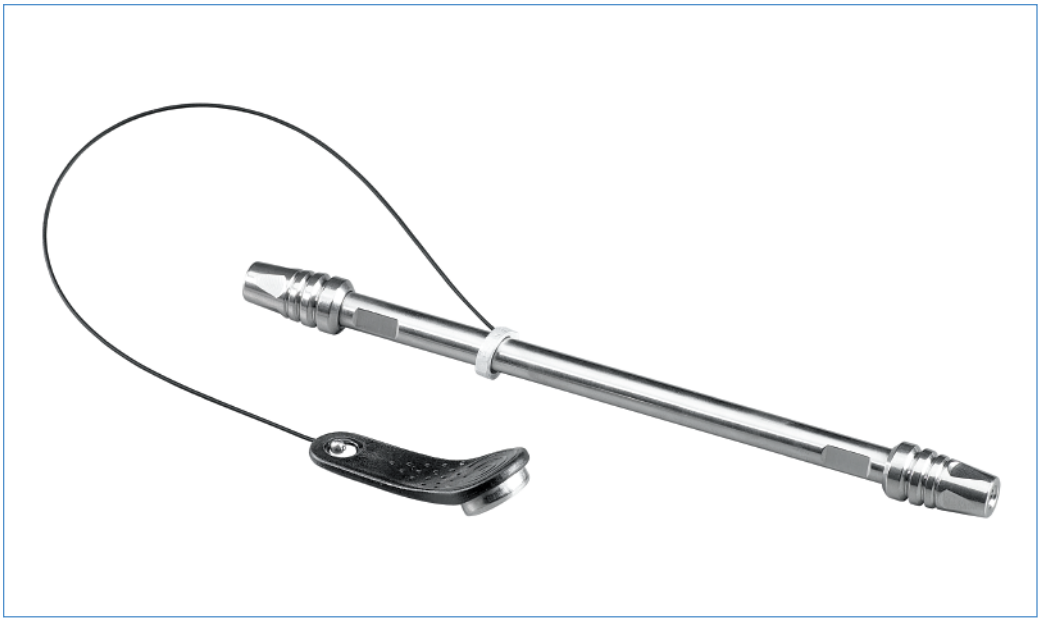
Waters

ACQUITY UPLC™ BEH Column

Care and Use Instructions



Thank you for choosing a Waters ACQUITY UPLC™ BEH column. The ACQUITY UPLC™ BEH packing materials were designed specifically for use with the Waters ACQUITY UPLC™ system and are manufactured in a cGMP, ISO 9002 certified plant using ultra pure reagents. Each batch of ACQUITY UPLC™ BEH material is tested chromatographically with acidic, basic and neutral analytes 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 are provided on the eCord™ intelligent chip.

Acquity
Ultra Performance LC™

Contents

I. GETTING STARTED

Each ACQUITY UPLC™ BEH column comes with 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™ BEH 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 (part number 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 (part number 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 1.7 µm column. For 1.0 mm i.d columns, scale the flow rate using the calculation shown in section III. Additional flow rate and pressure guides are provided in section VI (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™ BEH 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	Internal Diameter	
(mm)	1.0 mm	2.1 mm
20	0.016	0.07
30	0.024	0.1
50	0.04	0.2
100	0.08	0.4
150	0.12	0.5

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. 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.

e. 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.

II. COLUMN USE

To ensure the continued high performance of ACQUITY UPLC™ BEH columns, follow these guidelines:

a. Sample Preparation

1. Sample impurities often contribute to column contamination. One option to avoid this is to use Waters Oasis® solid-phase extraction cartridges/columns or Sep-Pak® cartridges of the appropriate chemistry to clean up the sample before analysis.
2. It is preferable to prepare the sample in the operating mobile phase or a mobile phase that is weaker (less organic modifier) than the mobile phase for the best peak shape and sensitivity.
3. 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.
4. Filter sample with 0.2 µm membranes to remove particulates. 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 8,000 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™ BEH 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.

