

ACQUITY UPLC Protein BEH SEC Columns and Standards

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I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY™ UPLC™ Protein BEH SEC Guard, Column, and/or Standard Mix that are integral parts of Waters' ACQUITY UPLC SEC System solution. The ACQUITY UPLC Protein BEH SEC 125 Å offering is best suited for the analysis of peptides and proteins in the molecular weight range from 1000–80,000 Daltons that include insulin and its aggregates. The ACQUITY UPLC Protein BEH SEC 200 Å Column was designed to characterize proteins ranging in molecular weight range from 10,000–450,000 Daltons that include monoclonal IgG monomers from aggregates, while our ACQUITY UPLC Protein BEH SEC 450 Å is best suited for the analyses of proteins and conjugates that range from 100,000–1.5 million Daltons (Figure 1). All of these SEC chemistries are based on Waters' Ethylene Bridged Hybrid (BEH)-based particle technology and diol-bonded surface that provide a stable chemistry with minimal non-desired secondary interactions for proteins and peptides. The columns are manufactured in a cGMP, ISO 9001 certified plant using ultra pure reagents. Each batch of Protein Separation Technology SEC packing material has been tested and qualified using a protein test mixture (also available for purchase), and the results are held to narrow specification ranges to ensure reproducible performance. Every column is also individually tested for efficiency. A Performance Test Chromatogram along with a Certification of Analysis (CofA) are available with each column.



See page 6 for important information
on Extending SEC Column Life

II. CONFIGURING AN ACQUITY UPLC SYSTEM FOR USE IN PROTEIN SEC SEPARATIONS

a. Calibrators

In order to obtain the best performance from your SEC column, it is important that your ACQUITY UPLC System is properly configured. It is recommended that only pre-cut tubing is used, and that the I.D. of all connecting tubing is 0.005" or less for optimal chromatographic performance.

Size-exclusion chromatography may require modifications to an existing ACQUITY UPLC System. Please refer to "Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC System" (p/n: [715002147A](http://www.waters.com/chemcu)) for specific recommendations that can be obtained at www.waters.com/chemcu.

The sample loop used may affect the performance of your separation. Optimally, select the smallest volume sample loop that is required for the application. Sample loops larger than 20 µL with the ACQUITY UPLC Protein BEH SEC Column are not recommended.

III. GETTING STARTED

A Performance Test Chromatogram and a CofA are available for each shipped ACQUITY UPLC Protein BEH SEC Column. The CofA is specific to each batch of packing material and includes the batch number and chromatographic separation obtained using defined protein standards. This document is available upon request at www.waters.com/coa.

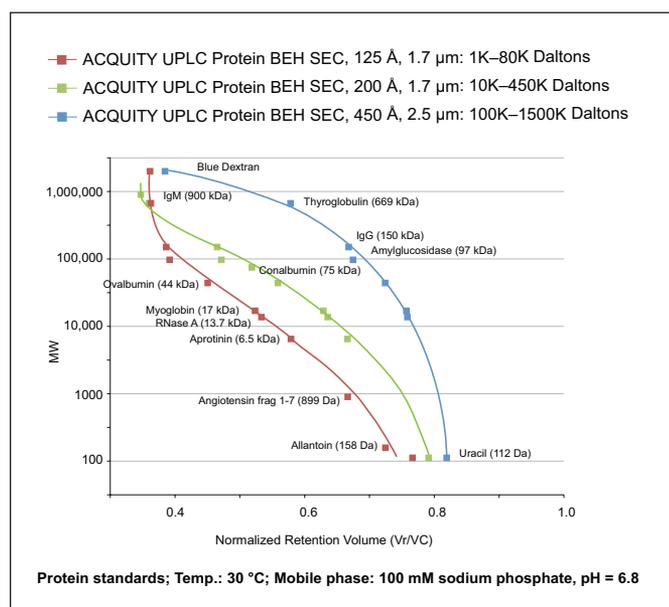


Figure 1. Calibration curves on ACQUITY UPLC Protein BEH SEC 125 Å, 200 Å, and 450 Å Columns.

