Preparative Column
Care and Use Manual

Dear User:

Thank you for choosing our preparative HPLC columns. We are committed to developing and manufacturing high-performance chromatographic packing materials and providing top-quality analytical and preparative HPLC columns to support your separation and purification challenges.

Our preparative column packing utilizes fully porous, spherical silica with a wide range of bonded phases. From raw material production to column packing, our manufacturing process is strictly controlled to ensure batch-to-batch reproducibility. Every column undergoes rigorous quality testing prior to shipment. To ensure optimal performance and maximum column lifespan, please read this user manual carefully before use.

Preparation Before Use

Column Identification

Each preparative column is supplied with a Certificate of Analysis (CoA), listing specifications such as: column dimensions, bonded phase, particle size and shape, silica type, pore size, factory test conditions, and test results (e.g., column efficiency and backpressure). You may reproduce factory test conditions to check parameters like column efficiency and pressure before use. Note that different instruments may result in varying test results.

To confirm authenticity and gain access to after-sales support, please verify the unique identification code on the column label. Upon receiving your column, please check the following:

1. Verify that the packaging is intact and matches the ordered column model.
2. Ensure the package contains a COA report with the inspector's signature.
3. Check the column surface for any damage and confirm that the protective plugs on both ends are intact.
4. Verify that the column body bears a Welch Materials identification label. Confirm that the model and serial number on the box match those on the column label.

Column Installationvv

1. Handle with care. Dropping or impact with hard surfaces may cause irreversible damage to the column bed.

2. When connecting a preparative column with HPLC system, use 1/16" or 1/8" stainless steel or PEEK tubing to minimize dead volume. Columns with an internal diameter of 50 mm or larger use 1/8" fittings and are provided with 1/16" adapters.

3. Ensure proper connection and secure tightening of tubing and fittings.

4. If leakage occurs between tubing and fittings, reconnect with suitable ones, and retighten.

5. If leakage occurs between fitting and column end, hold the column with a wrench and tighten the fitting clockwise gently. Avoid over-tightening, especially with stainless steel fittings, to prevent seizing.

6. Make sure the flow direction of the mobile phase matches the arrow marked on the column. Confirm there is no leakage during operation.

Column Equilibration

Unless otherwise noted, the column should be equilibrated using the mobile phase applied during factory testing. Prior to analysis, verify that your mobile phase is miscible with the factory testing solvent system.

For reversed-phase columns (e.g., ODS/C18, C8, phenyl, C4, CN), use water-miscible organic solvents.

For normal-phase silica columns, use n-hexane, dichloromethane, trichloromethane, isooctane, etc.

Equilibrate the column with at least 10 column volumes of appropriate mobile phase.

The column volume (V) can be calculated as:

$V = \pi r^2 L$

Where:

V = column volume

$\pi = 3.1415926$

r = column radius (cm)

L = column length (cm)

Specification	Column Volume	Mobile Phase Volume for Equilibration
10×250 mm	19.6 mL	196 mL
21.2×250 mm	88.2 mL	882 mL
30×250 mm	176.7 mL	1767 mL
50×250 mm	490.8 mL	4908 mL

Note:
Mobile phase volume for equilibration = 10 x column volume

Equilibration Steps:

1. After purging, connect the column inlet to the autosampler valve outlet.
2. Once a steady flow of mobile phase emerges from the column outlet, connect it to the detector inlet. This helps prevent air bubbles from entering the detector and shortens equilibration time.
3. When changing mobile phases, increase flow rate gradually.
4. Start the experiment only after pressure and baseline are stabilized (i.e., the column is equilibrated).

Note: If using low-concentration additives (e.g., 5–10 mmol/L ion-pairing agents), equilibrate with 100–200 column volumes.

Sample Preparation

1. Dissolve samples in the mobile phase or in a solvent weaker than the mobile phase.
2. If the sample is insoluble in the mobile phase, ensure mutual miscibility among the sample, solvent, and mobile phase to avoid precipitation.
3. Filter samples through a 0.22 μm membrane before injection.

Precautions During Use

To ensure optimal performance and lifespan, please following the guides:

1. Use of Guard Column

Impure samples may lead to reduced column life and performance. Two preventive strategies are recommended:

A. Perform appropriate SPE pretreatment.

B. Solid particles from unfiltered samples, solvent residues, pump wear, seal or tubing degradation may clog the frit, increasing pressure and reducing efficiency. Use an inline filter or guard column to prevent particulates from entering the preparative column. When using a guard column, ensure compatibility of its physical and chemical properties with the main column to avoid interference with separation.

2. pH Range

Each column type has a specific pH tolerance (see table blow). Operating outside the recommended pH range may lead to silica dissolution or bonded phase hydrolysis, causing irreversible damage. When operating near the pH limit, use a mobile phase containing >10% organic solvent.

Additionally, operating near pH limits may reduce column lifespan due to sensitivity to temperature and solvent composition. Therefore, flush immediately with a compatible solvent suitable for storage and miscible with the current mobile phase after running under extreme pH conditions.

Column Type	pH Range
XB-C4, C8, C18, Phenyl	2.0-8.0
SiO_2, Amide, Amino Acid	2.0-8.0
XB-CN	2.0-8.0
AQ-C18	2.0-8.0
LP-C8, LP-C18	0.5-8.0
Xtimate Series	1.0-12.5

3. Reagents

For better chromatographic performance, please use high-purity HPLC-grade solvents. All solvents must be properly filtered before use to prevent particle blockage and pressure buildup. Mobile phase solvents must be degassed before use to prevent bubble formation in the pump and detector.

