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# ACQUITY APC Columns

## CONTENTS



## I. INTRODUCTION

Thank you for choosing an ACQUITY APC™ Column. The ACQUITY APC Columns are designed to achieve maximum separation performance when used with the ACQUITY® Advanced Polymer Chromatography™ (APC™) System. Please take a few moments to read this manual carefully. Following the recommendations in this manual will prolong column life and enhance chromatographic reproducibility.

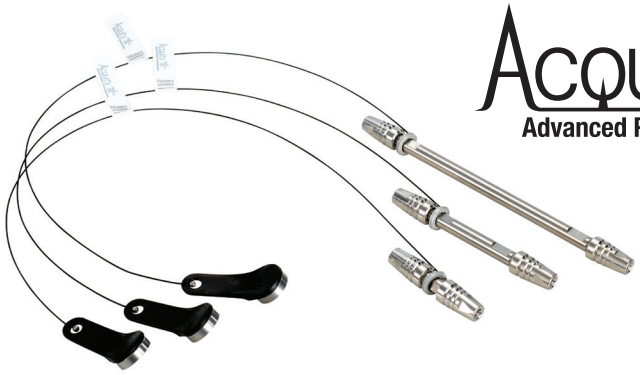
ACQUITY APC Columns are packed with sub-3-µm Ethylene Bridged Hybrid (BEH) particles that provide mechanical strength, packed-bed stability and high separation efficiency. They deliver superior chromatographic performance for all polymer classes, including low molecular weight aqueous and organic soluble polymers, up to a molecular weight of 2,000,000 g/mole (Table 1).

To maximize separation selectivity and performance ACQUITY APC Columns are available in two bonding technologies optimized for each solvent class:

**ACQUITY APC XT:** Packed with a high-coverage trimethyl silane bonded to an Ethylene Bridged Hybrid (BEH) substrate. These columns are recommended for extended temperature (< 90 °C) separations in organic solvents.

**ACQUITY APC AQ:** Feature unbonded Ethylene Bridged Hybrid (BEH) substrate. Recommended for room temperature (< 45 °C) separations in aqueous solvents.

ACQUITY APC Columns are manufactured in an ISO 9001 facility and are held to narrow specification ranges to ensure reproducible performance. Every column is individually tested and a Certificate of Batch Analysis is provided on the attached eCord™ Intelligent Chip.



Acquity
Advanced Polymer Chromatography™

**Table 1. Physical Characteristics**

| | Recommended Separation Solvent | Temperature Limit (°C) | Pore Size (Å) | dp (µm) | Linear Range (g/mole)* |
|---|---|---|---|---|---|
| ACQUITY APC XT 45 | Organic | 90 | 45 | 1.7 | 200 - 5,000 |
| ACQUITY APC XT 125 | Organic | 90 | 125 | 2.5 | 1,000 - 30,000 |
| ACQUITY APC XT 200 | Organic | 90 | 200 | 2.5 | 3,000 - 70,000 |
| ACQUITY APC XT 450 | Organic | 90 | 450 | 2.5 | 20,000 - 400,000 |
| ACQUITY APC AQ 45 | Aqueous | 45 | 45 | 1.7 | 200 - 5,000 |
| ACQUITY APC AQ 125 | Aqueous | 45 | 125 | 2.5 | 1,000 - 30,000 |
| ACQUITY APC AQ 200 | Aqueous | 45 | 200 | 2.5 | 3,000 - 70,000 |
| ACQUITY APC AQ 450 | Aqueous | 45 | 450 | 2.5 | 20,000 - 400,000 |

**Linear range based on polystyrene standards*

## II. GETTING STARTED

### a. Preparing the System

Before attaching the column, the system must be prepared as follows:

1. Remove the old columns and connect the tubing ends with a zero-dead-volume connector.
2. Convert the system to the solvent required for the separation by flushing the system and injector pathway to remove old solvents.

For additional information, refer to the ACQUITY APC System's operator guide.

### b. Column Connections

The ACQUITY APC System uses tubing and connectors that have been designed to meet stringent tolerances to minimize extra-column volume within the system. It is highly recommended that you use the column connection hardware that is supplied with the system, and, when needed, replace with original manufacturer's hardware. For applications that require a bank of columns connected in series, a u-shaped column-joining tube (which has been optimized to fit within the column heater compartment) is available separately.