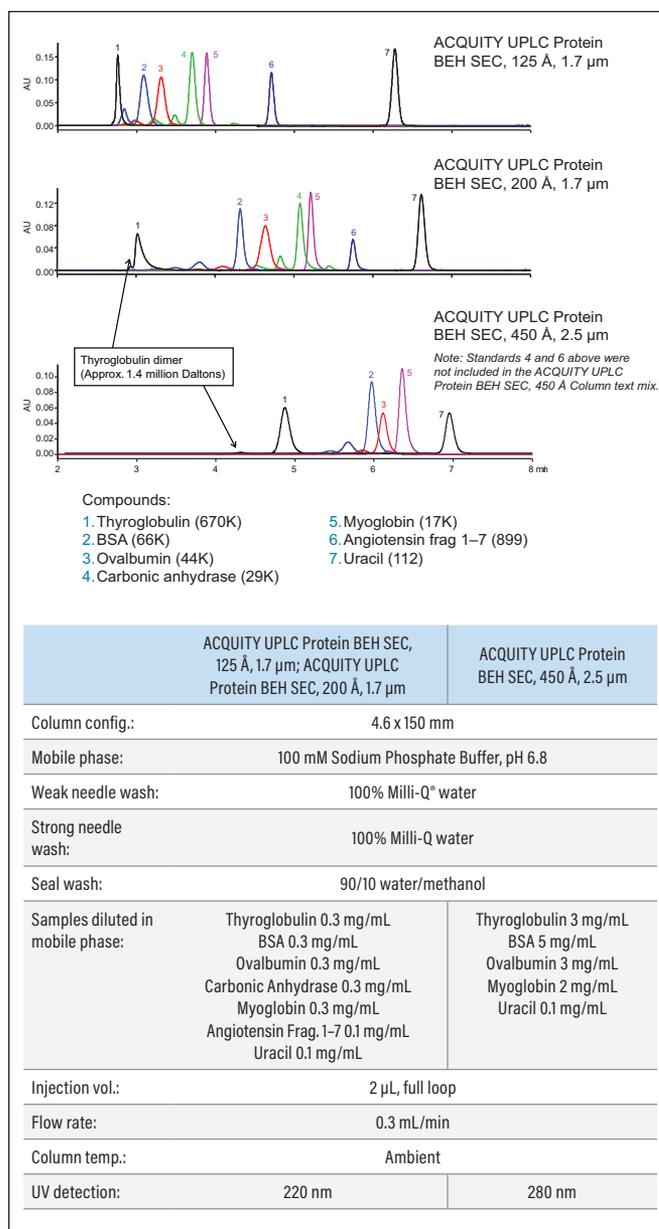


Figure 2. Separation of protein and peptide standards on ACQUITY UPLC Protein BEH SEC, 125 Å, 200 Å, and 450 Å Columns.

a. eCord installation

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

b. Column connectors

It is recommended to use the 150 mm column stabilizer (p/n: [205000365](#)) to connect the ACQUITY UPLC Protein BEH SEC Column to the ACQUITY UPLC System. A reusable fitting is supplied with the ACQUITY UPLC Column stabilizer.

c. Column installation

1. Prior to placing the column on the system, purge the solvent delivery system of any organic or water-immiscible mobile phases. When connecting the column, orient it in the proper direction as noted by the arrow on the column inlet side which indicates the correct direction of solvent flow.
2. Flush column with 100% aqueous buffer, by pumping at a flow rate of 0.2 mL/min.
3. Ensure that the mobile phase is flowing freely from the column outlet. Attach the column outlet to the detector using .004" I.D. tubing (p/n: [430001562](#)). Monitor the system pressure to ensure the column is within its pressure limitations.
4. Gradually increase the flow rate by not more than 0.1 mL/min at a time, as described in Step 2.
5. Once the system pressure has stabilized, ensure that there are no leaks at either the column inlet or outlet.

d. Column equilibration

ACQUITY UPLC Protein BEH SEC Columns are shipped in 20% methanol in water. 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 buffer to be used (refer to Table 1 for column volumes).

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

Column dimension	Approximate volume
4.6 x 150 mm	2.5 mL
4.6 x 300 mm	5.0 mL

e. Functional tests for benchmarking a new column

Waters recommends performing a benchmarking test upon receipt of your column and throughout the lifetime usage. By using a separation of common proteins with an appropriate method, you can:

- Verify the performance of the column upon receipt.
- Monitor the condition of the columns for extended use.
- Troubleshoot separation difficulties that may arise.

