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ACQUITY UPC² BEH, CSH, and HSS Columns

CONTENTS

I. INTRODUCTION

Thank you for choosing an ACQUITY UPC²® Column. The ACQUITY UPC² Columns feature Ethylene-Bridged Hybrid (BEH), Charged-Surface Hybrid (CSH™) and High-Strength Silica (HSS) particle technologies to provide a wide range of selectivities, excellent peak shape, and high efficiency. The ACQUITY UPC² packing materials are designed for use with the ACQUITY UPC² Systems and are manufactured in an ISO 9000 certified plant using ultra-pure reagents. Each batch of ACQUITY UPC² material is tested and the results are held to narrow specification ranges to assure excellent, reproducible performance. Every column is individually tested and a Performance Chromatogram and Certificate of Batch Analysis is provided on the eCord™ Intelligent Chip.

ACQUITY UPC² Columns are designed and tested specifically for use on ACQUITY UPC² Systems. ACQUITY UPC² Columns will exhibit maximum chromatographic performance and benefit ONLY when used on holistically-designed ACQUITY UPC² Systems since these systems and columns were created and designed to operate together. For these reasons, Waters cannot support the use of ACQUITY UPC² Columns on any system other than an ACQUITY UPC² System.

Chemistry	Particle Shape	Particle Size (µm)	Pore Size (Å)	Surface Area (m2/g)	Pore Volume (cc/g)	Carbon Load (%)	Endcapped	Particle Technology
BEH 2-Ethylpyridine (2-EP)	Spherical	1.7, 3.5	130	185	0.7	9	No	BEH
BEH (Unbonded)	Spherical	1.7, 3.5	130	185	0.7	N/A	N/A	BEH
CSH Fluoro-Phenyl	Spherical	1.7, 3.5	130	185	0.7	10	No	CSH
HSS C_{18} SB	Spherical	1.8, 3.5	100	230	0.7	8	No	HSS

Table 1. Physical Characteristics.

II. GETTING STARTED

Each ACQUITY UPC^2 Column comes with a Certificate of Analysis and a Performance Test Chromatogram. The Certificate of Analysis is specific to each batch of packing material and includes the gel batch number, physical characterization, analysis of unbonded particle, analysis of bonded particles, and a SFC chromatographic batch test. The Performance Test Chromatogram is specific to each individual column and contains the following information: gel batch number, column serial number, USP plate count, USP tailing factor, capacity factor, and chromatographic conditions under normal-phase LC conditions. These data should be stored for future reference.

a. Safety Considerations

An SFC column, while in use, is under pressure with CO_2 and possible modifiers as a supercritical fluid. A major safety concern is frostbite caused by adiabatic cooling when CO_2 decompresses from a fluid to a gas at atmospheric pressure. Pay attention to any frosting on the column or system connections. This indicates a leak, usually with temperatures far below 0 °C.

Any small leak could produce a situation where the LEL (lower exposure limit) is reached. Laboratories should be equipped with CO_2 and/or O_2 sensors when carbon dioxide (CO_2) is in use.

b. Column Connectors and Installation

ACQUITY UPC^2 Systems utilize tubing and connectors which have been designed to meet stringent tolerance levels and to minimize extra column volumes. For information on system tubing and connectors, please refer to the ACQUITY UPC^2 System Operator's Guide (Part Number 720004226EN).

Note: *Scale the flow rate up or down accordingly based upon the column i.d., length, particle size, and backpressure of the ACQUITY UPC^2 Column being installed.*

1. Make sure your co-solvent pump is primed and has an adequate solvent/modifier supply before performing injections. It is recommended to use CO_2 with purity level of 99.97% (food grade) and use high-quality chromatography grade solvents (typically methanol) as organic modifier.
2. Connect both the inlet and outlet of the column to the SFC system.
3. If the column is still filled with a solvent, use a low flow rate and back pressure setting (100 bar) to start pumping CO_2 and modifier through the column.
4. If you see frosting on the column at the inlet or outlet, tighten the finger-tight fitting or compression screw on that side. If you continue to see frosting, turn off the CO_2 and vent the system. Allow the column to depressurize fully before disconnecting the inlet or outlet to troubleshoot the leaking issue.

c. 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.

d. Column Equilibration

ACQUITY UPC^2 Columns are shipped dry. Equilibrate the column with a minimum of 10-column volumes of the mobile phase prior to use. (Refer to Table 2 for a listing of empty column volumes.)

e. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture to analyze the column upon receipt. We recommend that weaker solvents are selected as injection solvents. Peak distortion can occur due to strong solvent effects, resulting in lower efficiency values. (Methanol can be considered a strong solvent under SFC conditions.)