### c. Column Installations

Generally, analytical results are independent of the sequence in which a column bank is arranged. However, to improve resolution and column life, arrange the columns in order of decreasing pore size, with the largest pore size closest to the injector. This is recommended because the species with the highest molecular weight in the sample contributes the most to the viscosity of the sample. If the largest species are separated first, the viscosity of the sample plug decreases more quickly, placing less strain on the column bank. In the case of higher molecular weight polymers, there is less chance of shear degradation of the polymer sample.

To install the columns:

1. Remove the end plugs from each column and save them.
2. Connect the first column to the injector outlet tubing. Note the direction of flow. A flow direction arrow is etched on the inlet side end nut of the column.
3. Finger-tighten the fitting, then tighten with a wrench by another turn using the flats machined into the column end nut. Do not use a wrench on the column tubing. Figure 1 shows a proper tubing-to-column connection.
4. Connect the next column to the previous column using u-shaped tube connectors. Ensure that the solvent flow continues in the direction shown on the column end fittings. Thread the inlet and outlet fittings of the u-shaped tube until finger tight, then tighten with a wrench.
5. Repeat Step 4 until all columns in the bank are connected.
6. Connect the last column to the detector inlet tubing.

In a proper tubing/column connection (Figure 1), the tubing touches the bottom of the column endfitting, with no void between them.

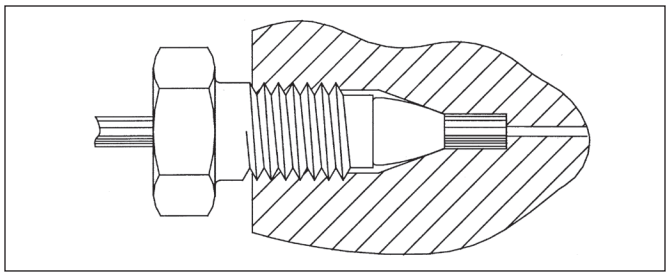


*Figure 1. Proper Tubing Column Connection.*

The presence of a void in the flow stream reduces column performance. This can occur if a Parker ferrule is connected to a Waters style endfitting (Figure 2).

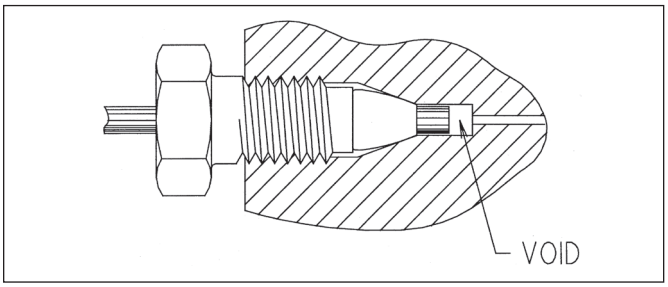



*Figure 2. Parker Ferrule in a Waters Style Endfitting.*

### d. eCord Installation

Attach the eCord button for each column to the side of the column heater module noting their sequence. Up to four columns can be installed at one time. The eCord button is magnetized and does not require specific orientation. Once connected, column identification and usage will be available though the instrument's control software.

### e. Column Equilibration

ACQUITY APC Columns are shipped dry giving the user full option of the solvent needed for the separation. Equilibrate the column with a minimum of 20-column volumes, or until a stable detector baseline is achieved. For a column bank, use the sum of the column volumes in series to determine the total equilibration volume. Refer to Table 2 for a listing of column volumes.

**Table 2. Empty Column Volumes in mL**

| Column Length (mm) | Column Internal Diameter (mm) | Volume |
|---|---|---|
| 30 | 4.6 | 0.50 |
| 50 | 4.6 | 0.83 |
| 75 | 4.6 | 1.25 |
| 150 | 4.6 | 2.50 |

The rigid hybrid particle bed used for the ACQUITY APC Columns allows the user to rapidly switch solvents without damaging the column packing material. Changing solvents works best between compatible solvents. For example, when changing between two immiscible solvents, an intermediate solvent/co-solvent that is miscible in both initial and final conditions should be used. For highly viscous solvents, reduce the flow rate to avoid over pressuring the system. Once the exchange is complete, equilibrate the column using the final solvent conditions with a minimum of 20-column empty volumes, or until a stable detector baseline is achieved.

