Waters™

ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing a Waters™ ACQUITY™ UPLC™ and/or ACQUITY Premier Oligonucleotide BEH™ C_{18} Column designed specifically for use on ACQUITY UPLC™ and ACQUITY Premier Systems. The separation of detritylated oligonucleotides on Waters second generation of hybrid-silica BEH Technology™ particles are based on the well established method on ion-pair, reversed-phase chromatography. ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns are available in several configurations to address different application needs. The ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} packing material is manufactured in a cGMP, ISO 9002 certified plant using ultra pure reagents. Each batch of ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} material is chromatographically tested with acidic, basic, and neutral analytes and the results are held to narrow specification ranges to assure excellent, reproducible performance. In addition, every column is individually tested and the associated Performance Test Chromatogram and Certificate of Acceptance information is available through the attached eCord™ Intelligent Chip Technology.

Note: Optimum performance of ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns is best assured using an appropriately configured Waters ACQUITY UPLC Systems (e.g., See Section II, g). Consequently, use of ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns on conventional HPLC systems is not recommended.

Acquity PREMIER

Acquity UPLC™

II. GETTING STARTED

Each ACQUITY UPLC and ACQUITY Premier Oligonucleotide 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 the batch of packing material contained in the ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} 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 that have been designed to meet stringent tolerance levels and 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

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 (acetonitrile with TEAA or methanol for TEA-HFIP ion-pairing method) 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 at 260 nm have been achieved, proceed to the next section.

c. Column Equilibration

ACQUITY UPLC and ACQUITY Premier Oligonucleotide 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 for the oligonucleotide separation.

Note: When mobile-phase additives are present in low concentrations (*e.g.*, TEAA or TEA-HFIP ion-pairing reagents), 100 to 200 column volumes may be required for complete ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Column equilibration.

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
100	0.4
150	0.5

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.

ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Column performance can be tested with the MassPREP™ Oligonucleotide Standard (p/n: 186004135), a quality controlled synthetic oligonucleotide sample consisting of 15, 20, 25, 30, and 35 mer deoxythymidine. Approximately 0.1 nmol of each oligonucleotide was injected onto the ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Column. Refer to p/n: 715001677 for more information on sample prep for the MassPREP Oligonucleotide Standard. Smaller peaks eluting prior to the main peaks are failure, by-product sequences from the synthesis.

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 www.waters.com.
3. Access technical and scientific information for the column (*i.e.*, certificate of analysis, application notes).

Chromatographic Conditions

UPLC system:	ACQUITY UPLC with installed 425 µL mixer (see Section II, g), PDA Detector with standard UV cell
Sample injected:	Approximately 0.1 nmol of MassPREP Oligonucleotide Standard (p/n: 186004135) diluted in 0.1 M TEAA
Column:	ACQUITY UPLC Oligonucleotide BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Mobile phases:	A: 0.1 M TEAA B: Acetonitrile/0.1 M TEAA, 20/80, v/v
Flow rate:	0.2 mL/min
Gradient delay:	0.45 mL
Gradient:	40 to 59.5% B in 26 minutes (8–11.9% acetonitrile, 0.15% acetonitrile per minute)
Detection:	260 nm, 5 scans per second

