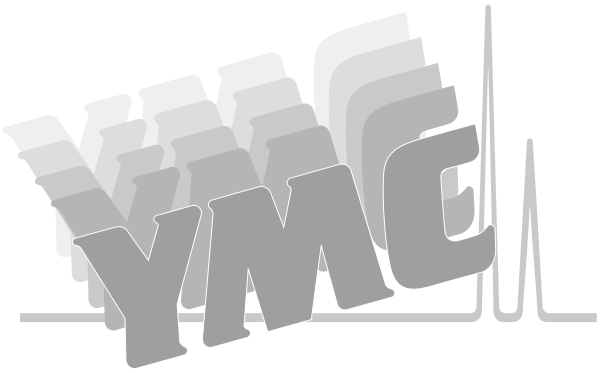


Column Care and Use instructions

Read carefully before using this column.

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column. We are sure you will find that YMC's built-in quality helps solve many of your challenging separation problems. We strive to provide products with the highest degree of lot-to-lot and column-to-column reproducibility to minimize variations in your chromatographic results. YMC manufactures each packing material and packed column under highly controlled conditions. Each must pass a series of stringent tests before being accepted for shipment. Included with each column is the final Quality Control Report.

Sample Preparation

1. It is preferable to prepare sample in the mobile phase or a weaker solvent than the mobile phase.
2. If the sample is not dissolved in the mobile phase, ensure sample, solvent and mobile phase are miscible to avoid sample or buffer precipitation.
3. Filter sample with 0.2μm membrane to remove particulates.

Installing the Column

1. Handle the column with care. Do not drop or hit column on a hard surface as it may disturb the bed and affect its performance.
2. Correct connection of 1/16" stainless steel tubing or PEEK tubing leading to and from the column is essential for high-quality chromatographic results.
3. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16" stainless steel tubing. When tightening or loosening the compression screw, place the 5/16" wrench on the compression screw and the other 3/8" wrench on the hex head of the column endfitting.

Note: If one of the wrenches is placed on the column flat during this process, the endfitting will be loosened and leak.

4. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing and ferrule must be assembled.
5. If a leak occurs between the endfitting and the column body, place a wrench on the column body flat and a wrench on the hex head end of the column endfitting and rotate the fitting 1/4 tum clockwise past finger-tight. **Do not over-tighten.**
6. The correct direction of solvent flow is indicated by an arrow on the column identification label.

Equilibration

YMC delivers the column in the test solvent shown on the performance report, with the exception of following products shipped in the solvents stated here.

100%ACN: J'sphere series, YMC CHIRAL CD BR series, YMC Carotenoid, YMCbasic

0.05% Sodium Azide: YMC-Pack Diol-GFC

It is important to ensure solvent compatibility before changing to a new solvent. Reversed-phase ODS (C_{18}), Octyl (C_8), Phenyl, Butyl (C_4), and Cyano utilize aqueous soluble organic mixtures. Use organic solvent such as hexane, methylene chloride, chloroform, isooctane and so forth with normal-phase silica, PVA-SIL, and amine. Equilibrate your column with a minimum of 10 times its internal volume with the mobile phase to be used (refer to Table 1 for some standard column volumes and the calculation of column volumes).

Table 1: Flush Amounts Equaling 10 Column Volumes

Column Size	Column Volume	Flush Volume
4.6×150mm	2.5mL	25mL
4.6×250mm	4.2mL	42mL
10 ×250mm	19.6mL	196mL
20 ×250mm	78.5mL	785mL

1. Purge your pumping system and then connect the inlet end of the column to the injector outlet. Turn on the pump flow at 0.1 mL/min and increase to 1 mL/min over 5 minutes.
2. When the solvent is flowing freely from the column outlet, attach the column to the detector. This procedure prevents entry of air into the detection system and gives more rapid equilibration.
3. When the mobile phase is changed, gradually increase the flow rate of the new mobile phase from 0 mL/min to 1.0 mL/min in 0.1 mL/min increments.
4. Once a steady backpressure and baseline have been achieved, the column is ready to be used.

Note: If mobile phase additives are present in low concentrations (such as ion-pairing reagents, at 5 to 10 mmol/L) 100 to 200 column volumes may be required for complete equilibration.