## III. COLUMN USE

### a. Guidelines

ACQUITY APC Columns have a finite lifetime directly related to their care and use. Column lifetime is reduced by contamination from samples and eluents; improper handling and storage; and exceeding operational conditions. To maximize ACQUITY APC Column lifetime, pay attention to these guidelines:

- For best resolution and maximum column life, do not exceed the flow rate recommendations found in Section III c.
- Protect the column from vibration and mechanical shock.
- Avoid precipitation by dissolving samples in the mobile phase. If the sample is not dissolved in the mobile phase, ensure that the sample, solvent and mobile phase are miscible to avoid precipitation.
- Always use high-quality, particle-free, HPLC grade solvents.
- Dedicate, whenever possible, the column to specific applications. Frequent switching of samples and solvents accelerates column deterioration and loss of resolution.
- Exceeding the upper temperature limit of the column.
- For aqueous mobile phases, take steps to avoid bacterial contamination. For additional information refer to "Controlling Contamination in UPLC/MS and HPLC/MS Systems", Waters part number 715001307.

## b. Calibration

Whenever replacing a single column of a complete column bank, generate a new calibration curve to ensure the reproducibility of the application. Figure 3 shows typical calibration curves for each column. The calibration curves were obtained with polystyrene standards. Figure 4 shows the expected linear range based on the calibration data.

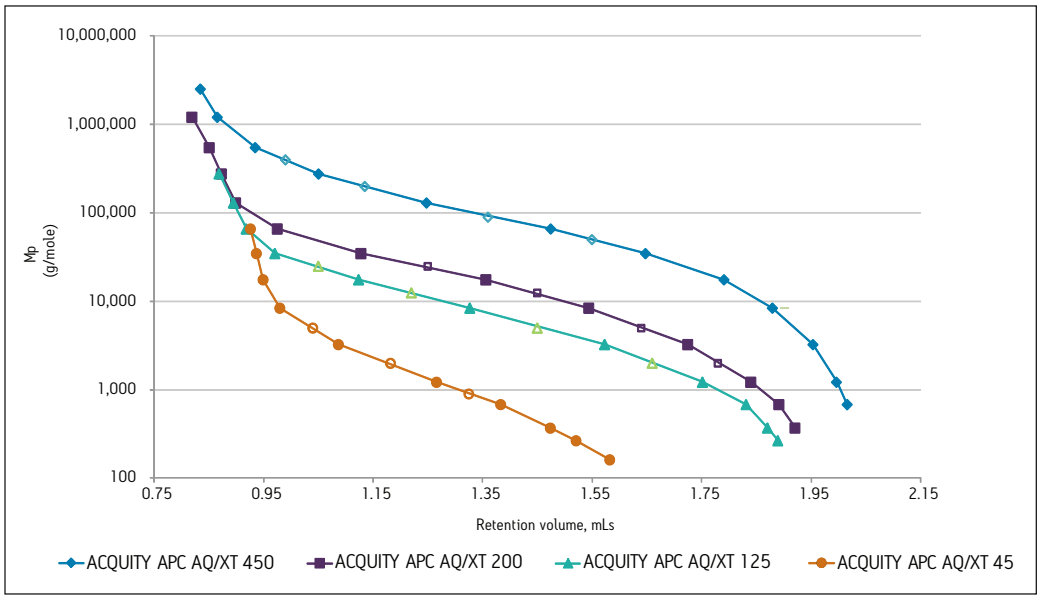



*Figure 3. Calibration Curves for ACQUITY APC XT and ACQUITY APC AQ Columns Using Polystyrene Standards.*

