ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18}, 130 Å and 300 Å Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY™ UPLC™ and/or ACQUITY PREMIER Peptide BEH C_{18}, 130 Å or 300 Å Column. The ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18}, 130 Å and 300 Å packing materials were designed specifically for use with the ACQUITY UPLC and ACQUITY PREMIER Systems and are manufactured in a cGMP, ISO 9002 certified plant using ultra pure reagents. Each batch of ACQUITY UPLC and ACQUITY PREMIER BEH C_{18} material is tested chromatographically with acidic, basic, and neutral analytes and has been qualified for peptide mapping. 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. Each batch of ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18}, 130 Å and 300 Å Columns is also QC tested with the separation of a protein digest.

II. GETTING STARTED

Each Peptide Separation Technology ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18} Column comes with a Certificate of Analysis and a 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 and ACQUITY PREMIER Peptide BEH C_{18} 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

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 ACQUITY UPLC or ACQUITY PREMIER Column and tighten the compression fitting using two 5/16–inch 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

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 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

ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18} 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)

Table 1. Empty Column Volumes in mL (Multiply by 10 for Flush Solvent Volumes)

Column Length (mm)	Internal Diameter 2.1 mm
50	0.2 mL
100	0.4 mL
150	0.5 mL

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. Procedure for Using New, Out-of-Box Columns

Prior to using a new column, it is important to confirm that it produces reproducible chromatography and the desired level of chromatographic resolution. To this end, it is useful to benchmark column performance with a sample that is representative of the intended application. The number of injections necessary to achieve reproducible performance may be dependent on sample characteristics and system type. Method variables like pH, mass load, ionic strength, and ion pairing, could also have impact. ACQUITY PREMIER Columns have MaxPeak™ High Performance Surfaces that reduce the number of injections necessary to achieve desired performance due to the improved hardware inertness.

e. 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.

f. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture, as found in the "Performance Test Chromatogram", to analyze the column upon receipt.
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 UPLC systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

g. Column QR Code

The quick reference (QR) code that is located on the column label provides column-specific information (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).

III. COLUMN USE

To ensure the continued high performance of ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18} Columns follow these guidelines:

a. Sample Preparation

1. Sample must be dissolved in a diluent compatible with initial strength of mobile phase.
2. Sample must be completely in solution and free of particulates.
3. To remove particulates the sample may be filted with a 0.2 µm membrane. If the sample is dissolved in a solvent that contained an organic modifier (e.g., acetronitrile, 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 UPLC and ACQUITY PREMIER Peptide BEH C_{18} Columns is 1 to 12. 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. For example, the use of phosphate buffer at pH 8 or above in combination with elevated temperatures will lead to shorter column lifetimes.

c. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use. Pall Gelman Laboratory Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poorer performance.

d. Pressure

ACQUITY UPLC and ACQUITY PREMIER Peptide BEH Columns can tolerate pressures of up to 15,000 psi (1034 bar or 103 Mpa).

e. Temperature

Temperatures between 20–55 °C are recommended for operating ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18} Columns in order to enhance selectivity, lower solvent viscosity, and increase mass transfer rates. Operating at the extremes of pH, temperature, and/or pressure will result in a shortened column lifetime.

Note: Under certain reversed–phase separation conditions (mobile phase, temperature, etc.) some proteins or peptides may exhibit secondary interactions with the column packing materials or hardware resulting in low recovery or poor peak shape. Repeating several injections of the sample or another protein (e.g., bovine serum albumin) until consistent chromatographic performance is achieved can resolve this issue. Additionally, in order to develop a robust separation method, the analyst should also optimize the separation conditions being used to minimize any observed secondary interactions.

Table 2. Buffer Recommendations for Using ACQUITY UPLC and ACQUITY PREMIER Peptide BEH C_{18}, 130 Å and 300 Å Columns from pH 1 to 12

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_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.
4-Methylmorpholine	~8.4	7.4–9.4	Volatile	Yes	Generally used at 10 mM or less.
Ammonia (NH_4OH)	9.2	8.2–10.2	Volatile	Yes	Keep concentration below 10 mM and temperatures below 30 °C.
Ammonium Bicarbonate	10.3 (HCO_3^-) 9.2 (NH_4^+) 6.3 (H_2CO_3)	6.8–11.3	Volatile	Yes	Used in the 5–10 mM range (for MS work keep source >150 °C). Adjust pH with ammonium hydroxide or acetic acid. Good buffering capacity at pH 10. *Note: Use ammonium bicarbonate (NH_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.
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	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).
(as acetate salt) Pyrrolidine	11.3	10.3–12.3	Volatile	Yes	Used as ion-pair for DNA analysis at pH 7–9. Mild buffer, gives long lifetime.

