# Analytical Columns and Cartridges

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## I. INTRODUCTION

Waters™ offers a large selection of analytical columns and cartridges packed with silica-based packing materials. Follow this instruction sheet to obtain the best performance, reproducibility, and longevity from your analytical columns and cartridges. Waters silica-based materials are packed in:

- Steel and PEEK™ (polyetheretherketone) Columns — The steel analytical columns are durable and highly efficient. PEEK Columns are used with nonmetallic systems.
- Steel Cartridge Columns — The cartridge columns feature reusable end fittings that permit you to replace the cartridge quickly and easily without tools.
- Polyethylene Radial-Pak™ Column Segments — The Radial-Pak Column segments (also referred to as Radial-Pak cartridges) employ Waters patented technology to improve efficiency by eliminating the wall effect and voiding common to rigid columns.

## II. START-UP TEST PROCEDURE

Follow the four step start-up procedure for successful operation of your Waters column or cartridge: A. The pH range for the mobile phase is pH 2 to 8. However, phosphate buffer at pH 8 in combination with high temperatures can lead to shorter column life. For high temperature work, this buffer at neutral pH is preferred.

### A. MEASURING INSTRUMENT BAND SPREADING

Poor column performance can sometimes be attributed to excessive instrument band spreading. Test the band spreading of your HPLC system before you install your column or cartridge. Band spreading of a properly operating analytical system should be less than 100 µL and <40 µL for 2.1 mm i.d. microbore columns.

Test the instrument band spreading using the procedure below.

1. Remove the column or cartridge from the system. Connect the inlet and outlet tubing with a low-dead-volume union.
2. Inject the test sample used for a plate count determination (see Section II. d.) using a fast chart speed or sampling rate. You may need to adjust the detector sensitivity or dilute the sample to keep the peak on scale.
3. Use the 5-Sigma method shown in Figure 2 to obtain the band spreading value in µL.

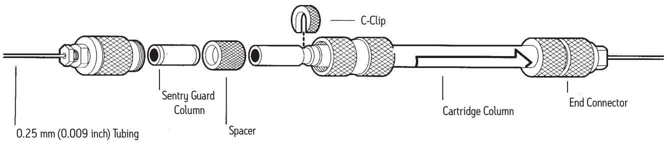

*Figure 1: Installing a Cartridge Column with a Sentry™ Guard Column*

Determine the width of the peak at 4.4% of the peak maximum. Determine how many microliters per minute your eluent is flowing. Multiply the two terms to yield the band spread of your instrument. If the band spreading is excessive, check all tubing and fittings. If it is necessary to repair fittings, refer to the procedure below. If you have a 2.1 mm i.d. microbore column, you may need to modify your HPLC system. Refer to the System Modifications Guidelines outlined in Section III.

Repair a damaged compression screw or a worn ferrule assembly using the procedure below.

1. Scribe the circumference of the tubing at the desired break point using a tube cutter or a file with a cutting edge.
2. Grasp the tubing on both sides of the scribe mark with cloth covered pliers. Gently work the tube back and forth until it separates at the scribe mark. Ensure that the tubing end is straight, open and free of burrs.
3. Slide the compression screw, followed by the ferrule (large end of the taper first), over the tube. Seat the tubing in the fitting. If the tubing is not completely seated, the resulting dead volume can lead to poor chromatographic results.

## B. INSTALLING THE CARTRIDGE OR COLUMN

For Column Installation: Connect the column with the direction of flow arrow pointing toward the detector. You will need a 5/16-inch open-end wrench.

For Steel Cartridge Column Installation: Refer to Figure 1. Unscrew the end connectors from the old cartridge column, leaving them connected to the inlet and outlet lines of the instrument. Attach the new cartridge column between the connectors so that the flow is in the direction of the arrow on the label. Finger tighten all fittings.

For Radial-Pak Column Segments: The column segments are installed in a Waters 8 x 10 Cartridge Holder. Please refer to the RCM 8 x 10 Cartridges Holder Manual for installation and operation instructions. Tighten the piston until the pressure gauge needle is in the correct compression zone, referring to the table below. Radial-Pak column segments are shipped dry. They should be prewetted with 100% organic solvent before equilibration with the mobile phase.

## C. EQUILIBRATING THE COLUMN

1. Equilibrate your column when it is first installed and after storage. Verify that your mobile phase is miscible in the column shipping solvent.
2. When you change the mobile phase of your system, gradually increase the flow rate of the new mobile phase from 0 mL/min to 1.0 mL/min in 0.1ml/mm increments.
3. Purge the column with at least 10 column volumes of your mobile phase until you achieve a stable baseline. If mobile phase additives are present (such as ion-pairing reagents, at 5 to 10 mmol/L) 100 to 200 column volumes may be required for complete equilibration.

## D. TESTING THE COLUMN EFFICIENCY AND CALCULATING COLUMN BACK PRESSURE

Perform an efficiency test on your column before you use it. Prepare the test sample and test the column using the conditions for your particular column configuration. Test sample preparation and column test conditions are enclosed in a separate sheet. Repeat the efficiency test periodically to track column performance aver time.