The BEH125 SEC Protein Standard Mix (p/n: [186006519](#)), BEH200 SEC Protein Standard Mix (p/n: [186006518](#)), and BEH 450 SEC Protein Standard Mix (p/n: [186006842](#)) were specifically designed for this purpose with carefully chosen proteins and/or peptides to provide a good representation of the intended application. Figure 3 is an example of the Performance Test Chromatogram using p/n: [186006519](#), Figure 4 is an example of the Performance Test Chromatogram using p/n: [186006518](#), and Figure 5 is an example of the Performance Test Chromatogram using p/n: [186006842](#). These are typical results obtained in a Waters laboratory using 4.6 x 150 mm columns on an ACQUITY UPLC System.

Reconstitution, Filtration, and Storage of Waters BEH SEC Protein Standard Mix

The performance and lifetime of an SEC column can deteriorate by the accumulation of sample and/or mobile phase derived >0.2 µm particulates. These include any injected protein containing materials such as microbial or insoluble protein aggregates. As a result, it is recommended to filter the reconstituted Waters BEH SEC Protein Standard Mix with a 0.2 µm filtration device prior to its use in the evaluation of our Waters BEH SEC Column performance. Filtration devices, such as sterile, low-bind, 0.2 µm syringe filters from EMD Millipore (13 mm catalog no. SLGV013SL or 4 mm catalog no. SLGV004SL) can be successfully used for this purpose. It is also advised to avoid freeze-thaw cycles of the reconstituted BEH SEC Protein Standard since this can result in the generation of protein aggregates. It is recommended that the reconstituted and filtered BEH SEC Protein Standard be stored at 4-8 °C for no more than a week before considering discarding.

Summary:

- Store the lyophilized BEH SEC Protein Standard in freezer.
- To use, reconstitute in 500 to 1000 µL of buffer (see below).
- Filter the reconstituted protein standard through an appropriate 0.2 µm filter.
- Store reconstituted protein standard at 4-8 °C.
- Discard reconstituted protein standard after 7 days.

Buffer preparation for standards:

Chemicals

- Sodium phosphate monobasic, monohydrate
- Sodium phosphate dibasic, anhydrous
- Milli-Q water

Preparation of 100 mM sodium phosphate buffer (500 mL)

1. Weigh out 500 ± 0.02 grams of H₂O into a 500 mL beaker.
2. Weigh out 3.55 ± 0.02 grams of sodium phosphate dibasic and add to the 500 mL beaker.
3. Weigh out 3.45 ± 0.02 grams of sodium phosphate monobasic and add to the 500 mL beaker.
4. Stir for a minimum of 30 minutes then filter the solution through a 0.2 µm Millipore GV filter.

Take the pH and record the value for reference purposes (approximate pH: 6.8).

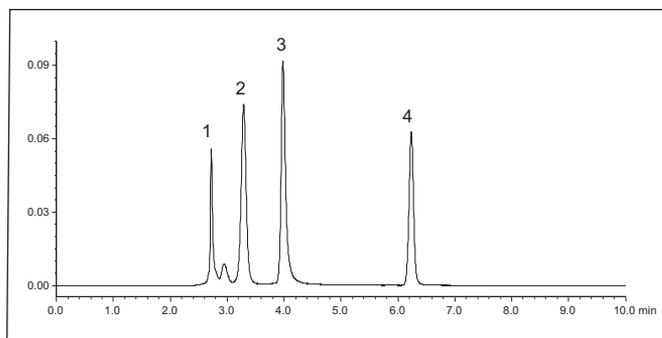


Figure 3. Protein mixture separation on an ACQUITY UPLC Protein BEH SEC, 125 Å, 1.7 µm Column.

