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CORTECS UPLC Columns

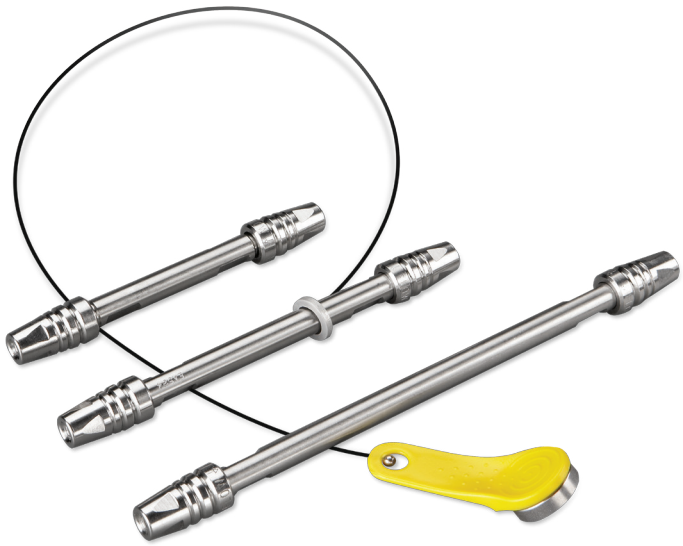
CONTENTS

I. INTRODUCTION

Thank you for choosing a CORTECS UPLC Column. The CORTECS 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 CORTECS 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.

CORTECS UPLC Columns will exhibit maximum chromatographic performance and benefits when used on the holistically-designed ACQUITY UPLC System since they were created and designed to operate with it.

CORTECS®
COLUMNS



II. GETTING STARTED

Each CORTECS UPLC Column comes with a Certificate of Analysis, which includes the bonded phase batch number and the analytical test results for the unbonded and bonded particle. The included Performance Test Chromatogram summarizes the performance of each individual column and provides batch number, column serial number, USP plate count, USP tailing factor, retention factor, and chromatographic test conditions. These data should be recorded and stored for future reference. When available, the information can be accessed via the ACQUITY UPLC System's console using the attached eCord.

a. Column Connectors

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

Optimized column inlet tubing (p/n 430001084) is supplied with the ACQUITY UPLC System. The inject valve end of the tubing is clearly marked with a blue shrink tube marker. Insert the opposite end of the tubing into the CORTECS UPLC Column and tighten the compression fitting using two 5/16" wrenches.

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

b. Column Installation

Note: The flow rates given in the procedure below are for a typical 1.6 µm, 2.1 mm I.D. x 50 mm length column. Scale the flow rate up or down accordingly based upon the column pressure and system limits.

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

Note: If mobile phase additives are present in low concentrations (e.g. ion-pairing reagents), 100 to 200 column volumes may be required for complete equilibration. In addition, mobile phases that contain formate (e.g. ammonium formate, formic acid, etc.) may also require longer initial column equilibration times.

c. Column Equilibration

CORTECS UPLC 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. Column Volumes (mL)

Column length (mm)	I.D.	
	2.1 mm	3.0 mm
30	0.1	0.2
50	0.2	0.4
75	0.3	0.5
100	0.4	0.8
150	0.5	1.0

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

For CORTECS UPLC HILIC Columns, flush with 50 column volumes of 50:50 acetonitrile/water with 10 mM final buffer concentration. Prior to the first injection, equilibrate with 20 column volumes of initial mobile phase conditions (refer to Table 1 for a list of column volumes). See "Getting Started with CORTECS UPLC HILIC Columns" for additional information.

d. eCord Installation

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

e. Initial Column Efficiency Determination

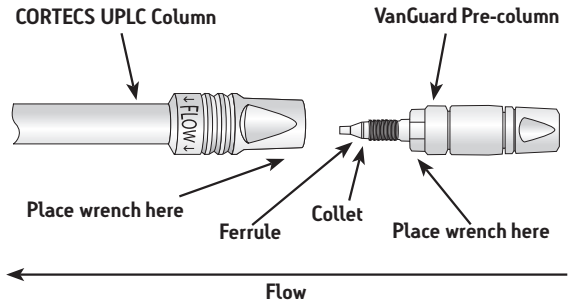
1. Perform an efficiency test on the column before using it. This test may consist of:
   a. an analyte test mixture that is commonly used in your laboratory, and/or
   b. an analyte mixture as found on the "Performance Test Chromatogram" which accompanied your column.