Use the following equation to calculate the back pressure of your column or cartridge with water as a mobile phase at 1 mL/min flow rate. Multiply the result by your mobile phase viscosity in (cP) and your flow rate to obtain your operating back pressure. A viscosity table for common solvents and a viscosity curve for aqueous mixtures is enclosed on a separate sheet.

$$\Delta p = \frac{2.1 \bullet L \bullet f}{dp \bullet D^2}$$

Where Δp ($H_2O$, 1 mL/min) is the pressure (MPa); L is the column length (mm); f is a constant, 20 for Radial-Pak Column segments or 10 for steel columns; dp is the particle diameter (µm) and D is the column diameter.

## III. NARROW-BORE COLUMNS (2.1 mm I.D.)

This section describes how to measure extra column effects and gives some guidelines on how to maximize the advantages of your Waters narrow-bore column. Modifications to your HPLC system will probably be required in order to avoid system band spreading. Without proper system modifications, system band spreading causes peak broadening and has a large impact on peak width as peak volume decreases. To measure the band spreading of your HPLC system, refer to the procedure, Measuring Instrument Band Spreading, in Section II. a. and refer to Figure 2.

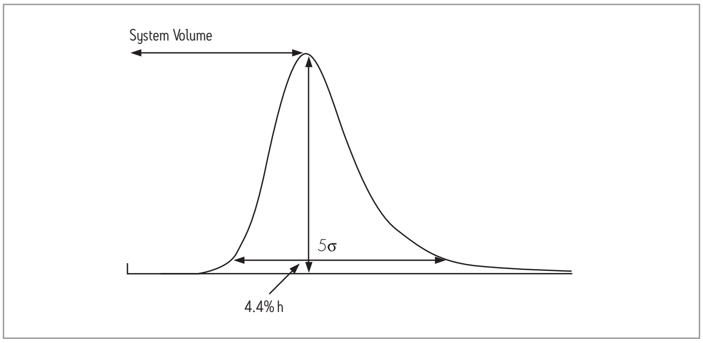

*Figure 2: Determination of System Bandspreading Using the 5-Sigma Method*

A second effect, called dwell volume is observed with gradient analysis. In this case, the lower flow rate causes the gradient front to take longer to reach the head of the column. To measure the dwell volume for your system, refer to the procedure, For Gradient Analysis, below and refer to Figure 3.

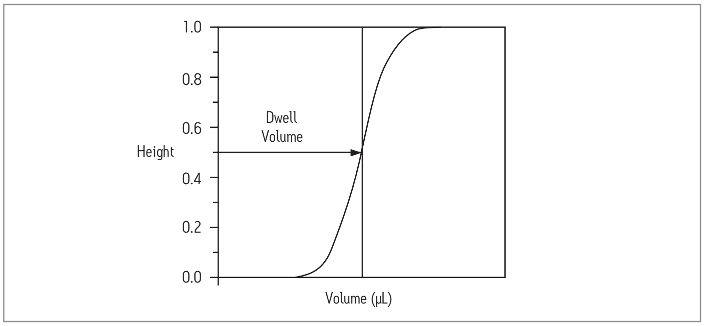

*Figure 3: Determination of Dwell Volume*

For Gradient Analysis:

1. Replace the column with a zero dead volume union.
2. Determine the gradient-delay or dwell volume for your system by performing a dwell volume test. Prepare eluent A (pure solvent), and eluent B (solvent plus sample).
3. Equilibrate system with eluent A until a stable baseline is achieved. Switch to 100% eluent B and record the time at half height of the step. Refer to Figure 3 for an illustration.
4. Modify your HPLC system in order to minimize the dwell volume according to the System Modifications Guidelines section. The dwell volume should be less than 1 mL for small volume columns.

System Modification Guidelines:

1. Use 0.25 mm (0.009-inch) tubing between pump and injector.
2. Use a microbore detector flow cell, for example, Waters Tuneable UV-VIS Absorbance Detector with a 2.6 μL volume) for the 2.1 mm columns. Recall that due to the shorter path length, detector sensitivity is reduced to achieve lower band spread.
3. Use 0.12 mm (0.005-inch) i.d. tubing for the 2.1 mm column to minimize dead volume. With careful plumbing, standard 0.25 mm (0.009-inch) i.d. tubing can be used with the 3.0 mm column.
4. Use a manual injector with a low dead volume, for example, Rheodyne Model 7725.