Validating the Column

Each prepacked column has an individual quality control report which provides significant information about the column. This report is available as a ready reference and should be kept in your files. It indicates the column specifics: column dimensions, bonding chemistry type, particle shape, particle size, porosity, gel lot, and chromatographic test conditions. Perform an efficiency test on your column before you use it. YMC recommends using a suitable solute mixture, such as found in the Quality Control Report, to immediately analyze the column once you receive it. Determine the number of theoretical plates (N) and use for periodic comparison. Repeat the test periodically to track column performance over time. Slight variations may be obtained on tow different HPLC systems due to system electronics, the quality of the connections, operating environment, reagent quality, column condition and operator technique. Please report any column problems observed upon receipt of the column.

Listed on the performance report are the test results: plate count, K, and asymmetry. Additional information includes test conditions such as solvent system, flow rate, and backpressure.

Column Care

To ensure the continued high performance of your columns and cartridges, follow these guidelines:

1. Guard columns

Samples: Sample impurities very often contribute to column contamination. Two ways to avoid this are:

a. Use of YMC solid-phase extraction sample clean-up cartridges of matching chemistry

b. Use of a YMC guard cartridge of matching chemistry and particle size between the injector and main column. It is important to use a high-performance matching guard column to protect the main column while not compromising analytical resolution.

2. pH range

Recommended pH ranges for solvent and buffer combinations are between 2.0 and 7.5 for most YMC columns, unless otherwise indicated here. A pH less than 2.0 may cause hydrolysis of the bonded phase. At a pH greater than 7.5, the alkaline solvent buffers will attack the silica substrate resulting in void formation in the column as the silica solubilizes.

pH 2.0-13.0:YMC-Pack PolymerC18
pH 5.0-7.5: YMC-Pack Diol-GFC
pH 3.5-6.5: YMC CHIRAL CD BR series
pH 2.0-6.5: YMC CHIRAL NEA

3. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all buffers before use. 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.

4. Pressure

The column backpressure is a function of several parameters.

- Particle size and distribution.
- Packing porosity and bonded phase coating levels.
- Column dimensions (diameter and length).
- Solvent viscosity, flow rate, and temperature.

The operation pressure on columns should be maintained according to the following directions in order to maximize column lifetime.

For columns in length up to 150mm: Max. 20 MPa or 3000 psi.
For columns in length of 250 mm or more: Max 25 MPa or 3700 psi.

Notes

1. For inner diameter in 10 and 20 mm I.D.: Max. 10 MPa or 1500 psi.
2. For inner diameter in 30 mm I.D. or more:
   Please contact at sales@ymc.co.jp for the information.
3. Combinatorial Chemistry columns (CC columns): Max. 25 MPa or 3700 psi.
4. PolymerC18: Max. 15 MPa or 2000 psi.

5. Temperature

30-50℃ temperatures are recommended to enhance selectivity, lower solvent viscosity, increase injection-to-injection reproducibility, and increase mass transfer within the column.

Column Cleaning, Regenerating, and Storage

1. Cleaning and Regeneration

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

Guard columns need to be replaced at regular intervals as determined by sample contamination. When system backpressure steadily increases above a set pressure limit, it is usually an indication that the guard column should be replaced.
When the system backpressure increases above a set pressure limit, run columns in reverse flow direction with the outlet disconnected from the detector. Also, replacement frits or frit assemblies are available. The frit will trap any particulate contaminants before they reach the column bed.

2.Storage

Do not store columns in buffered, acidic or basic eluents. Flush the column with 10 column volumes of 50/50 acetonitrile/water or methanol/water (see **Table 1)** and replace with shipping solvent for storage. Completely seal column to avoid evaporation and drying out of the bed.

YMC supplies each column with sealing end plugs.

For preparative separations, use low residue solvents and volatile buffers to minimize post-chromatography sample handling problems.

Troubleshooting

Changes in retention time, resolution, or backpressure are often due to column contamination. See the Column Cleaning, Regeneration, and Storage section of this instruction sheet.

Problem-Action

1. Backpressure increases

Contamination: reverse the flow direction of column, pump with 10-20 column volumes of mobile phase. If the inlet frit is blocked, replace frit.

2. Loss of peak resolution

Flush with 100% organic to remove any contamination. Check that system solvent composition hasn't changed. Flush system with fresh solvent for at least 20 column volumes to ensure full equilibration.

3.Peak shape deterioration

Peaks broaden and lose efficiency: check column connections/tubing to ensure correct sealing of nuts and ferrules. Voids: replace the column or cartridge.

Scaling Up/Down

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

①To maintain the same linear velocity: $F_2 = F_1(r_2/r_1)^2$

②To maintain the same retention time: $F_2 = F_1 \times (L_2/L_1) \times (r_2/r_1)^2$

③To adjust new sample loading values: $W_2 = W_1 \times (L_2/L_1) \times (r_2/r_1)^2$

Where: L = Length of column, in mm

r = Radius of the column, in mm

F = Flow rate, in mL/min

W = sample loading values.