   Note: If b. is performed, the isocratic efficiencies measured in your laboratory may be less than those given on the Waters "Performance Test Chromatogram". This is normal. CORTECS UPLC Columns are QC tested on ACQUITY UPLC I-Class Systems which have extremely low system volumes. This presents a more challenging test of how well the column was packed and guarantees the highest quality packed column.
2. Determine the number of theoretical plates (N) and use this value for periodic comparisons.
3. Repeat the test at predetermined intervals to track column performance over time. Slight variations may be obtained on two different ACQUITY UPLC Systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

f. VanGuard Pre-columns

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

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



Installation Instructions

1. Remove 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 CORTECS UPLC Column perpendicular to the work surface so that the column inlet is on the bottom (column outlet on top).
4. From below, insert the VanGuard Pre-column into the CORTECS 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 CORTECS 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.

III. COLUMN USE

To ensure the continued high performance of CORTECS UPLC Columns, follow these guidelines:

a. Sample Preparation

1. Sample impurities often contribute to column contamination. One option to avoid this is to use Waters Oasis® Solid-Phase Extraction Cartridges/Columns or Sep-Pak® Cartridges of the appropriate chemistry to cleanup the sample before analysis. For more information, 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 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 "Getting Started with CORTECS UPLC HILIC Columns".

b. pH Range

The recommended operating pH range for CORTECS UPLC Columns is 2 to 8 for the C_{18}, C_{18}+, C_8, and Phenyl chemistries, and 1 to 5 for the HILIC chemistry. A listing of commonly used buffers and additives is given in Table 2. Additionally, the column lifetime will vary depending upon the operating temperature, the type, and concentration of buffer used. See Table 3 for more characteristic details of CORTECS chemistries.

Table 2. Buffer Recommendations for Using CORTECS UPLC Columns

Additive/buffer	pK_a	Buffer range	Volatility (±1 pH unit)	Used for mass spec	Comments
TFA	0.30		Volatile	Yes	Ion pair additive, can suppress MS signal, used in the 0.02–0.10% 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	7.20	6.20– 8.20	Non-volatile	No	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime.

Table 3. CORTECS Chemistry Characteristics

Name of column	Ligand type	Surface charge modification	End cap style	Carbon load	Ligand density	pH limits	Temp. limits
C_{18}	Trifunctional C_{18}	None	Proprietary	6.6%	2.7 µmol/m^2	2–8	45 °C
C_{18}+	Trifunctional C_{18}	+	Proprietary	5.7%	2.4 µmol/m^2	2–8	45 °C
C_8	Trifunctional C_8	None	Proprietary	4.5%	3.4 µmol/m^2	2–8	45 °C
T3	Trifunctional C_{18}	None	Proprietary	4.7%	1.6 µmol/m^2	2–8	45 °C
Shield RP18	Monofunctional Embedded Polar C_{18}	None	Proprietary	6.4%	3.2 µmol/m^2	2–8	45 °C
Phenyl	Trifunctional phenyl	None	Proprietary	5.9%	3.2 µmol/m^2	2–8	45 °C
HILIC	None	None	None	Unbonded	n/a	1–5	45 °C

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 VI for more information.

d. Pressure

CORTECS UPLC Columns can tolerate operating pressures up to 18,000 psi (1241 bar or 124 MPa).

e. Temperature

Temperatures between 20–45 °C are recommended for operating CORTECS UPLC 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.

IV. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

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

Use the cleaning routine that matches the properties of the samples 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.

