ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3, 100 Å Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY™ UPLC™ and/or ACQUITY PREMIER Peptide HSS T3, 100 Å Column that contains our High Strength Silica particle technology to deliver increased peptide retentivity and different selectivity compared to results generated on the Peptide CSH™ C_{18}, 130 Å or Peptide BEH C_{18}, 130 Å and 300 Å column offerings. The manufacture of our Peptide HSS T3 particles begins with ultrapure reagents to control the chemical composition and purity of the final product. Peptide HSS T3 columns are manufactured in a cGMP, ISO 9001:2000 certified plant with each step being conducted within narrow tolerances. Each batch of ACQUITY UPLC and ACQUITY PREMIER HSS T3, 100 Å material is chromatographically tested with acidic, basic, and neutral analytes, as well part of Waters standard batch qualification procedure. In addition, each batch of HSS T3, 100 Å material is QC tested with a gradient separation of a tryptic digest of cytochrome *c* and must pass stringent performance criteria before acceptance for use in an ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3, 100 Å Column. A final QC test for packed bed efficiency is also performed on each column with this as well as the batch test results provided on the column's attached eCord™ Intelligent Chip.

ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3, 100 Å Columns were designed and tested specifically for use on ACQUITY UPLC Systems and will deliver maximum chromatographic performance when used on the holistically-designed ACQUITY Systems.

II. GETTING STARTED

Each ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 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 HSS T3 Column 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 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

Note: The flow rates given in the procedure below are for a typical 2.1 mm I.D. by 50 mm length 1.8 µm column. 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 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 HSS T3 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)

Column length (mm)	Column internal diameter (mm)	
	1.0 mL	2.1
50	0.04 mL	0.2 mL
100	0.08 mL	0.4 mL
150	0.12 mL	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. The ACQUITY PREMIER Columns have MaxPeak™ High Performance Surfaces that can 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. 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).

g. 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.

h. 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 UPLC Chemistries and frits as our 2.1 mm I.D. UPLC Columns. VanGuard Pre-Columns are designed to be attached directly to the inlet side of an ACQUITY UPLC and ACQUITY PREMIER Column.

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

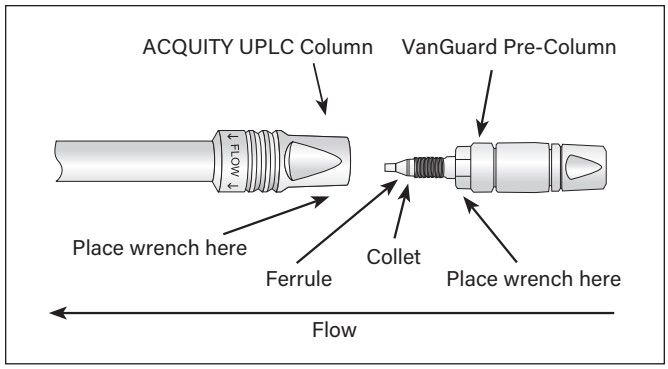

Figure 1. VanGuard Pre-Column installation diagram.

i. Installation Instructions

1. Remove the VanGuard Pre-Column from its box and shipping tube and remove plastic plug.
2. Orient the pre-column so that male end is facing up and carefully remove rubber O-ring that holds collet and ferrule in place during shipping (collet and ferrule are not yet permanently attached).
3. Orient the ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 Column perpendicular to the work surface so that column inlet is on the bottom (column outlet on top).
4. From below, insert the VanGuard Pre-column into the ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 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 the pre-column is now on top.
6. Tighten with two 5/16" wrenches placed onto the ACQUITY UPLC and ACQUITY PREMIER Column flats and the VanGuard Pre-column hex nut (male end) as shown.
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 and ACQUITY PREMIER 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 and ACQUITY PREMIER Peptide HSS T3 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 filtered with a 0.2 µm membrane.