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

IV. COLUMN CLEANING, REGENERATION, AND STORAGE

a. Cleaning and Regeneration

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

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 3). Flush columns with 20-column volumes of HPLC-grade solvents. Increasing mobile-phase temperature to 35–55 °C increases cleaning efficiency. If the column performance is poor after regenerating and cleaning, call your local Waters office for additional support.

Table 3. Column Cleaning Sequence

Polar Samples	Proteinaceous Samples
Water	Option 1: Inject repeated 100 µL aliquots of dimethylsulfoxide (DMSO) using a reduced flow rate delivering 50% Eluent A and 50% Eluent B
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 (CH3CN)
Isopropanol	Option 3: Flush column with 7M guanidine hydrochloride, or 7M urea

Note: To avoid potentially damaging precipitation within your column (e.g., if your separation eluent contains phosphate buffer), be certain to flush column with 5–10 column volumes of water BEFORE using suggested organic eluent column wash procedures.

b. Storage

For periods longer than four days at room temperature, store the column 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.

V. eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip is a technology that provides the history of a column's performance throughout its lifetime. The eCord is 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.

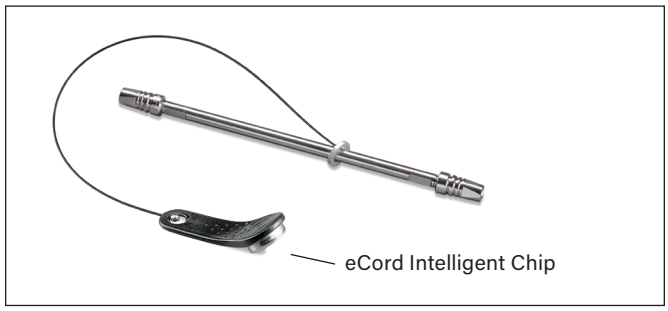


Figure 1. eCord Intelligent Chip.

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. The eCord provides a solution to easily track the history of column usage.

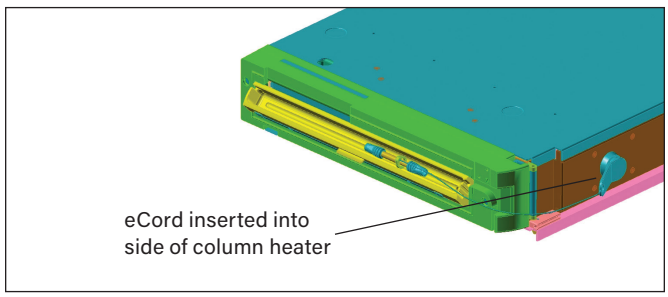


Figure 2. eCord inserted into side of column heater.

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 in Empower™ and MassLynx™ Software allowing the user to access column information on their desktop.

c. Manufacturing Information

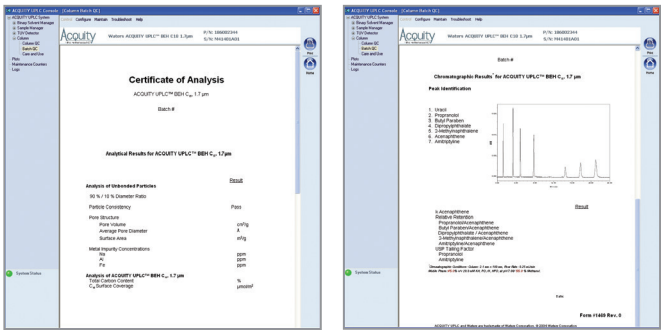


Figure 3. The eCord chip provides the user with an overview of the bulk material QC test results.