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

Table 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. 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. 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 (p/n 205000971) has been installed, running solvents such as THF or hexane should only be considered when the column cannot be cleaning by running neat, reversed-phase organic solvents such as acetonitrile. Reduce flow rate, lower operating temperatures, and limit system exposure to THF and/or hexane.*

b. Storage After Reversed-Phase and HILIC Use

For periods longer than four days, store the column in 100% acetonitrile. For separations utilizing elevated temperature, store immediately after use in 100% acetonitrile. 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 column volume information) followed by 10 column volumes of acetonitrile. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced. Completely seal the column to avoid solvent evaporation and drying out of the chromatographic bed.

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

V. INTRODUCING 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.

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 eCord. 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 users the reassurance that a well-performing column is installed onto their instruments.

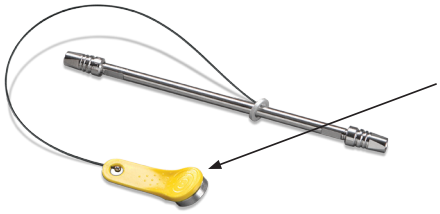


Figure 1. eCord Intelligent Chip.

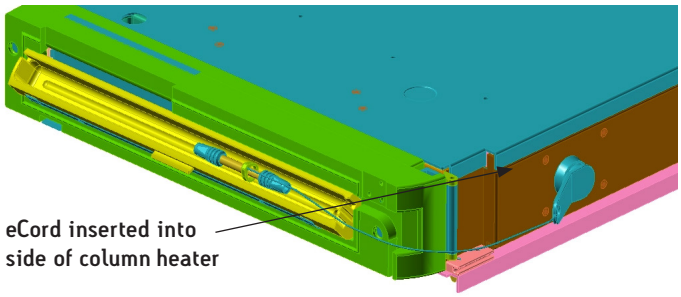
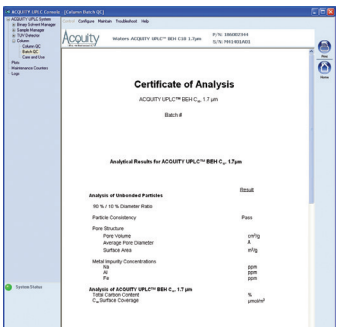


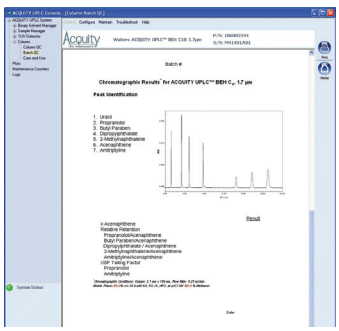
Figure 2. eCord inserted into side of column heater.

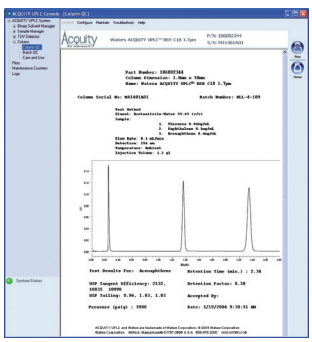
b. Installation

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





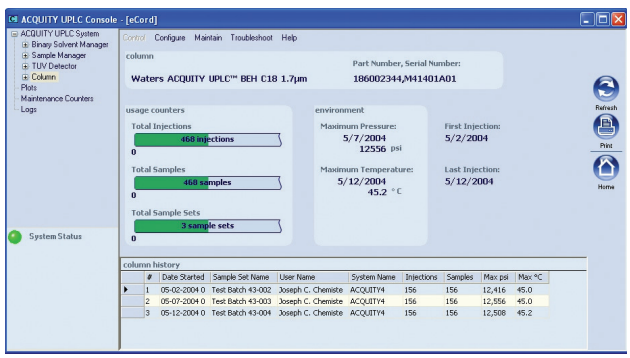


The eCord provides QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition the QC results and acceptance is placed onto the column.

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

d. Column Use Information

The eCord provides the user 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 the column.