Table 2. Buffer Recommendations for Using ACQUITY UPLC™ BEH Columns from pH 1 to 12

Additive/Buffer	pKa	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.
Borate	9.2	8.2 – 10.2	Non-Volatile	No	Reduce temperature/concentration and use a guard column to maximize lifetime.
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 (as acetate salt)	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). Used as ion-pair for DNA analysis at pH 7-9.
Pyrrolidine	11.3	10.3 – 12.3	Volatile	Yes	Mild buffer, gives long lifetime.

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.

Degas all solvents thoroughly before use to prevent bubble formation in the pump and detector. The use of an on-line degassing unit is also recommended. This is especially important when running low pressure gradients since bubble formation can occur as a result of aqueous and organic solvent mixing during the gradient.

d. Pressure

ACQUITY UPLC™ BEH columns can tolerate pressures of up to 15,000 psi (1034 bar or 103 Mpa).

e. Temperature

Temperatures between 20 °C – 55 °C are recommended for operating ACQUITY UPLC™ BEH 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.

III. SCALING UP/DOWN ISOCRATIC METHODS

The following formulas will allow scale up or scale down, while maintaining the same linear velocity, and provide new sample loading values:

If column i.d. and/or length are altered:

$$F_2 = F_1 (r_2/r_1)^2$$

$$Load_2 = Load_1 (r_2/r_1)^2(L_2/L_1)$$

$$\text{Injection volume}_2 = \text{Injection volume}_1 (r_2/r_1)^2 (L_2/L_1)$$

Where:

r = Radius of the column

F = Flow rate

L = Length of column

1 = Original, or reference column

2 = New column

IV. 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 below). 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	Non-polar Samples	Proteinaceous Samples
1. water	1. isopropanol (or an appropriate isopropanol/water mixture*)	Option 1: Inject repeated aliquots of dimethyl sulfoxide (DMSO)
2. methanol	2. tetrahydrofuran (THF)	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)
3. tetrahydrofuran (THF)	3. dichloromethane	
4. methanol	4. hexane	
5. water	5. isopropanol (followed by an appropriate isopropanol/water mixture*)	Option 3: Flush column with 7M guanidine hydrochloride, or 7M urea
6. mobile phase	6. mobile phase	

** Use low organic solvent content to avoid precipitating buffers.*

b. Storage

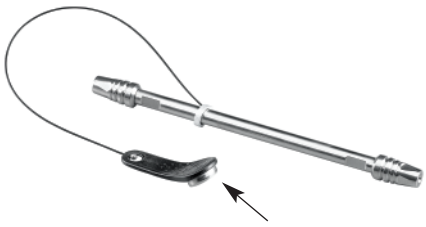
For periods longer than four days at room temperature, store the column in 100% acetonitrile. For elevated temperature applications, store immediately after use in 100% acetonitrile for the best column lifetime. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush 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™

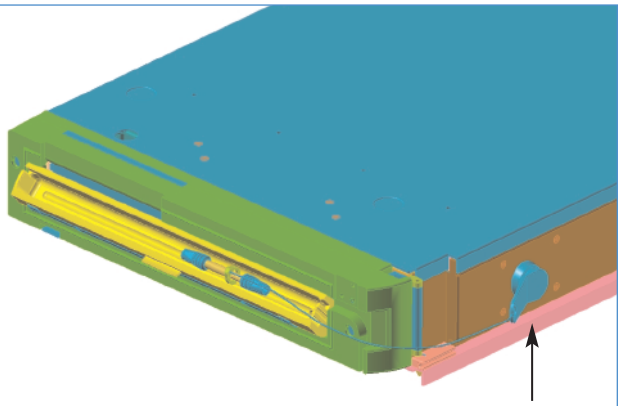
a. Introduction

The eCord™ intelligent chip is a new technology that 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.



Waters 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.