Table 2. BEH125 SEC Test Mix

Analyte	pI	MW
1 Thyroglobulin, 0.1 mg/mL	4.6	660,000
2 Ovalbumin, 0.3 mg/mL	4.5	44,200
3 Ribonuclease A, 0.3 mg/mL	9.6	13,700
4 Uracil, 0.05 mg/mL	N/A	112

- Instrument:** ACQUITY UPLC System with Tunable UV (TUV) detector
- Column:** ACQUITY UPLC Protein BEH SEC, 125 Å, 1.7 µm, 4.6 x 150 mm that includes BEH125 SEC Protein Standard Mix (p/n: [176003906](#))
- Sample:** BEH125 SEC Protein Standard Mix (p/n: [186006519](#))
- Protein standard preparation:** Dissolve the BEH125 SEC Protein Standard in 500 µL of 100 mM sodium phosphate buffer pH 6.8. Once solubilized, do not freeze sample, store at 2–8 °C for no more than a week.
- Mobile phase:** 100 mM sodium phosphate, pH 6.8
- Weak needle wash:** 100% Milli-Q water
- Strong needle wash:** 100% Milli-Q water
- Seal wash:** 90/10 water/methanol
- Injection type:** Full loop
- Injection volume:** 2 µL
- Flow rate:** 0.3 mL/min
- Column temp.:** 30 °C
- UV detection:** 220 nm

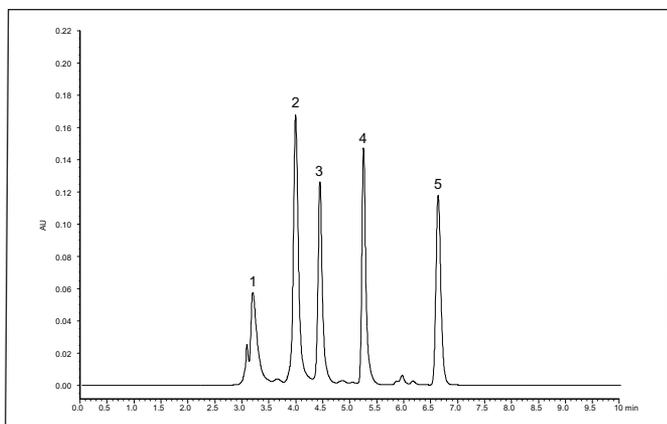


Figure 4. Protein mixture separation on an ACQUITY UPLC Protein BEH SEC, 200 Å, 1.7 µm Column.

Table 3. BEH200 SEC Test Mix

Analyte	pI	MW
1 Thyroglobulin, 3 mg/mL	4.6	660,000
2 IgG, 2 mg/mL	6.7	150,000
3 BSA, 5 mg/mL	4.6	66,400
4 Myoglobin, 2 mg/mL	6.8, 7.2	17,000
5 Uracil, 0.1 mg/mL	N/A	112

Instrument: ACQUITY UPLC with Tunable UV (TUV) detector

Column: ACQUITY UPLC Protein BEH SEC, 200 Å, 1.7 µm, 4.6 x 150 mm that includes BEH200 SEC Protein Standard Mix (p/n: [176003904](#))

Sample: BEH200 SEC Protein Standard Mix (p/n: [186006518](#))

Protein standard preparation:
Dissolve the BEH200 SEC Protein Standard in 500 µL of 100 mM sodium phosphate buffer, pH 6.8. Once solubilized, do not freeze sample, store at 2–8 °C for no more than a week.

Mobile phase: 100 mM sodium phosphate, pH 6.8

Weak needle wash: 100% Milli-Q water

Strong needle wash: 100% Milli-Q water

Seal wash: 90/10 water/methanol

Injection type: Full loop

Injection volume: 2 µL

Flow rate: 0.3 mL/min

Column temp.: 30 °C

UV detection: 280 nm

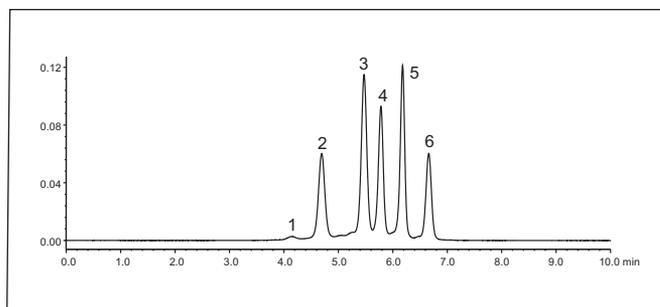


Figure 5. Protein mixture separation on an ACQUITY UPLC Protein BEH SEC, 450 Å, 2.5 µm Column.