VI. ADDITIONAL INFORMATION

a. Tips for Maximizing CORTECS UPLC Column Lifetimes

1. To maximize CORTECS UPLC Column lifetime, pay close attention to:
   - Water quality (including water purification system)
   - Solvent quality
   - Mobile phase preparation, storage, and age
   - Sample, buffer, and mobile phase solubilities
   - Sample quality and preparation.
2. When problems arise, often only one improper practice must be changed.
3. Always remember to:
   - Use in-line filter unit 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) and the highest quality solvents possible. HPLC grade water is not ultra pure water.
   - Consider sample preparation (e.g. solid-phase extraction, filtration, etc).
4. Avoid (where possible):
   - 100% aqueous mobile phases.
   - HPLC grade bottled water.
   - "Topping off" your mobile phases.
   - Old aqueous mobile phases. Remember to rinse bottles thoroughly and prepare fresh every 24 to 48 hours
   - Using phosphate salt buffer in combination with high ACN concentrations (e.g. >70%) due to precipitation.
5. Do not assume a "bad" column is the culprit when high backpressure or split peaks are observed. Investigate the cause of the column failure by checking:
   - Backpressure
   - Mobile phase(s), bacteria, precipitation, and/or samples
   - Sample quality
   - Injection solvent strength
6. Remember: the diameter of CORTECS UPLC Column (2.1 mm 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.

b. Troubleshooting Questions

- Are you using 100% aqueous mobile phases? Are you able to add a small amount of organic modifier to your mobile phase A?
- Is the mobile phase filtered through a 0.2 µm membrane?
- What is the age of the mobile phase?
- Is the bottle labeled with the preparation date?
- Was the mobile phase prepared fresh or topped off?
- Is the water source of adequate quality?
- When was the last time the water system was serviced or was the bottle of water unopened?
- Is bacterial growth a possibility (pH 7 phosphate buffer is susceptible to bacterial growth within 24 hours)?
- If the sample is filtered/purified (i.e. SPE, filtration... etc.) is the problem still observed?
- Has the quality of the samples changed over time?

c. Getting Started with CORTECS UPLC HILIC Columns

1. Due to the fact that CORTECS UPLC HILIC Columns do not possess a bonded phase, the pH operating range is 1 to 5, and they can be operated at temperatures up to 45 °C.
2. As with any LC column, operating at the extremes of pH, pressures and temperatures will result in decreased column lifetime.

Column Equilibration

1. When 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.

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 CORTECS 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 must be used instead of a buffer, use 0.2% (v:v) instead of 0.1%.
5. For best peak shape, maintain a buffer concentration of 10 mM in your mobile phase/gradient at all times.

Injection Solvents

1. If possible, injection solvents should be 95% acetonitrile. The polar solvent (i.e. water, methanol, isopropanol) should be minimized to 25% of the total volume.
2. A generic injection solvent is 75:25 acetonitrile/methanol. This is a good compromise between analyte solubility and peak shape.
3. Avoid water and dimethyl sulfoxide (DMSO) as injection solvents. These solvents will produce very poor peak shapes.
4. Exchange water or DMSO with acetonitrile by using reversed-phase solid-phase extraction (SPE). If this is not possible, dilute the water or DMSO with organic solvent.

Miscellaneous Tips

1. CORTECS UPLC HILIC Columns are designed to retain very polar bases. Acidic, neutral, or non-polar compounds will have limited retention.
2. Optimal flow rates for small (<200 daltons) very polar bases are in the 0.4 to 0.8 mL/min range with the CORTECS UPLC HILIC Columns.
3. In HILIC, it is important to remember that water is the strongest solvent. Therefore, it must be eliminated or minimized in the injection solvent.
4. 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.
5. Alternate polar solvents such as methanol, acetone, or isopropanol can also be used in place of water to increase retention.
6. If using an ACQUITY UPLC System, the weak needle wash should closely match the percent organic present in the initial mobile phase conditions, otherwise, analyte peak shape or retention may suffer.

VII. CAUTIONARY NOTE

Some products may be hazardous during and after use and are to be used by professional laboratory personnel trained in the competent handling of such materials. The responsibility for the safe use of products rests entirely with the purchaser and user. The safety data sheets (SDS) for these products are available at www.waters.com/sds.

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