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 15,000 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 HSS T3 Columns is pH 2 to 8. A listing of commonly used buffers and additives is given in Table 2. Additionally, the column lifetime will vary depending upon the operating temperature, and the type and concentration of buffer used. *Note: Working at the extremes of pH, temperature and/or pressure will result in shorter column lifetimes.*

c. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use through a 0.2 µm filter. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poorer performance.

d. Pressure

ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 Columns can tolerate operating pressures up to 18,000 psi (1241 bar or 124 MPa). *Note: Working at the extremes of pressure, pH and/or temperature will result in shorter column lifetimes.*

e. Temperature

The maximum recommended temperature for ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 Columns is 60 °C. Higher temperatures can be used, but may result in shorter column lifetimes. When operating with mobile phases that are close to the lower pH limit, using temperatures above 60 °C may result in shorter column lifetimes due to hydrolysis of the bonded phase. High temperatures should also be avoided when operating close to the upper pH limit, where the dissolution of silica particles starts to occur. To maximize column lifetime near the upper pH limit, temperatures lower than 45 °C are recommended. The rate of silica dissolution also varies with the type and concentration of the buffer, with carbonate and phosphate buffers giving some of the highest rates. As a result, when using these buffers near the upper pH limit, lower temperatures and lower concentrations should be considered. *Note: Working at the extremes of temperature, pressure and/or pH will result in shorter column lifetimes.*

Table 2. Buffer Recommendations for Using ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3, 100 Å Columns from pH 2 to 8

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.

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 3). 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.

b. Storage

For periods longer than four days at room temperature, store reversed-phase ACQUITY UPLC and ACQUITY PREMIER HSS Columns in 100% acetonitrile. For elevated temperature applications, store immediately after use in 100% acetonitrile for the best column lifetime. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10-column volumes of HPLC-grade water (see Table 1 for common column volumes) and replace with 100% acetonitrile for storage. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced. Completely seal column to avoid evaporation and drying out of the bed.

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

Table 3. Reversed-Phase Column Cleaning Sequence

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 7 M guanidine hydrochloride, or 7 M 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 to 10 column volumes of water BEFORE using suggested organic eluent column wash procedures.

IV. 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.

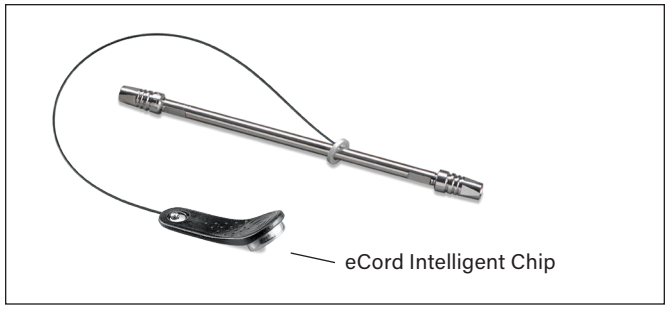

Figure 2. eCord Intelligent Chip.

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.

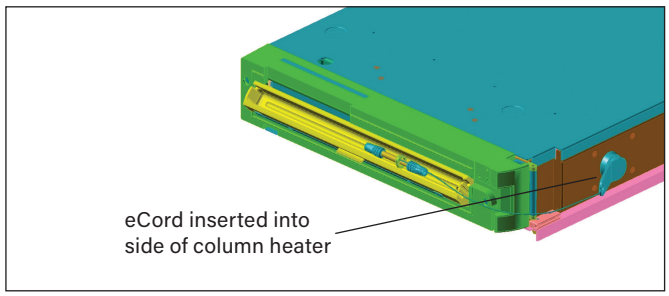

Figure 3. eCord inserted into side of column heater.