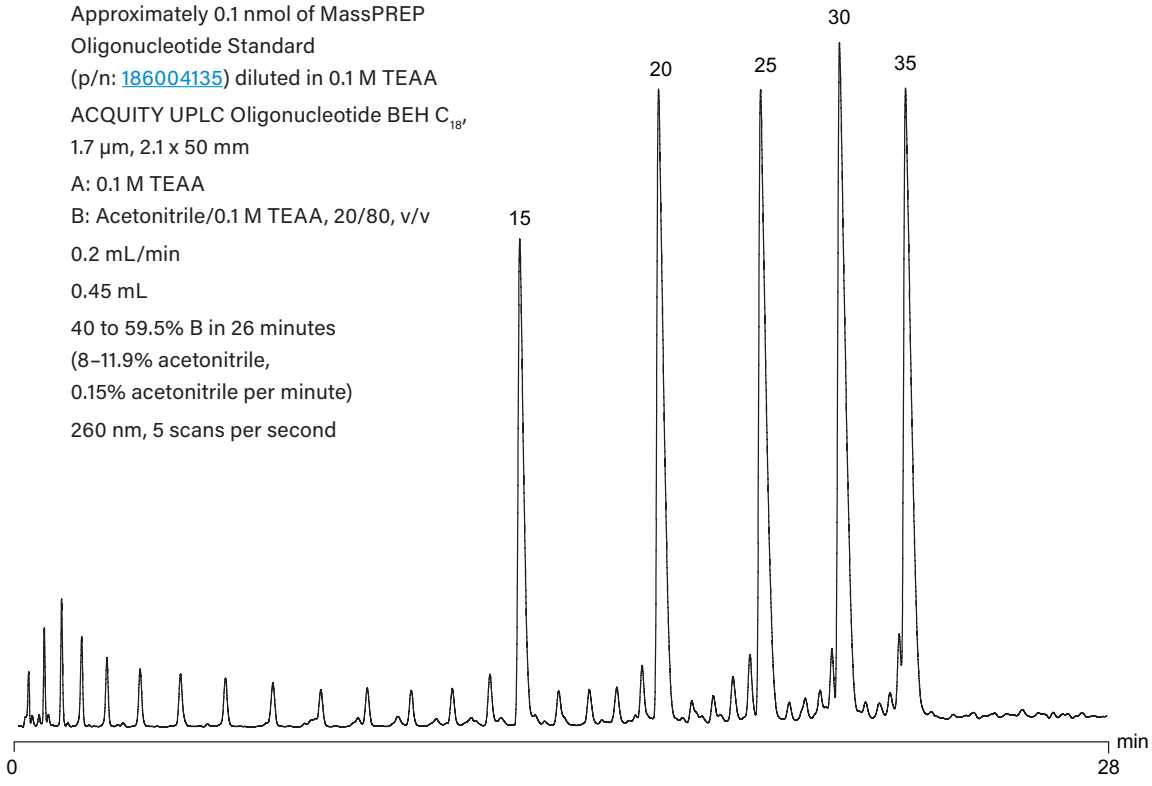

Figure 1. Separation using the MassPREP Oligonucleotide Standard on an ACQUITY UPLC Oligonucleotide BEH C_{18} Column .

h. Vanguard Fit Column Format

Waters Premier Oligonucleotide BEH C_{18} 130 Å and BEH C_{18} 300 Å columns are also now available in our VanGuard™ Fit column format. This column format available only from Waters includes an integrated and easily replaceable 5mm pre-column that serves to protect and extend the life of the full-length analytical column. Unlike when using a separate pre-column with a connector, the VanGuard FIT integrated pre-column design adds no dispersion and has no impact on the resolution of your analytical separations. When chromatography degrades it is often due to fouling wherein particulates from samples, mobile phases and/or the LC system start clogging pores and channels at the head of the column. The VanGuard FIT Cartridge replacements contain the same qualified sorbent as the analytical column and act like a shield protecting the analytical column from these particulates. Should fouling occur, they can easily be replaced and by doing so, restore column performance, extending the life of the analytical column.

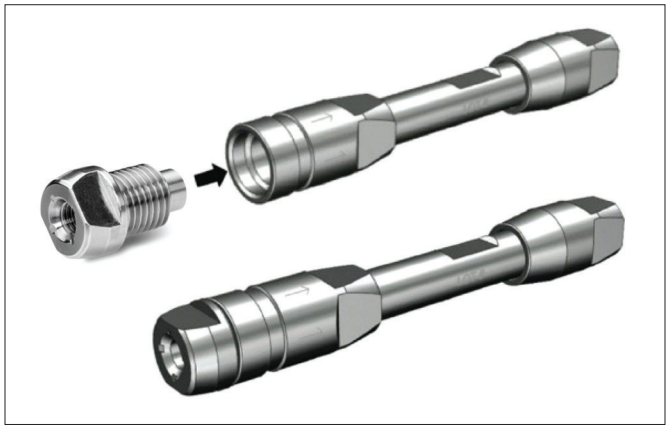

Figure 2: A drawing of a VanGuard FIT Column. It is recommended to use a 3/8" wrench to remove the VanGuard FIT Cartridge.