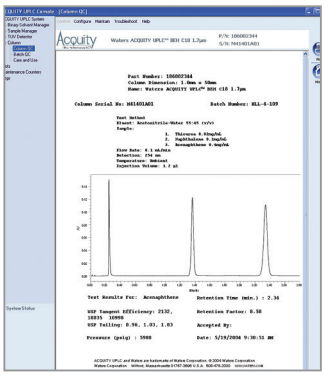


Figure 4. The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition, the QC results and acceptance is placed onto the column.

d. Customer Use Information

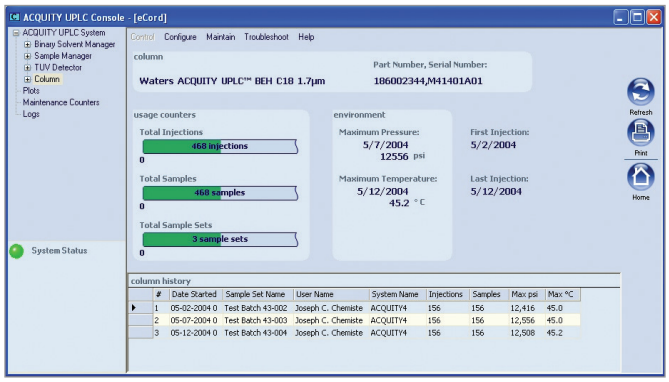


Figure 5 An example of column use information provided by the eCord chip.

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.

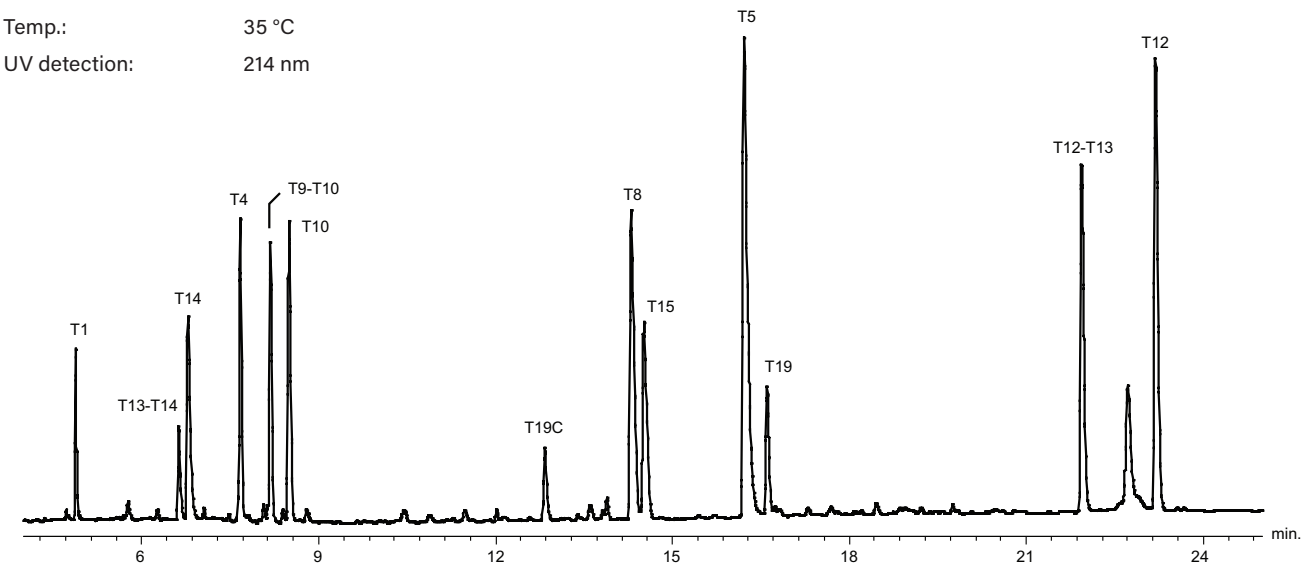
VI. REPRESENTATIVE TEST CHROMATOGRAPH AND CONDITIONS FOR SEPARATION OF PROTEIN DIGEST

Chromatographic Conditions:

Column:	2.1 x 100 mm
Sample:	Tryptic digest of cytochrome *c*
Flow rate:	0.20 mL/min
Mobile phase A:	0.045% TFA in water
Mobile phase B:	0.045% TFA in acetonitrile
Gradient:	from 0–15% B in 6 min, from 15–36% B in 20 min
Temp.:	35 °C
UV detection:	214 nm

Peak Identification:

T1	N–AcGDVEK	T8	TGPNLHGLFGR
T13–T14	KYIPGTK	T15	MIFAGIK
T14	YIPGTK	T5	CAQCHTVEK (heme attached)
T4	IFVQK	T19	EDLIAYLK
T9–T10	KTGQAPGFSYTDANK	T12–T13	GITWGEETLMEYLENPKK
T10	TGQAPGFSYTDANK	T12	GITWGEETLMEYLENPK
T19C	EDLIAY		



VII. 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/sds.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com