Table 4. BEH450 SEC Test Mix

Analyte	pI	MW
1 Thyroglobulin, 0.1 mg/mL	4.6	1.4 million
2 Thyroglobulin, approx. 3 mg/mL	4.6	660,000
3 IgG, 2 mg/mL	6.7	150,000
4 BSA, 5 mg/mL	4.6	66,400
5 Myoglobin, 2 mg/mL	6.8, 7.2	17,000
6 Uracil, 0.1 mg/mL	N/A	112

Instrument: ACQUITY UPLC with Tunable UV (TUV) detector

Column: ACQUITY UPLC Protein BEH SEC, 450 Å, 2.5 µm, 4.6 x 150 mm that includes BEH450 SEC Protein Standard Mix (p/n: [176002996](#))

Sample: BEH450 SEC Protein Standard Mix (p/n: [186006842](#))

Protein standard preparation:
Dissolve the BEH450 SEC Protein Standard in 500 µL of 100 mM sodium phosphate buffer, pH 6.8. Once solubilized, do not freeze sample, store at 2–8 °C for no more than a week.

Mobile phase: 100 mM sodium phosphate, pH 6.8

Weak needle wash: 100% Milli-Q water

Strong needle wash: 100% Milli-Q water

Seal wash: 90/10 water/methanol

Injection type: Full loop

Injection volume: 2 µL

Flow rate: 0.3 mL/min

Column temp.: 30 °C

UV detection: 280 nm

IV. COLUMN SPECIFICATIONS AND USE

Note: Particulates impact SEC column lifetime.

The performance and lifetime of any UPLC- or HPLC-based SEC column can be significantly compromised by the injection and accumulation of sample and/or mobile phase derived particulates onto bed of the packed column. Particulate can include protein aggregates in the sample or microbial contaminants contained in prepared SEC eluent.

Consequently, it is highly recommended that one use high quality, filtered water (i.e., MilliQ Millipak 0.22 μm filtered water) when preparing the SEC mobile phase and that it be filtered with a $<0.2 \mu\text{m}$ filter (e.g., through a single use, sterile disposable filter).

It is also recommended to avoid use of silica-based filter supports when filtering mobile phases of $\text{pH} > 6.8$ due to the potential of introducing soluble and/or insoluble silicates into the SEC eluent which could alter column performance. Sterile 0.2 μm nylon, nalgene units have been used for this purpose (i.e., Fisher Scientific catalog no. 09-740-46).

Protein samples should be centrifuged or passed through a sterile 0.2 μm low-bind filter to ensure they do not contain significant amounts of particulates. Prior to routine use, a filter should be evaluated to ensure it is able to quantitatively yield analytes of interest. If not filtered, samples can be centrifuged at 16,000 $\times g$ for 5 minutes in a temperature-controlled microcentrifuge. Where it is not possible to filter or centrifuge samples, it should be accepted that the lifetime of SEC columns will decrease as a result of their exposure to $>0.2 \mu\text{m}$ particulates.

To check the peak shape performance of an SEC column and to monitor its lifetime, it is recommended to perform intermittent injections of 0.2 μm filtered Waters BEH SEC Protein Standard Mixes and/or 0.05 mg/mL buffered solutions of uracil.

To help ensure the continued high performance of your ACQUITY UPLC Protein BEH SEC Columns, follow these guidelines:

a. SEC eluent and needle wash preparation

- Use HPLC-grade buffers, water, and organic solvents when possible.
- Filter solutions through a compatible 0.2 μm or smaller pore size filter. The use of a sterile filtration apparatus is recommended for buffers capable of supporting microbial growth.
- Solutions that are susceptible to microbial growth should be replaced at regular intervals to avoid column

contamination. Do NOT refill partially full SEC eluent bottles with new eluent. Rather, when required use new bottle containing freshly prepared SEC eluent.