1 designates the original, or reference column

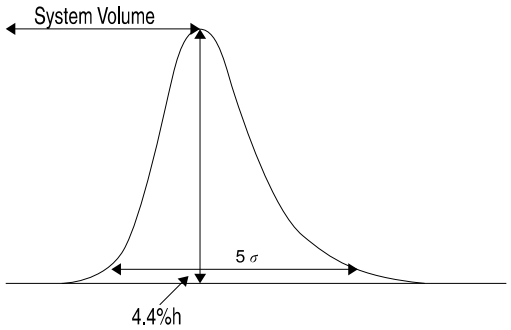
2 designates the new dimension column

Use of Narrow-Bore Columns (≦3.0 mm i.d.)

This section describes how to measure extra column effects and gives some guidelines on how to maximize the advantages of your narrow-bore columns. The 3.0 mm i.d. narrow-bore column usually requires no system modifications. With the 2.1 mm i.d. column, however, modifications to your HPLC system may be required in order to eliminate excessive system band spread volume (refer to System Modification Guidelines in the next section for tips on system modifications).

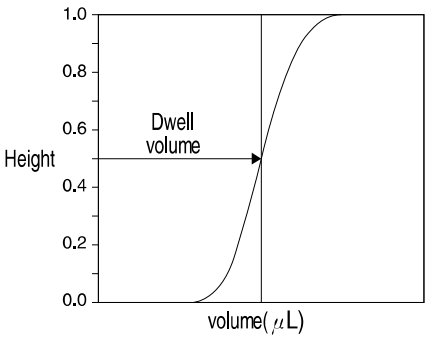
Without proper system modifications, excessive system band spread volume causes peak broadening and has a large impact on peak width as peak volume decreases. To measure the bandspreading of your HPLC system, refer to the procedure, Measuring System Bandspread Volume, in the next section and refer to Figure 1.

Figure 1:Determination of System Band Spreading Using the 5-Sigma Method



A second effect, called dwell volume is observed when operating in the gradient mode. 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, Measuring Gradient Delay Volume, in the following section and refer to Figure 2.

Figure 2: Determination of Dwell Volume



Measuring System Bandspread Volume

1. Replace the column with a zero dead volume union.
2. Determine the band spread volume for your HPLC system. Inject 1 μL of acetone in methanol at 0.2 mL/min.

3. Measure the peak width at 4.4% (5 sigma) of the peak height. Refer to Figure 1 for an illustration of the system band spread volume measurement.
4. Modify your HPLC system to minimize band spread volume according to the System Modifications Guidelines section. The bandspread volume should be less than 90 μL for 3.0 mm columns and less than 40 μL for 2.1 mm colums.

Measuring Gradient Delay Volume

1. Replace the column with a zero dead volume union.
2. Determine the gradient-delay or dwell volume for your system by performing the following test. Prepare eluent A (pure solvent, such as methanol) and eluent B (solvent plus sample, such as 5.6 mg/mL propylparaben in methanol).
3. Equilibrate the system with eluent A until a stable baseline is achieved. Switch to 100% eluent B and record the half height of the step. Refer to Figure 2 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.

System Modification Guidelines

1. Use a microbore detector flow cell with the 2.1mm columns. Recall that due to the shorter pathlength, detector sensitivity is reduced to achieve lower band spread volume.
2. Use 0.12mm (0.005") i.d. connecting tubing for the 2.1mm column to minimize dead volume. With careful plumbing, standard 0.25mm (0.009") i.d.tubing can be used with the 3.0mm i.d. column.
3. Use 0.25 mm (0.009) tubing between pump and injector.

YMC Small Particle Size (3μm) Columns -Fast Chromatography

The YMC column with 3μm packing provides faster and more efficient separations without sacrificing column lifetime. This section describes four parameters to consider when performing separations on the 3μm column.

- **Flow Rate** — Compared with the 5μm columns, the 3μm columns have a higher optimum flow rate. The 3μm columns are used for high efficiency and short analysis times. The higher flow rates, however, lead to increased backpressure. Use a flow rate that is practical for your system.
- **Backpressure** — The backpressure on the 3μm columns is higher than for the 5μm columns of the same dimension. Use a shorter column to compensate for increased backpressure and obtain a shorter analysis time or increase temperature (See Column Care & Use Section #5).
- **Sampling Rate** — Use a sampling rate of about 10 points per second.
- **Detector Time Constant** — Use a time constant of 0.1 seconds for fast analysis.

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