2. Determine the number of theoretical plates (N) and use this value as a benchmark for periodic comparisons.

3. Repeat the test at predetermined intervals to track column performance over time. (Slight variations may occur if performance tests are performed on two different ACQUITY UPC^2 Systems due to the quality of the connections, operating environment, system electronics, reagent quality and column condition.)

Empty Column Volumes (mL)	Column Internal Diameter (mm)	
Column Length	**2.1 mm**	**3.0 mm**
50 mm	0.2	0.4
75 mm	0.3	0.6
100 mm	0.4	0.8
150 mm	0.5	1.0

Table 2. Empty Column Volumes in mL (multiply by 10 for f lush solvent volumes).

f. VanGuard Pre-Columns

VanGuard™ Pre-columns are 2.1 mm i.d. x 5 mm length guard column devices designed specifically for use on the ACQUITY UPC^2 Systems. VanGuard Pre-columns are packed with the same chemistries and frits as our 2.1 mm i.d. ACQUITY UPC^2 Columns. VanGuard Pre-columns are designed to be attached directly to the inlet side of an ACQUITY UPC^2 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.*

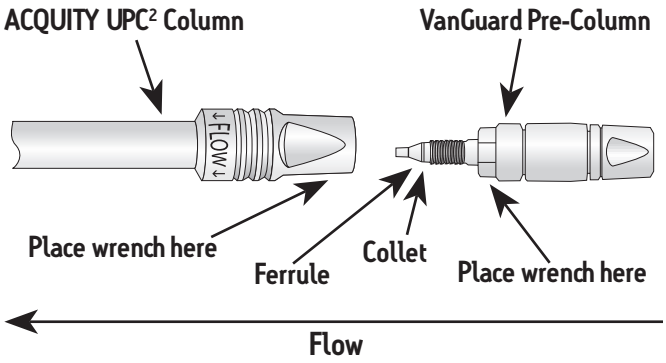

Figure 1. Connecting a VanGuard Pre-column to an ACQUITY UPC^2 Column.

Installation Instructions

1. Remove VanGuard Pre-column from box and shipping tube and remove plastic plug.

2. Orient 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 ACQUITY UPC^2 Column perpendicular to work surface so that column inlet is on the bottom (facing down), with column outlet on top (facing up).

4. From below, insert VanGuard Pre-column into ACQUITY UPC^2 Column inlet and hand-tighten (collet and ferrule are not yet permanently attached).

5. While pushing the VanGuard Pre-column into the column inlet, turn assembled column and pre-column 180° so that pre-column is now on top.

6. Tighten with two 5/16" wrenches placed onto ACQUITY UPC^2 Column flats and VanGuard Pre-column hex nut (male end) as shown above.

7. Tighten 1/4 turn to set collet and ferrule.

8. Check that ferrule is set by loosening connection and inspecting the ferrule depth. A properly set ferrule depth will resemble other connections in the ACQUITY UPC^2 System.

9. Reattach pre-column, connect VanGuard Pre-column and analytical column combination to the ACQUITY UPC^2 System, apply mobile-phase flow and inspect for leaks.

III. COLUMN USE

To ensure the continued high performance of ACQUITY UPC^2 Columns, follow these guidelines:

a. Sample Preparation

1. Sample impurities often contribute to column contamination. Waters offers both solid-phase extraction (SPE) and supercritical fluid extraction (SFE) options. For SPE, use Oasis® Solid-Phase Extraction Cartridges/Columns or Sep-Pak® Cartridges of the appropriate chemistry to cleanup the sample before analysis. For more information, visit to www.waters.com/sampleprep. Alternatively, Waters offers the MV-10 ASFE Supercritical Fluid Extraction System for high throughput extractions from a wide variety of sample matrices.

2. Consider preparing the sample in a weak solvent (such as heptane) for the best peak shape and sensitivity. Using weak sample diluents may avoid peak distortion due to "strong solvent effects" In particular, stronger solvents can impact peak shapes of low retaining analytes.