- Select solvent inlet filters that are compatible with solutions used, and clean or replace filters regularly when using solutions that are susceptible to microbial growth.

b. Sample preparation

- Ensure that samples are free of particulates before injecting onto the SEC column. If samples appear cloudy or turbid, they should not be injected, as this could lead to column pressure increases. Sample preparation such as filtration or centrifugation may be used, if appropriate.
- 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.

c. Column specifications

- **Shipping solvent:** 20% methanol in water
- **Recommended flow rate range for maximum column lifetime:** 0.1–0.4 mL/min
- **Maximum recommended pressure drop across column:**

Column Name	Length	Maximum Suggested Back Pressure
ACQUITY UPLC BEH SEC, 125 Å, 1.7 μm	4.6 x 150 mm	7000 PSI (483 Bar)
	4.6 x 300 mm	10,000 PSI (690 Bar)
ACQUITY UPLC BEH SEC, 200 Å, 1.7 μm	2.1 x 150 mm	7000 PSI (483 Bar)
	4.6 x 150 mm	7000 PSI (483 Bar)
	4.6 x 300 mm	10,000 PSI (690 Bar)
ACQUITY UPLC BEH SEC, 450 Å, 2.5 μm	4.6 x 150 mm	3000 PSI (207 Bar)
	4.6 x 300 mm	5000 PSI (345 Bar)

The maximum recommended pressure drop across the column is provided as recommendations to ensure longest possible column lifetimes. This guidance should not be interpreted as an absolute upper pressure limit. For example, some methods may exceed the suggested maximum pressure yet deliver desired SEC separation performance, although shorter column lifetimes may be expected.

Additionally, the stated maximum recommended pressure drop across the column refers only to the column itself and NOT from the added pressure contribution from the LC System. To determine pressure drop across column: Install a zero dead volume union in place of the column and determine the system pressure at operating conditions. Subtract that value from the system back pressure observed with the column installed.

- **Mass load:** <100 µg for a 4.6 x 150 mm
- **Volume load:** <20 µL for 4.6 x 150 mm
- **Recommended pH range:** 2-8. The column lifetime will vary depending upon the operating temperature as well as the type and concentration of buffer used.
- **Recommended salt conc.:** ≤0.5 M
- **Recommended organic conc.:** <20% acetonitrile (Caution: Many proteins are insoluble at elevated organic concentrations. Prior to chromatography, test to ensure the sample does not precipitate at the organic concentration to be used for the chromatography. Also, if the column is run under denaturing conditions [greater than 10% organic], subsequent column performance under 100% aqueous conditions may be affected.)
- **Recommended temperature:** 4–60 °C. Reduce flow rate when operating at low temperatures (e.g., 10 °C) to avoid excessive column pressure.
- **Recommended storage:** For overnight storage, continuously flush the column with the mobile phase at 10–20% of the maximum recommended flow rate. Store the column in the HPLC-grade water when it will be used within 24 hrs or in 10–20% methanol for long-term storage.

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

V. TROUBLESHOOTING

The first step in systematic troubleshooting is the comparison of the column performance in its current state to the performance when it was functioning properly. The functional tests with the protein mixture may reveal subtle changes in surface chemistry that affect the application.

There are several common symptoms of change in the column.

1. **An increase in pressure is often associated with decreased performance in the application.** The first step in diagnosis is to ensure that the elevated pressure resides in the column rather than somewhere else in the system. This is determined by monitoring the pressure of the system as each connection is broken from the outlet end to the inlet. If the system is occluded, the blockage should be identified and removed. If the pressure increase resides in the column, it is helpful to know whether the problem was associated with a single injection or whether it occurred over a series of injections. If the pressure gradually built up, it is likely that the column can be cleaned as described in Section VI.

If a single sample caused the pressure increase, it likely reflects particulates or insoluble components, such as lipids or higher order aggregates. Cleaning is still an option, but using the more aggressive options. If samples appear cloudy or turbid, they should not be injected, as this will lead to pressure increases. Sample preparation such as filtration or centrifugation may be used, but one should first check whether this impacts the results.

2. **Loss of resolution and increased peak tailing can be caused by microbial contamination.** It is important to follow good standard laboratory practices to prevent microbial contamination. This includes changing buffer bottles frequently, using high purity water, using a sterile filtration apparatus, and storing system and column under recommended conditions. If microbial contamination has occurred, cleaning the column will have no effect on performance.

The following protocol can be effectively used to clean a microbial contaminated ACQUITY UPLC H-Class System prior to reuse for SEC separations.