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

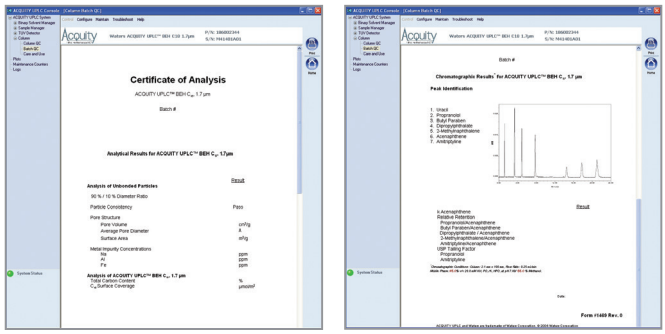

Figure 4. The eCord chip provides the user with an overview of the bulk material QC test results.

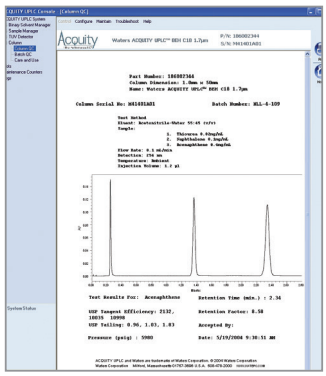

Figure 5. The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition, the QC results and acceptance is placed onto the column.

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.

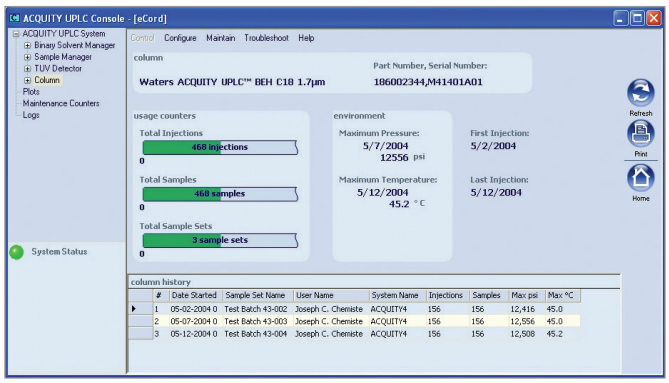

Figure 6. An example of column use information provided by the eCord chip.

V. ADDITIONAL INFORMATION

a. Tips for Maximizing ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 Column Lifetimes

1. To maximize ACQUITY UPLC and ACQUITY PREMIER Peptide HSS T3 Column lifetime, pay close attention to:
   - Water quality (including water purification system)
   - Solvent quality
   - Mobile-phase preparation, storage, and age
   - Sample, buffer, and mobile-phase solubilities
   - Sample quality and preparation
2. When problems arise, often only one improper practice must be changed.
3. Always remember to:
   - Use in-line filter unit or, preferably, a VanGuard Pre-column.
   - Discourage bacterial growth by minimizing the use of 100% aqueous mobile phases where possible.
   - Change aqueous mobile phase every 24–48 hours (if 100% aqueous mobile phase use is required).
   - Discard old 100% aqueous mobile phases every 24–48 hours to discourage bacterial growth.
   - Add 5–10% organic modifier to mobile phase A and adjust gradient profile.
   - Filter aqueous portions of mobile phase through 0.2 µm filter.
   - Maintain your water purification system so that it is in good working order.
   - Only use ultra pure water (18 megohm-cm) water and highest quality solvents possible. HPLC-grade water is not UPLC grade water.
4. Avoid (where possible):
   - 100% aqueous mobile phases (if possible)
   - HPLC-grade bottled water
   - "Topping off" your mobile phases
   - Old aqueous mobile phases. Remember to rinse bottles thoroughly and prepare fresh every 24 to 48 hrs
   - Using phosphate salt buffer in combination with high ACN concentrations (e.g., >70%) due to precipitation
5. Don't: assume a "bad" column is the culprit when high backpressure or split peaks are observed:
   - Investigate cause of column failure
   - Backpressure
   - Mobile phase(s), bacteria, precipitation, and/or samples
   - Peak splitting
   - Sample quality
   - Injection solvent strength
6. Remember: the diameter of UPLC columns (1.0, 2.1, and 3.0 mm I.D.) are often lower than that of a conventional HPLC column and therefore, mobile phases last much longer. To reduce the chances of mobile-phase contamination or degradation, only prepare what you need for analysis or store excess bulk quantities in a refrigerated environment.
7. Mobile-phase-related questions to ask:
   - Am I using 100% aqueous mobile phases? Am I able to add a small amount of organic modifier to my mobile phase A?
   - Do I filter my aqueous mobile phases through 0.2 µm filters?
   - How old is my mobile phase? Do I label the bottle with preparation date?
   - Do I "top off" or do I prepare fresh mobile phases every 24–48 hrs?
   - What is the quality of my water? Has the quality recently changed? How is my water purification system working? When was it last serviced?
   - Am I working with pH 7 phosphate buffer (which is VERY susceptible to bacterial growth)?
8. Sample-related questions to ask:
   - If I inject neat standards prepared in mobile phase do I observe these problems?
   - If I prepare my standards in water and prepare them like samples (e.g., SPE, filtration, etc.) do I still observe these problems?
   - Has the quality of my samples changed over time?