3. If the sample is not dissolved in the mobile-phase modifier, ensure that the sample, solvent and mobile phases are miscible in order to avoid sample precipitation. Filter sample with 0.2 µm membranes to remove particulates. If the sample is dissolved in a solvent, ensure that the membrane material does not dissolve in the solvent. Contact the membrane manufacturer with solvent compatibility questions. Acrodisc® filters are recommended (refer to the Waters Quality Parts,® Chromatography Columns and Supplies Catalog for additional information). Please consider that some analytes can be retained on certain membrane materials and result in lower recovery (or lower detector signal) than expected. Alternatively, centrifugation for 20 minutes at 8000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial could be considered.

b. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. 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, reduced column lifetime, and compromised performance.

c. Additives

ACQUITY UPC^2 BEH, CSH, and HSS Columns can safely be used with commonly-used acidic and basic additives used in supercritical fluid chromatography (SFC) such as trifluoroacetic acid (TFA), formic acid, ammonium acetate, ammonium formate, ammonium hydroxide, organic amines (such as diethylamine and triethylamine) and ammoniated methanol. Typical concentrations of 20 mM or 0.2% can be used safely. Consider the volatility, solubility and detector compatibility when choosing an appropriate additive. Additives tend to improve peak shape and control the retention characteristics of analytes, but can also impart different selectivities. It is recommended to flush and remove additives and salts from the column before placing the column into storage.

d. Pressure

ACQUITY UPC^2 Column hardware and packing materials can tolerate pressures of up to 18,000 psi (1241 bar or 124 Mpa). Pressures greater than 5000 – 6000 psi should be avoided in order to maximize column and ACQUITY UPC^2 System lifetimes.

e. Temperature

The recommended maximum operating temperature for ACQUITY UPC^2 BEH, CSH, and HSS Columns is 60 °C.

f. Useable Flow-Rate Ranges and Expected ACQUITY UPC^2 System Pressures

Table 3 estimates maximum flow rate and system pressure which can be achieved without over-pressuring the ACQUITY UPC^2 System. The ACQUITY UPC^2 System has a maximum flow rate of 4 mL/minute and maximum pressure of 6000 psi (~400 Bar).

The predicted flow rates and expected backpressure information is based on the ACQUITY UPC^2 System BPR (Backpressure Regulator) set at 1800 psi (~120 Bar).

ACQUITY UPC^2 Columns, 1.7 µm

Dimension	100% CO_2		60/40 CO_2/MeOH	
	Predicted Max Flow Rate (mL/min)	Predicted Max Pressure (psi)	Predicted Max Flow Rate (mL/min)	Predicted Max Pressure (psi)
2.1 x 50 mm	3.45	5581	2.00	6011
2.1 x 75 mm	2.65	5970	1.35	5938
2.1 x 100 mm	2.05	5969	1.05	6060
2.1 x 150 mm	1.40	5951	0.70	6002
3.0 x 50 mm	4.00	4179	3.60	5946
3.0 x 75 mm	3.75	4897	2.60	5959
3.0 x 100 mm	3.45	5499	2.00	5919
3.0 x 150 mm	2.70	5977	1.40	6023

ACQUITY UPC^2 Columns, 3.5 µm

Dimension	100% CO_2		60/40 CO_2/MeOH	
	Predicted Max Flow Rate (mL/min)	Predicted Max Pressure (psi)	Predicted Max Flow Rate (mL/min)	Predicted Max Pressure (psi)
2.1 x 50 mm	4.00	3549	4.00	4963
2.1 x 75 mm	4.00	4096	3.75	5973
2.1 x 100 mm	3.85	4530	3.00	5979
2.1 x 150 mm	3.50	5386	2.10	5946
3.0 x 50 mm	4.00	3042	4.00	3703
3.0 x 75 mm	4.00	3275	4.00	4283
3.0 x 100 mm	4.00	3528	4.00	4906
3.0 x 150 mm	4.00	4063	3.80	5965

Table 3. Useable Flow Rate Ranges and Expected ACQUITY UPC^2 System Pressures.

g. Band Spreading Minimization

The ACQUITY UPC^2 System has been designed to minimize band spreading. Deviation from Waters specified tubing could result in deterioration of chromatographic performance due to band spreading induced by inappropriate tubing i.d. Figure 2 shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen, the larger tubing diameter causes excessive peak broadening and lower sensitivity.

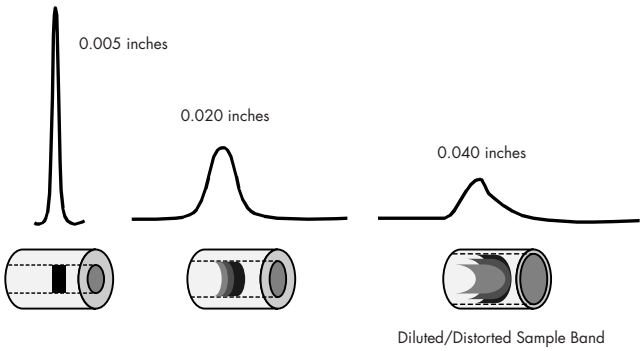

Figure 2: Effect of connecting tubing on system.