- Replace the ACQUITY UPLC Protein BEH SEC Column with a zero volume union (p/n: [700002636](#)).
- Program system startup function with the following parameters:
 - a. Prime each solvent line and seal wash for 5 min.
 - b. Wash solvent: 150 seconds
 - c. Purge solvent: 50 cycles
- **Solvent sequence:**
 - a. **Water:** All solvent lines, purge, needle wash, and seal wash. Run system startup, then equilibrate for 30 minutes (1.0 mL/min 25% each line).
 - b. **70% IPA:** All solvent lines, purge, needle wash, and seal wash. Run system startup, then make 30 injections of 70% IPA (2 min run time, 10 µL injection volume, 1.0 mL/min 25% each line).
 - c. **Water:** All solvent lines, purge, needle wash, and seal wash. Run system startup, then make 10 injections of water (2 min run time, 10 µL inj. volume, 1.0 mL/min 25% each line).
 - d. **30% phosphoric acid: Lines A, B, C, and D only. Keep the purge, needle wash, and seal wash lines in water!** Run system startup, then make 30 injections of 30% phosphoric acid (2 min run time, 10 µL injection volume, 1.0 mL/min 25% each line).

- e. **Water:** All solvent lines, purge, needle wash, and seal wash. Run system startup, then make 10 injections of water (2 min run time, 10 μ L inj. volume, 1.0 mL/min 25% each line).
 - f. **10% MeOH:** All solvent lines, purge, needle wash, and seal wash. Run system startup, then make 30 injections of 10% MeOH (2 min run time, 10 μ L injection volume, 1.0 mL/min 25% each line). Keep flow of 0.1 mL/min overnight.
3. Increased peak tailing can be caused by failure of a tubing connector or a buildup of material on the column inlet frit. Before proceeding with diagnostic or corrective measures, check all connections that the mobile phases have been correctly prepared and that the correct method has been selected. Then repeat the protein standard test. If the proteins show increased peak tailing, it is likely that there is significant buildup of material on the column inlet and the column will require replacement.
 4. Carryover is defined as the appearance of the constituents of one sample in the next analysis. In size-exclusion chromatography, carryover is typically due to system components or improper wash solvents. Run a blank injection. If the protein peaks only appear when an injection is made, they likely originate from system components or inadequate wash solvents. Adsorption in the system components most likely occurs in the loop or needle. In these instances, the component may need to be changed.

Note: Useful, general information on column troubleshooting problems may be found in "HPLC Columns Theory, Technology and Practice", U.D. Neue, (Wiley-VCH, 1997), the Waters HPLC Troubleshooting Guide (p/n: [720000181EN](#)), and the Waters website, www.waters.com

VI. COLUMN CLEANING, REGENERATION, AND STORAGE

a. Cleaning and regeneration

Changes in peak shape (i.e., increased tailing, shoulders on the peak, shifts in retention, change in resolution, ghost peaks, or increased backpressure) may indicate contamination of the column. Choose a cleaning option that may be expected to dissolve the suspected contaminant.

1. It may be useful to conduct cleaning procedures at one-half the flow rate typically used with that column. In this way, the possibility of high-pressure events is reduced.

2. Recommended cleaning solvents:
 - a. A concentrated salt solution at low pH (e.g., 0.5 M Na_2SO_4 , pH 2.7).
 - b. A low concentration of methanol (e.g., 20%) in HPLC-grade water.
 - c. Use of ionic detergents and other surfactants should be avoided if the SEC column is to be subsequently used to analyze native proteins.

Note: Choose a cleaning solvent based on sample properties (e.g., use [a] to remove basic protein and [b] to remove hydrophobic proteins). Chaotropic agents can solvate strongly adsorbed proteins via hydrogen bond disruption.

3. As a last resort, flow reversal or back flushing can be tried at a low flow rate (e.g., 0.1 mL/min). However, this approach may further damage the column or only provide short-lived improvement in performance.

b. Storage

For overnight storage, when the SEC column is on the LC system:

Continuously flush the column with the used SEC mobile phase at 10–20% of the flow rate used for separations.

For short-term storage (<2 weeks), when the SEC column is removed from the LC system:

Tightly cap column and store at 4 °C to prevent microbial growth. DO NOT FREEZE.