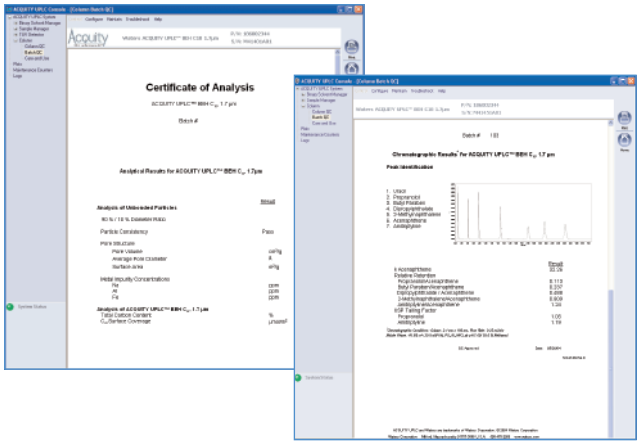


eCord™ inserted into side of column heater

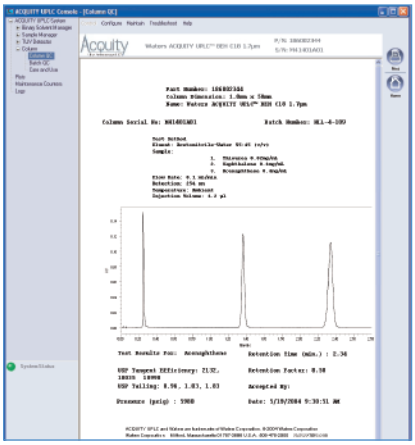
b. Installation

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

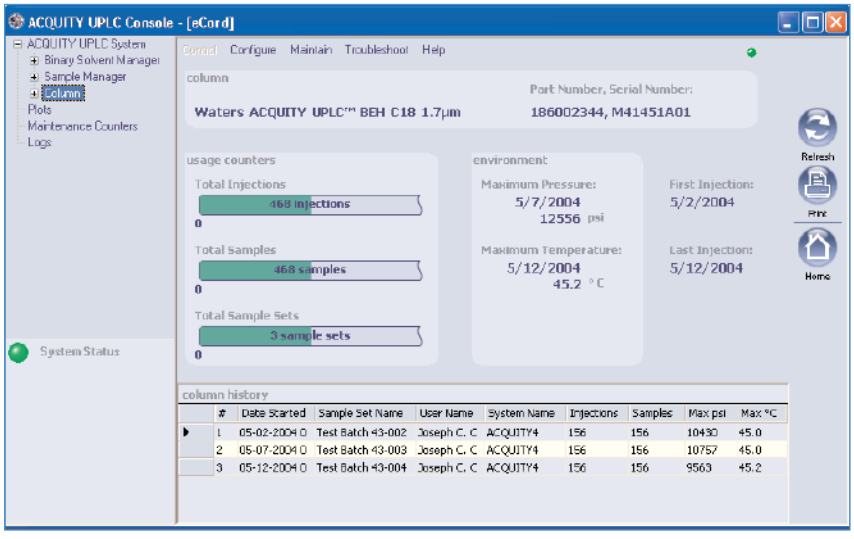


The eCord™ chip provides the user with an overview of the bulk material QC test results.



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



The eCord™ chip 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. Recommended flow rates and expected backpressures for ACQUITY UPLC™ BEH 1.7µm columns.

1.0 mm ID Columns (40 °C)

UPLC Linear Velocity (mm/sec)	3		4		5		6	
Column Dimensions	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)
1.0 x 20 mm	0.10	1800	0.13	2300	0.17	3100	0.20	3600
1.0 x 30 mm	0.10	2600	0.13	3400	0.17	4500	0.20	5300
1.0 x 50 mm	0.10	4300	0.13	5600	0.17	7400	0.20	8700
1.0 x 100 mm	0.10	8600	0.13	11200	0.17	14600	0.20	17200

2.1 mm ID Columns (40 °C)

UPLC Linear Velocity (mm/sec)	3		4		5		6	
Column Dimensions	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)	Flow Rate (mL/min)	Backpressure (psi)
2.1 x 20 mm	0.45	2200	0.60	2900	0.75	3600	0.90	4400
2.1 x 30 mm	0.45	3000	0.60	4100	0.75	5100	0.90	6100
2.1 x 50 mm	0.45	4800	0.60	6400	0.75	8000	0.90	9500
2.1 x 100 mm	0.45	9100	0.60	12100	0.75	15200	0.90	18200