i. Replacing the VanGuard FIT Cartridge

1. When desired, the single-use, VanGuard Fit Cartridge can be replaced using two 3/8" wrenches. Simply apply the wrenches to the flats on the guard and column end nut and turn in a counterclockwise direction (see Figure 5). This will allow the VanGuard Fit Cartridge to be removed and appropriately discarded following good laboratory practices.
2. A new VanGuard FIT Cartridge can now be used to replace the discarded one. Two additional cartridges are provided with each VanGuard FIT Cartridge shipment and extra cartridges can be obtained separately as needed. Hand-tighten the new cartridge in a clockwise direction, then tighten using two 3/8" wrenches. Proper sealing should not require more than a 1/4 turn past the hand-tightened position.

III. COLUMN USE

To ensure the continued high performance of your ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns, follow these guidelines:

a. Sample Preparation

1. Dissolve the detritylated synthetic oligonucleotide sample in mobile phase A (*e.g.*, 0.1 M TEAA). For example, a 0.05–0.2 µmole scale synthesis can be prepared in 0.1 mL of 0.1 M TEAA. Proportionately larger or smaller volumes of 0.1 M TEAA is required when dissolving samples from different scale syntheses. Due to the nature of gradient separations, relatively large volumes of sample (in low organic strength eluent) can be injected and concentrated onto the head of the column before beginning the gradient elution program.
2. Samples must be completely in solution and free of particulates. Remove all particles from the sample (Controlled Pore Glass Synthesis Support, etc.), which may block the inlet column frit, increase the operating pressure, and shorten the column life time. Sample contamination with high concentration of salts and/or detergents may also interfere with analysis.
3. To remove particulates the sample may be filtered with a 0.2 µm membrane. Be sure that the selected membrane is compatible and does not dissolve with the selected mobile phase diluent. Contact the membrane manufacturer with solvent compatibility questions. An alternative method of particulate removal involves centrifugation for 20 minutes at 8000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial.

b. Recommended Mobile Phases

The most common ion-pair mobile phase for synthetic oligonucleotide separations is based on triethylammonium acetate (TEAA). This mobile phase can be prepared by titrating glacial acetic acid aqueous solution with triethylamine (TEA).

Note: To maximize column life, it is ESSENTIAL that all prepared oligonucleotide mobile phases be filtered through a solvent compatible, 0.2 µm membrane and contained in bottles that are clean and particulate free.

TEAA

One liter of 0.1 M TEAA may be prepared as follows:

1. Perform work in a hood.
2. Add 5.6 mL of glacial acetic acid into 950 mL of water and mix well.
3. Slowly add 13.86 mL of TEA.
4. The pH should be adjusted to pH 7 +/- 0.5 by careful addition of acetic acid.
5. Adjust final volume to 1 L with water.

Alternatively, premixed TEAA can be used (*e.g.*, Sigma 1 M TEAA [p/n: 90357]). Mix 100 mL with 900 mL of water to prepare 1 L of 0.1 M TEAA mobile phase.

Alternative ion-pairing reagents are recommended for improved separation of phosphorothioates or when performing LC-MS analyses. An ion-pairing mobile phase based on triethylamine (TEA) and hexafluoroisopropanol (HFIP) as the buffering acid produces an efficient eluent system for improved separations involving these application types.

As indicated below, two ion-pairing systems are useful.

For routine detritylated oligonucleotide applications, aqueous buffer consisting of 8.6 mM TEA and 100 mM HFIP is effective. For applications such as those involving the separation of G-rich oligonucleotides, it is advisable to use aqueous buffer consisting of 15 mM TEA and 400 mM HFIP (pH 7.9).

TEA-HFIP System 1

One liter of 8.6 mM TEA/100 mM HFIP is prepared as follows:

1. Perform work in a hood.
2. Add 10.4 mL of HFIP (16.8 g) into 988.4 g of water and mix well.
3. Slowly add 1.2 mL of TEA.
4. The pH is approximately 8.3 +/- 0.1.

TEA-HFIP System 2

One liter of 15 mM TEA/400 mM HFIP is prepared as follows:

1. Perform work in a hood.
2. Add 41.56 mL (67.17 g) of HFIP into 956.36 g of water and mix well.
3. Slowly add 2.08 mL (1.52 g) of TEA.
4. The pH of final buffer is approximately 7.9 +/- 0.1.

c. Recommended Injector Wash Solvents

Between analyses, the ACQUITY UPLC System injector and seals can and should be washed with two separate solvents. A 90% water/10% acetonitrile mixture is the recommended strong solvent injector wash solution for the TEAA ion-pairing based method.