For long-term storage, when the SEC column is removed from the LC system:

Flush column (approx. 10-column volumes) and store in a mobile phase containing 5–25 mM phosphate buffer, pH 6.5–7.0, and 20–100 mM (KCl or NaCl) with the addition of 10% acetonitrile, 20% methanol, or 0.05% sodium azide to prevent microbial growth. This storage solution can be a dilute version of the mobile phase with an antimicrobial added to it. To further mitigate the risk of microbial growth, tightly cap column and store at 4 °C. DO NOT FREEZE.

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

VII. eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip Technology 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.

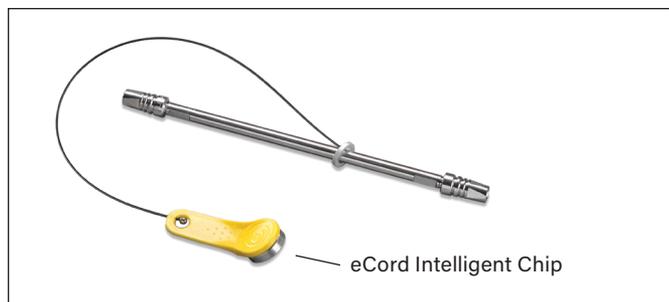


Figure 6. eCord Intelligent Chip.

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.

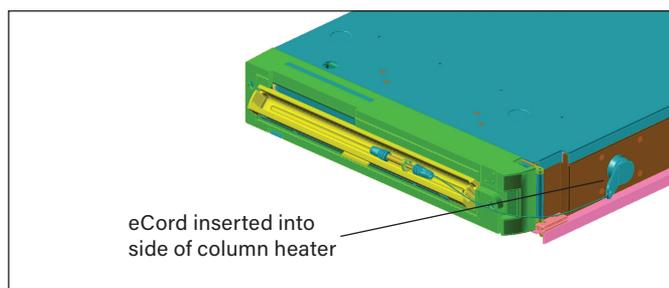


Figure 7. eCord inserted into side of column heater.

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

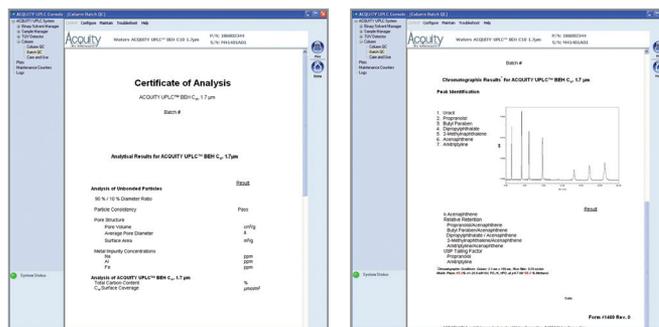


Figure 8. The eCord chip provides the user with an overview of the bulk material QC test results.

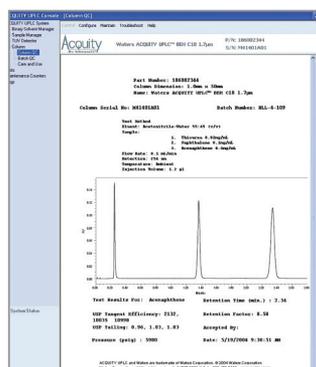


Figure 9. The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition, the QC results and acceptance is placed onto the column.

d. Column Use Information

The eCord will automatically capture 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.

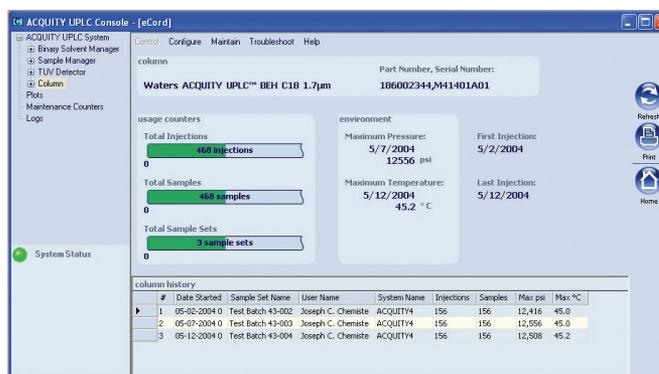


Figure 10. An example of column use information provided by the eCord chip.

VIII. CAUTIONARY NOTE

Depending on 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.

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