etc.) do I still observe these problems?
   - Has the quality of my samples changed over time?

b. Recommended Flow Rates and Backpressures for ACQUITY UPLC CSH 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

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

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

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