A 90% water/10% methanol mixture is the recommended strong solvent injector wash solution for the TEA-HFIP based method.

0.20 µm membrane filtered, LC grade water is the recommended weak wash solvent solution for all ACQUITY oligonucleotide separation methods.

Note: Do not use oligonucleotide separation mobile phases A and B for the respective weak and strong injector wash solvents especially with TEA-HFIP ion pairing systems due to seal incompatibility issues with HFIP.

d. pH Range

The recommended operating pH range for ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns is 1 to 12.

e. Pressure

ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns can tolerate pressures of up to 15,000 psi (1034 bar or 103 Mpa).

f. Temperature

Temperatures between 20 °C–90 °C are recommended for operating ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Columns in order to enhance selectivity, lower solvent viscosity, and increase mass transfer rates.

Note: Operating at elevated pH, temperature, and/or pressure may potentially result in shortened column life.

g. ACQUITY UPLC Mixer Options

The standard Waters ACQUITY UPLC System is equipped with 50 µL in-line mobile phase mixer. For demanding biopolymer separations (*e.g.*, peptide mapping), use of a shallow gradients (*e.g.*, 0.15% mobile phase B change per minute) is required. In these situations, it is recommended that the organic solvent concentration in mobile phase B be reduced by "premixing" with a measured amount of mobile phase A (*e.g.*, mobile phase A= 0.1 M TEAA and mobile phase B= acetonitrile/0.1 M TEAA, 20/80, v/v).

Use of a 425 µL mixer (p/n: 205000403) specifically designed for shallow UPLC gradient separations is recommended when the solvent premixing technique (detailed above) is not used and when mobile phase B contains either 100% acetonitrile (for TEAA ion-pairing method) or 100% methanol (for TEA-HFIP ion-pairing method).

In addition, the Solvent Deliver System Outlet Tube Assembly (p/n: 430001486) is required for 425 µL mixer installation onto a standard ACQUITY UPLC System.

Note: The 425 µL mixer introduces an additional delay volume to gradient separations. For ultra-fast oligonucleotide analyses, the smaller 50 µL mixer should be used with the described premixed mobile-phase technique.

h. Flow Rate

The recommended flow rate for oligonucleotide separations performed on a 2.1 x 50 mm ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Column is 0.2 mL/min. When faster flow rates are desired for separations, use of the 425 µL mixer with installed Outlet Tubing Assembly is recommended.

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 approximately 20-column volumes of 0.2 µm membrane filtered, neat organic solvent (e.g., acetonitrile with the TEAA method or methanol with the TEA/HFIP protocol) is usually sufficient to remove the contaminant.
If the neat organic solvent flushing procedure does not solve the problem, purge the column with 20-column volumes of oligonucleotide mobile phase A followed by 20-column volumes of either 7 M guanidine hydrochloride or 7 M urea. Be sure to flush column with an additional 20-column volumes of 0.2 µm membrane filtered, LC-grade water prior to reuse of oligonucleotide mobile phases. 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 the column in 100% acetonitrile. Immediately after use at elevated temperature and/or pH, store column in 100% acetonitrile for the best column lifetime. Do not store column in highly aqueous (<20% organic) mobile phase, as this may promote bacterial growth. Completely seal column to avoid evaporation and drying out of the packed bed.

V. eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip 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.

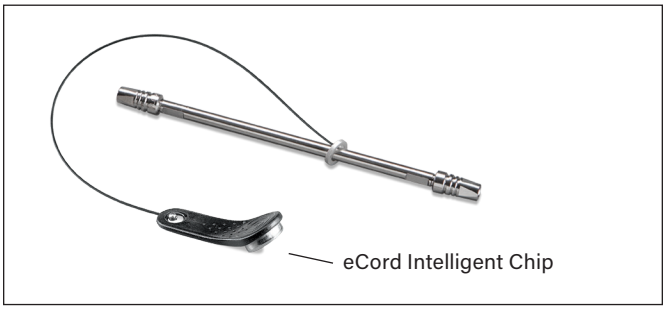

Figure 2. eCord Intelligent Chip.