## IV. COLUMN MAINTENANCE

This section describes routine column maintenance procedures that should be followed to maximize the lifetime of your Waters column. The procedures are described in detail below:

### A. CARING FOR THE COLUMN

To ensure the continued high performance of your Waters column, follow these guidelines:

- Protect steel columns, cartridge columns and column segments from contaminants with a Waters Sentry Guard Column or a Waters Guard-Pak™ Insert.
- Use Waters Sep-Pak™ Solid-phase Extraction Cartridges or Waters Oasis™ Cartridges to clean up your sample prior to analysis.
- Make sure that solvents are miscible when changing mobile phases. This will prevent phase separation or precipitation.
- Always degas and filter mobile phases through a 0.22 μm membrane filter. Acrodisc® Filters by Gelman Sciences are recommended.
- Do not exceed an operating pressure of 40 MPa (400 atm or 6000 psi) for steel columns, or 13 MPa (130 atm or 2000 psi) for Radial-Pak Column segments.

## B. CLEANING AND REGENERATING THE COLUMN

A shift in retention or resolution may indicate contamination of the column. Flushing with an organic solvent is usually sufficient to remove the contaminant.

If system back pressure increases with usage, replace the guard column. If you are not using a guard column, replace the inlet frit. If the problem continues, the column may be plugged with a contaminant that needs to be dissolved with an appropriate solvent. For example, proteinaceous material can be removed with repeated 200 µL injections of dimethylsulfoxide.

If the flushing procedure does not solve the problem, wash the column with a sequence of progressively more nonpolar solvents. For example, switch from water to tetrahydrofuran (THF) to methylene chloride. Return to the standard mobile phase conditions by reversing the sequence.

## C. STORING THE COLUMN

For Column and Cartridge Columns: Leaving the column unused for less than four days does not require special storage procedures. For longer storage, store the column in shipping solvent which is identical to the column test solvent with a few exceptions. Store Nova-Pak™ CN HP and µBondapak™ CN Columns in 0.1 M ammonium sulfate. Store all µBondapak $C_{18}$ Columns in methanol and store all µBondapak Phenyl Columns in acetonitrile/water. If the mobile phase contains a buffer salt, first flush the column with 10 column volumes of 18 megohm water. Make sure that the end plugs are firmly in place. Never let the column dry out.

For Radial-Pak Column Segments: Wash Radial-Pak column segments with water to remove all buffer salts, then with the organic component of the mobile phase. Use a miscible intermediate if necessary. Remove the cartridges from the 8 x 10 holder and replace the end caps.

## V. TROUBLESHOOTING

Changes in retention time, resolution or back pressure are often due to column contamination. This is discussed in Section IV. b., Cleaning and Regenerating the Column.

Poor chromatography is often the result of a system problem. For information on troubleshooting your system refer to the Guide to Successful Operation of your LC System, available from Waters. Ask for part number WAT022378.

Our experienced team of chromatographic specialists provide outstanding customer support for corrective, preventive, and quality maintenance. Our technical support staff is always available to help you with questions and provide solutions to your applications needs. In the U.S., call us at 1-800-252-4752. If you are outside the U.S., contact your local Waters representative.

## VI. ORDERING AND ADDITIONAL INFORMATION

If you would like new product information or a copy of the Waters Parts and Supplies Catalog, complete the reply card and mail. For more detailed information about Waters columns and cartridges, consult the Waters Chromatography Columns and Supplies Catalog or contact your Waters representative. To obtain a copy of the catalog, complete the enclosed reply card. The catalog is also available on the Internet at waters.com.

### A. AVAILABILITY AND USE OF ELECTRONIC COLUMN TAGS

‘Smart columns’ are those enabled with electronic devices (tags) permanently affixed to enable identification and/or tracking of column history and use information. These tags contain unique product identifying information, such as part and serial numbers, and product descriptions. Data residing on these devices is automatically imported by complementary Waters’ systems, whether LC instrumentation or the Empower™ Chromatographic Data System. This type of product data is used to aid in tracking and decision-making and troubleshooting, especially valuable for GLP/GMP compliant laboratories.

Tags come in two types: tethered like eCord™ and untethered like eConnect™. The eCord is a standard feature on ACQUITY UPLC and some UHPLC columns for use with Waters ACQUITY and ARC UHPLC systems. The eConnect column tag is an optional feature for HPLC columns and are designed to be used with the Waters Alliance iS HPLC system.

Although the eCord chip and eConnect electronic column tag designs differ, their designed to be robust and durable. When in operation, the eCord enabled column is connected via a cable tether to the system, allowing the electronic tag to be physically located outside the column manager. This placement reduces exposure to variable operating temperatures and solvents. In contrast, the eConnect tag is designed to be within the column manager, it is exposed to the full range of operating temperatures and may be exposed to solvents. Extensive testing has been conducted to ensure the performance of the eConnect tag in this environment. The durability of the eConnect tag was confirmed with various solvents and solutions with pH between 2 and 11. In the case of significant solvent exposure, communication between the tag and system can be temporarily interrupted. Allowing the eConnect tag to dry following solvent exposure, evaporation should be suitable in most cases, will allow the tag performance to recover.

In addition, eConnect tags also offer a convenient, easy-to-use connection to the Waters website. Using an NFC enabled internet connected device to scan the eConnect tag will provide the user with a web address which will take them directly to the Waters product page for that consumable. This page provides access to product information, like Care and Use or Certificate of Analysis, as well as reorder and related product support.

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