IV. TROUBLESHOOTING

1. One of the most common problems with regards to columns is incorrect or insufficient priming of the co-solvent/modifier pump. If no peaks are observed after an injection or unusually long retention times are observed, check the priming of the co-solvent/modifier pump first.
2. If you see frosting on the column at the inlet or outlet, tighten the compression screw on that side. If tightening doesn't work, depressurize the system and the column, and then replace the fitting that is not sealing correctly.
3. If you continue to see frosting on the column, turn off the CO_2 and vent the system. Allow the column to depressurize fully before disconnecting the inlet or outlet. Please contact your Waters representative for additional support.

V. COLUMN CLEANING, AGING, REGENERATING, AND STORAGE

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 high concentrations of 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.

a. Cleaning

Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (see Table 4). Placing the column on an ACQUITY UPLC® System (or HPLC system) will enable the use of a wide range of solvents and water to improve cleaning. Flush columns with 20 column volumes of solvent. Increasing column temperature increases cleaning efficiency. If the column performance is poor after cleaning and regenerating, call your local Waters office for additional support.

Note: *Using an ACQUITY UPC² System is not recommended for the cleaning and regeneration procedures because recommended solvents and high concentrations of water are immiscible with supercritical CO_2. Rather, the use of an ACQUITY UPLC System is recommended for cleaning and regeneration.*

Polar Samples	Non-Polar Samples
1. Water (could also be a mixture of acetonitrile and water)	1. Isopropanol (or an appropriate water/ Isopropanol mixture)
2. Methanol	2. Tetrahydrofuran
3. Tetrahydrofuran	3. Dichloromethane
4. Methanol	4. Hexane
5. Water	5. Isopropanol (followed by an appropriate water/ isopropanol mixture)
6. Mobile Phase	6. Mobile Phase

Table 4: Column Cleaning Sequence.

Retest the column after using the cleaning procedure to determine if the specific problem has been fixed. If so, continue using the column, avoiding samples and solvents that may clog the column inlet.

b. Aging

Research at the Waters Corporation confirms that there may be changes in retention with SFC columns due to packing material surface changes. This is commonly referred to by SFC industry experts as "Aging." Mobile-phase additives and methanol can cause changes to the silica or hybrid surface resulting in retention shifts. When working with a new column, the column can be flushed with CO_2/methanol to eliminate or reduce further shifts in retention. Storage in 100% CO_2 is recommended to ensure the column retention and selectivity will be the same from the end of one test to the beginning of another. The 100% CO_2 will halt the aging process and results in no additional retention shifts.

c. Regeneration

If you have noticed a significant change in retention or selectivity, an additional regeneration process may restore your column's performance. There are two main sources of retention or selectivity change for ACQUITY UPC² Columns, assuming no damage has been done to the column bed. Mobile-phase additives and methanol can cause changes to the silica or hybrid surface. The effects can be gradual or expeditious and may be permanent.

Columns showing peak shape, retention or selectivity changes are best cleaned and regenerated on an ACQUITY UPLC System. Before placing the column to be regenerated on a LC system, it should be free of mobile-phase co-solvents and additives. To do this, flow 100% CO_2 through the column (3.0 x 100 mm) at 1 mL/min for five minutes (40 °C and 2000 psi at the ABPR). For other column dimensions, the flow rate should be recalculated appropriately to avoid overpressuring the system.

Based on the stationary phase to be regenerated, different rinsing agents should be used as to not damage the packing material. High-pH rinsing agents (pH > 8) should be avoided for cleaning silica-based materials. Low-pH rinsing agents (pH < 1) should be avoided for cleaning silica-based materials with ligand bondings.

Step 1. Unbonded and bonded silica-based materials should be rinsed with 200+ column volumes of pure water. All hybrid-based materials (e.g., BEH and CSH) can be rinsed with either 200+ column volumes of pure water or 30+ column volumes of 0.1% N,N-Dimethylbutylamine (or similar tertiary amine) in water.

Step 2. All columns should be rinsed with an additional 10-column volumes of 90/10 IPA/water to remove the mobile-phase additives.

Step 3. All columns should be rinsed with 10-column volumes of isopropanol to remove any residual water before returning the column to an ACQUITY UPC^2 System.

After regeneration, please reinstall and equilibrate the ACQUITY UPC^2 Column on the ACQUITY UPC^2 System. Retest the column after using the cleaning procedure to determine if the specific problem has been fixed.

d. Storage

Purging and storing a column in pure CO_2 is recommended. This will eliminate or minimize retention changes of the column during storage. Storing the column in methanol is not recommended.