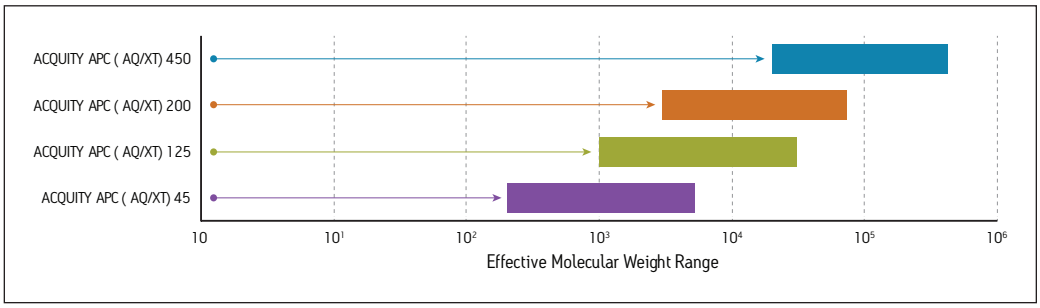



*Figure 4. Effective Molecular Weight Range for ACQUITY APC Columns.*

## c. Useable Flow-Rate Ranges

Excessive mobile-flow rates can create backpressure that can damage the column packing material. For any given particle size, as the pore volume increases, the base particle loses mechanical strength due to the reduction in particle wall thickness surrounding the open pore. Table 3 shows the maximum flow rate recommendation for a single column for each pore size, independent of column length. When connecting multiple columns in series, the column that specifies the lowest flow rate will dictate the maximum flow rate for the column bank. Higher viscosity solvents will limit the flow rate due to flow restriction. Table 4 provides viscosity data for common solvents.

For example, if you were to select a three-column bank using 45Å, 200Å and 450Å columns and require that DMF to be used as the mobile-phase solvent, you would be limited to a maximum flow rate of 1.0 mL/min. In this case, DMF has a solvent viscosity of 0.92 cP at 25 °C. Under these conditions the 450Å column limits the flow to 1.0 mL/min, even though the other columns can support a higher flow.

*Note: When connecting multiple columns in series, the maximum flow rate may not be achievable due to pressure limitations of the instrumentation. Please refer to the system's owner manual for more information.*

**Table 3. Recommended Mobile Phase Flow Rate for a Single ACQUITY APC Column**

| Pore Size (Å) | Maximum Flow Rate at Solvent Viscosity < 0.6 cP | Maximum Flow Rate at Solvent Viscosity > 0.6 cP |
|---|---|---|
| 45 | 1.8 mL/min | 1.1 mL/min |
| 125 | 2.0 mL/min | 1.6 mL/min |
| 200 | 2.0 mL/min | 1.4 mL/min |
| 450 | 1.7 mL/min | 1.0 mL/min |

**Table 4. Viscosity of Common Solvents at Different Temperatures ****

| Solvent | Viscosity (cP) | |
|---|---|---|
| | 20 °C | 40 °C |
| Acetone | 0.32 | 0.26 |
| Chloroform | 0.54 | 0.45 |
| Dimethyl formamide (DMF) | 0.92 | 0.68 |
| Dimethyl sulfoxide (DMSO) | 2.00 | 1.50 |
| Ethyl Acetate | 0.44 | 0.36 |
| Hexane | 0.31 | 0.26 |
| Methanol | 0.56 | 0.44 |
| Methylene chloride | 0.11 | 0.087 |
| N-Methylpyrrolidone (NMP) | 1.70 | 1.30 |
| Tetrahydrofuran (THF) | 0.48 | 0.39 |
| Toluene | 0.59 | 0.46 |
| Water | 1.00 | 0.67 |

**References:

1. Reid, R.C., Prausnitz, J.M., Poling, B.E. The Properties of Gases and Liquids, 4th Edition, McGraw Hill, 1987, Table 9-8.
2. http://www.wolframalpha.com
3. Yang, J. *Chem Eng Data* (2008), 53, 1639-1642.

### d. Minimizing Band Spread

The ACQUITY APC System was designed to minimize band spreading. Deviation from Waters specified tubing could result in deterioration of chromatographic performance. Figure 5 shows the influence of tubing inner diameter. Using larger tubing causes excessive peak broadening, lower sensitivity, and loss in resolution.