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

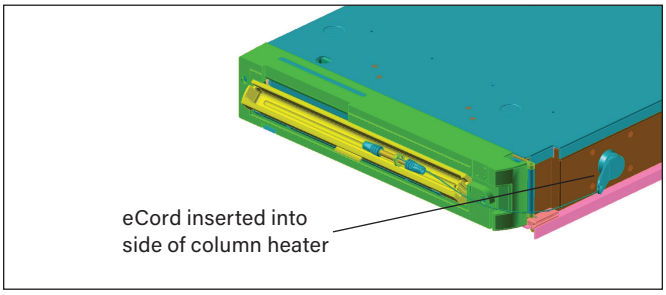

Figure 3. eCord inserted into side of column heater.

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

c. Manufacturing Information

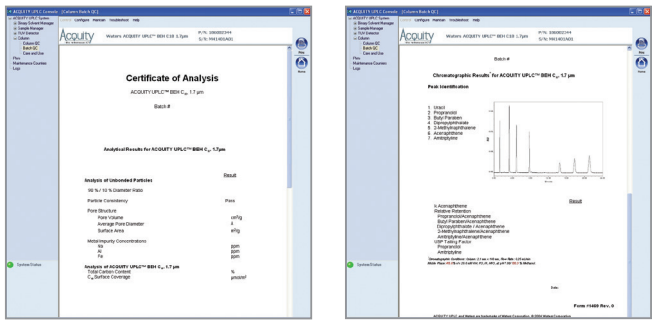

Figure 4. The eCord chip provides the user with an overview of the bulk material QC test results.

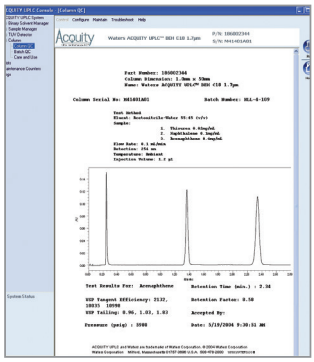

Figure 5. The eCord chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions, and analytes used to test the columns. In addition, the QC results and acceptance is placed onto the column.

d. Customer Use Information

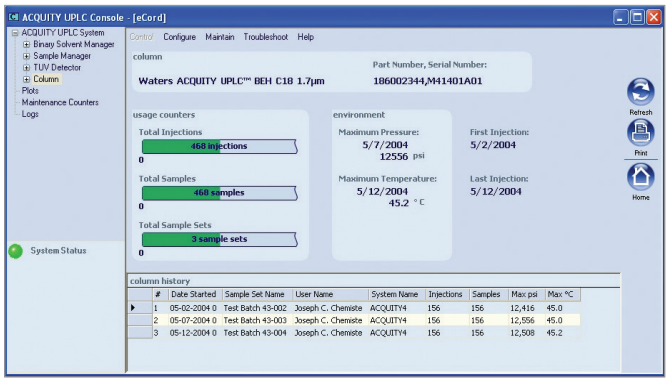

Figure 6. An example of column use information provided by the eCord chip.

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. ADDITIONAL INFORMATION

a. Band Spreading Minimization

Waters ACQUITY UPLC System is designed to have a minimal post column band spreading. If desirable, the mass spectrometer can be connected either directly or in series with a UV (PDA) detector. The connecting tubing internal diameter should be 100 µm or less in order to preserve the achieved separation. Length of the tubing should also be kept to a minimum.

Detritylated synthetic oligonucleotide separations are almost exclusively performed using gradient elution techniques. As such, the effect of pre-column sample band broadening can be minimized by allowing the sample to bind to the column before beginning the actual separation gradient. However, proper connection from the ACQUITY UPLC and ACQUITY Premier Oligonucleotide BEH C_{18} Column outlet to the detector is critical in order to minimize the deleterious effect of p-column sample band spreading. Use of appropriate internal diameter tubing (e.g., 0.005 inch PEEK™ tubing for UV detector applications) is recommended.

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 rest entirely with the purchaser and user. The Safety Data Sheet (SDS) for this product is available at www.waters.com/sds.

Waters™

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