VI. INTRODUCING ECORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip will provide 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 3. 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.

b. Installation

Install the column into the ACQUITY® Column Manager. 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.

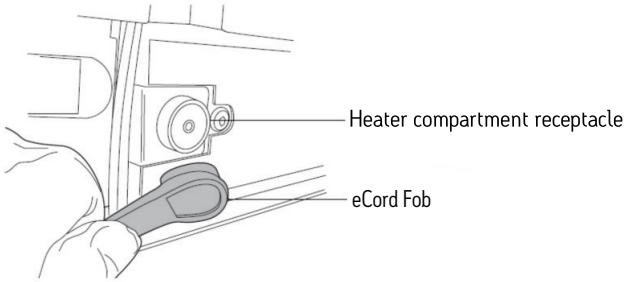

Figure 4. Installing the eCord Intelligent Chip.

c. Manufacturing Information

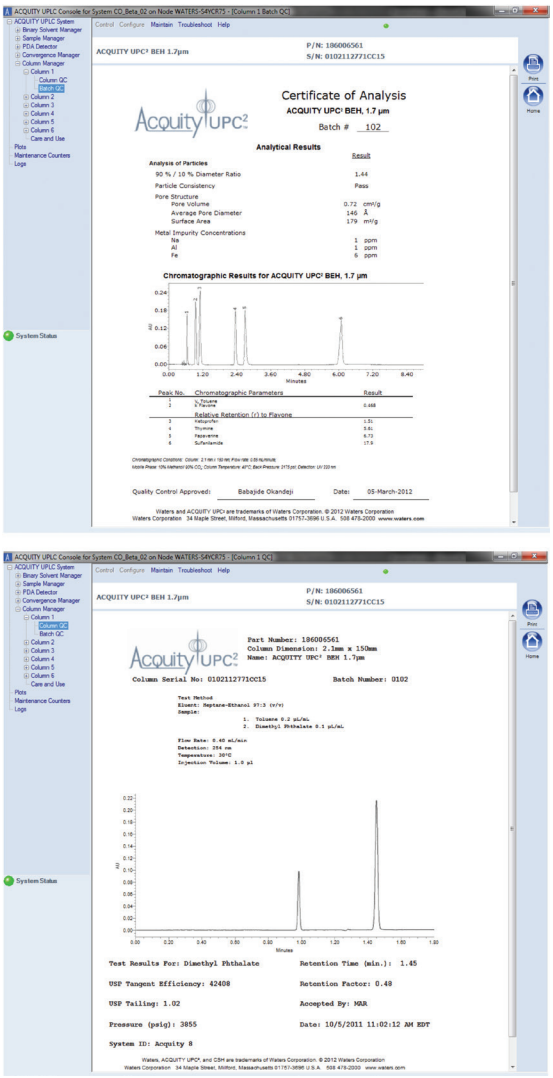

Figure 5: Manufacturing results stored on an eCord.

d. Column Use Information

The eCord Chip provides the customer with column use data, 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. Up to 50 sample sets can be stored on the eCord Chip. In addition, the eCord provides two-way communications between the eCord Chip and Empower® Software.

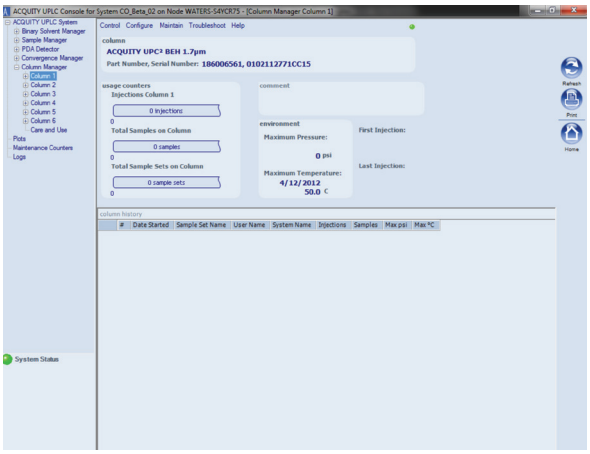

Figure 6. Column use information.

VII. ACQUITY UPC² COLUMN FAMILY

UPC² BEH, CSH, and HSS Columns Care & Use Manual is one of three manuals for the ACQUITY UPC² Column family. The other two Care & Use Manual are available at www.waters.com/CU

ACQUITY UPC² Trefoil™ Columns Care & Use Manual
- 720004828EN

ACQUITY UPC² Torus™ Columns Care & Use Manual
- 720005203EN

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THE SCIENCE OF WHAT'S POSSIBLE.®

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