VI. REPRESENTATIVE TEST CHROMATOGRAM

Chromatographic Conditions:

Column:	ACQUITY UPLC Peptide HSS T3, 100 Å, 1.8 µm, 2.1 × 150 mm (p/n: 186008756)
Sample:	Cytochrome c Digestion Standard (p/n: 186006371)
Inject:	7.5 µL
Mobile phase A:	0.045% TFA in water
Mobile phase B:	0.045% TFA in acetonitrile
Temp.:	35 °C
Wavelength:	214 nm

Gradient:

Time	Flow	%A	%B1	Curve
0	0.2	100	0	–
9.0	0.2	85	15	6
39.0	0.2	64	36	6
43.0	0.2	40	60	11
58.0	0.2	100	0	11
67.0	0	100	0	11

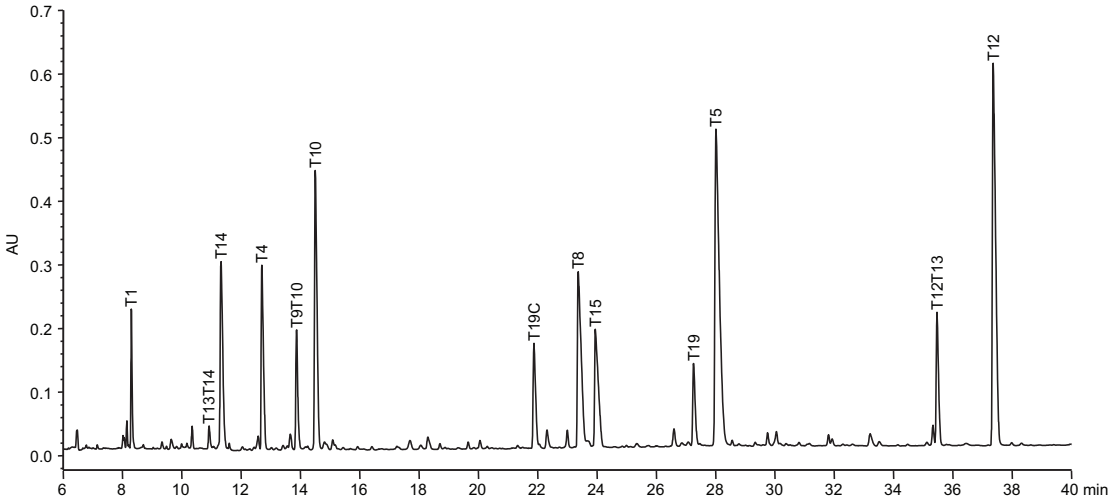

Figure 4. Separation of tryptic digest of cytochrome c on an ACQUITY UPLC HSS T3, 1.8 µm Column.

IX. 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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