Note: 1) ACQUITY UPLC™ BEH 1.7 µm particle columns

2) ACN/Aqueous gradient, P_{max} at ~30% ACN

3) Approximate maximum total system backpressure given

4) Actual backpressures may vary

Sales Offices:

Australia
Waters Australia Pty. Limited
Tel: 2 9933 1777
Fax: 2 9898 1455

Austria and European Export (Central South Eastern Europe, CIS and Middle East)
Tel: 431 877 18 07
Fax: 431 877 18 08

Belgium
Waters S.A.-N.V.
Tel: 32 2 726 1000
Fax: 32 2 726 1100

Brazil
Waters Technology
Tel: 55 11 5543 7788
Fax: 55 11 5093 1624

Canada
Waters Limited
Tel: 800 252 4752
Fax: 905 678 9237

China
Waters China Limited
Tel: 86-10-84518918
Fax: 86-10-84518928

CIS/Russia
Tel: (7) 095 931 9193
Fax: (7) 095 336 7000

Czech Republic
Tel: 42 02 617 11384
Fax: 42 02 617 11386

Denmark
Waters A/S
Tel: 45 46 59 8080
Fax: 45 46 59 8585

Finland
Waters Oy
Tel: +358 9 506 4140
Fax: +358 9 506 41411

France
Waters S.A
Tel: (33) 1 30 48 72 00
Fax: (33) 1 30 48 72 11

Germany
Waters GmbH
Tel: 49 6196 400600
Fax: 49 6196 482388

Hungary
Tel: 36 1 350 5086
Fax: 36 1 350 5087

Hong Kong
Waters China Ltd.
Tel: 852 29 64 1800
Fax: 852 25 49 6802

India
Waters India Pvt. Ltd.
Tel: 91 80 2 837 1900
Fax: 91 80 2 839 2157

Ireland
Waters Chromatography Ireland Ltd.
Tel: 353 1 448 1500
Fax: 353 1 448 1510

Italy
Waters S.P.A.
Tel: 39 02 274 211
Fax: 39 02 250 1827

Japan
Nihon Waters K.K.
Tel: (81) 3 3471 7191
Fax: (81) 3 3471 7116

Korea
Waters Korea Limited,
Tel: (82) 2 820 2700
Fax: (82) 2 820 8730

Mexico
Waters S.A. DE C.V.
Tel: 5255 5524 7636
Fax: 5255 5524 9376

The Netherlands
Waters Chromatography B.V.
Tel: +31 (0)76-50 87 200
Fax: +31 (0)76-50 87 280

Norway
Waters AS
Tel: 47 63 84 60 50
Fax: 47 63 84 60 51

Poland
Tel: (48) 22 833 4400
Fax: (48) 22 833 0987

Puerto Rico
Waters Technologies Corporation
Tel: 787 747 8445
Fax: 787 747 8448

Singapore
Waters Asia Ltd.
Tel: 65 6278 7997
Fax: 65 6278 7557

Spain
Waters Cromatografía, S.A.
Tel: 34 93 600 93 00
Fax: 34 93 600 93 60

Sweden
Waters Sverige AB
Tel: 46 8 555 11500
Fax: 46 8 555 11550

Switzerland
Waters AG
Tel: 41 62 889 2030
Fax: 41 62 889 2059

Taiwan
Tel: 886 2 2543 1898
Fax: 886 2 2543 1918

United Kingdom
Waters U.K. Ltd.
Tel: 44 208 238 6100
Fax: 44 208 207 7070

All Other Countries

Waters Corporation
34 Maple Street
Milford, MA 01757 USA
Tel: 508 478 2000
800 252 4752
Fax: 508 872 1990

http://www.waters.com

The quality management system of Waters' manufacturing facilities in Taunton, Massachusetts and Wexford, Ireland complies with the International Standard ISO 9001:2000 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.

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