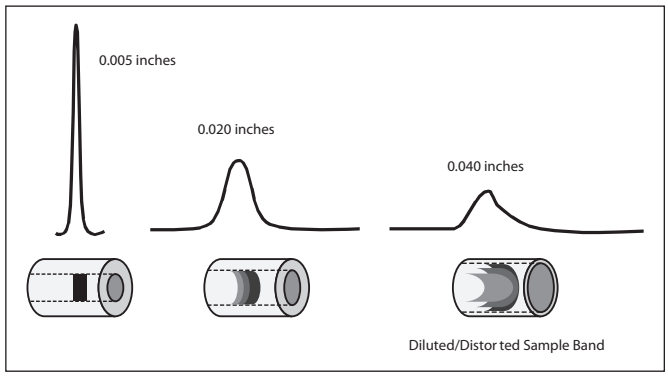



*Figure 5. Effect of Connecting Tubing on System.*

## IV. TROUBLESHOOTING

Changes in retention time, resolution or backpressure are often due to column contamination. See the Column Cleaning, Regeneration and Storage section of the care and use manual. Information on column troubleshooting problems may be found in **HPLC Column Theory, Technology and Practice**, U.D. Neue, (Wiley-VCH, 1997); Waters HPLC Troubleshooting Guide (Literature Code # 720000181EN); or by visiting www.waters.com

## V. COLUMN CLEANING, REGENERATION AND STORAGE

Assuming that there is no damage to the column bed, 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 and Regeneration

Use a cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column. The columns are stable using a wide range of solvents to improve the dissolution of the contaminant. Before beginning any cleaning procedure it is best to isolate the column from downstream

connections to protect further contamination post column. Increasing column temperature increases cleaning efficiency. If the column performance is poor after cleaning and regenerating, call your local Waters office for additional support.

If the inlet for the first column in the column bank series is plugged with precipitated material or sample, it is possible to disconnect the column and reverse the flow to dislodge the blockage. This must be done extremely carefully at a low flow rate (not to exceed 0.1 mL/min) to prevent disruption of the packed sorbent bed. Once the blockage is removed, the column must be returned to its proper flow direction. If inlet plugging is a concern, a column in-line filter unit is available (Waters part number 205000343).

*Note: The addition of an in-line filter increases the likelihood of shear degradation, especially for large molecular weight polymer species.*

If the column performance is poor after cleaning and regenerating, call your local Waters office for additional support.

### b. Storage

If you will be using the column again within 24 hours, special storage procedures are unnecessary. For longer storage periods, return the column to its box with the end plugs firmly in place. Do not leave a column at elevated temperature without solvent flow.

For maximum column life, avoid temperature cycling. Maintain operating temperature and reduce the flow rate to 0.1 mL/min when columns are not in use.

## VI. 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.

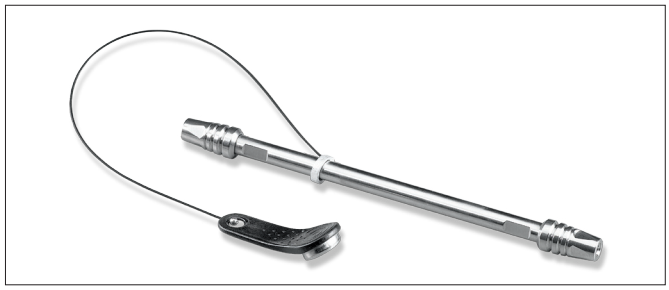


*Figure 6. eCord Intelligent Chip.*

### b. Installation

Install the column into the ACQUITY APC Column Manager. Plug the eCord into the side of the column heater noting the order of the attachment point. Once the eCord is inserted into the column heater (see Figure 7) the identification and overall column usage information will be available allowing the user to access column information on their desktop. Up to four columns can be connected at one time.

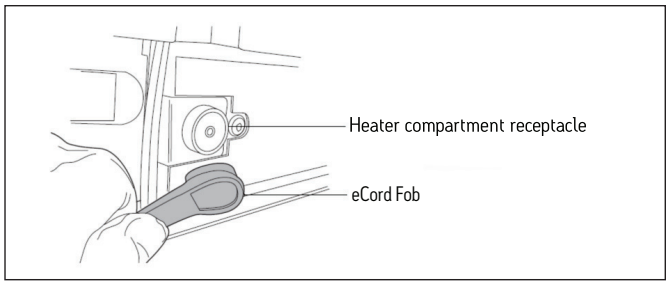



*Figure 7. Installing the eCord Intelligent Chip.*

### c. 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.

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