4. Pressure Control

Column backpressure depends on:

A. Particle size and distribution

B. Column dimensions (ID and length)

C. Viscosity, flow rate, and temperature of the solvent

When adjusting flow rates, do so gradually to prevent abrupt pressure fluctuations that may damage the column. Avoid exceeding the maximum column pressure as it shortens column lifespan:

Particle Size (µm)	Max. Pressure (MPa)
5	40
10	25
15	15
20	10
20-40	5
40-70	4

For pressure limits unlisted above, please contact our technical support.

5. Temperature

Maintain column temperature between 30–50°C. Appropriate heating improves selectivity, reduces solvent viscosity, enhances reproducibility, and increases mass transfer efficiency.

Column Storage

Do not store the column with mobile phases containing buffer salts, acids, or bases. If the column was used with such solvents, follow the recommended column cleaning procedure before replacing with the factory mobile phase for storage.

Ensure the protective plugs are tightly sealed to prevent evaporation and drying of the packing material.

Note: Every column is shipped with compatible protective plugs for storage.

Scaling Up: Flow Rate and Sample Load

Flow rate and sample loading depend on column dimensions. Refer to the table below or calculate as follows:

A. Linear scaling of flow rate is proportional to the square of the column radius:

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

B. Flow rate scaling with retention time unchanged:

$$F_2 = F_1 \times (L_2/L_1)(r_2/r_1)^2$$

C. Sample loading:

$$W_2 = W_1 \times (L_2/L_1)(r_2/r_1)^2$$

D. Scaling factor:

$$F_{scal} = (L_2/L_1)(r_2/r_1)^2$$

Where:
L = column length (mm)
r = column radius (mm)
F = flow rate (mL/min)
W = sample load
"1" = previous column size
"2" = target column size

Specification	4.6×250 mm	10×250 mm	21.2×250 mm	30×250 mm	50×250 mm
Packing Material Mass (g)	2.5	11.8	53.1	106.3	295.4
Scaling Factor	1	4.73	21.2	42.5	118
Sample Load (mg)	0.25-25	10.63 - 1063	50.31 - 5031	10.63 - 1063	29.54 - 2954
Flow Rate (mL/min)	0.5-2	3-5	10-20	20-45	70-130

Column Maintenance

Preparative columns are consumables whose performance gradually degrades with usage, often manifesting as peak broadening, reduced resolution, and increased backpressure. When these symptoms appear, replacement of the column should be considered.

Routine maintenance following proper operating procedures can significantly extend column life, while improper handling will shorten it. Below are common maintenance issues encountered during the use of preparative columns.

1. Increased Column Backpressure

A gradual rise in column backpressure over long-term use is normal, as sample injection inevitably introduces contaminants. However, a sudden increase in pressure over a short period typically indicates an abnormal condition. After ruling out instrument malfunctions, consider the following causes and solutions:

A. Contaminated Inlet Frit

If caused by complex samples containing fine particulates, install an inline filter or guard column upstream to intercept particulates. If caused by insoluble substances, replace with suitable solvent or filter samples through a membrane before injection.

If the inlet frit is clogged, flush the column in reverse at low flow for 20–30 column volumes. If reverse flushing fails, replace the inlet frit or contact technical support.

B. Contaminated Inlet Packing

Cause: Accumulation of sample contaminants at the column inlet over extended use.

Solution: Flush the column in reverse at low flow with a solvent that dissolves the contaminants for 20–30 column volumes. If reverse flushing is ineffective, replace the column or contact technical support.

C. Damage Due to Improper pH Use

Using eluents outside the recommended pH range may lead to stationary phase degradation. Column recovery is unlikely in such case; replacement is advised.

2. Proper Use of Buffer Salts

Buffer salts are typically water-soluble but poorly soluble in organic solvents. High content or improper use of organic solvent may result in salt precipitation, which can:

- Accelerate wear on pump pistons, seals, and valves.
- Clog the column’ s inlet frit.
- Penetrate the packing bed and block the pores on the particle surface or interstitial spaces between particles,
- Interfere with stationary phase chain mobility, leading to bed collapse and increased backpressure.
- As a result, deteriorate column retention capacity, efficiency, and resolution significantly, and greatly shorten the lifespan.

Salt precipitation is difficult to reverse. Correct handling is essential for maximizing column lifespan.

Recommended Practices:

A. Isocratic Conditions:

Flush the column with a salt-free transition mobile phase for 20–30 column volumes before and after each run, or flush overnight at 2 mL/min (adjust per column specs) post-run.

B. Gradient Conditions:

Before the run, flush with the initial mobile phase composition at 1/4 of the method flow rate for ⩾20–30 column volumes.

After the run, flush with transition mobile phase (same organic/water ratio as in the gradient or with higher aqueous content, but no buffer salts) for ⩾20–30 column volumes. Use shallow gradients to minimize salt precipitation.

C. In Case of Salt Precipitation:

Salt crystallization may quickly raise pressure and irreversibly damage the column. Attempt recovery with the following:

Method 1: Flush in reverse with methanol/water = 10/90 at 1/4 method flow rate, 35 °C, for 20–30 column volumes.

Method 2: Flush in reverse overnight with methanol/water = 10/90 at 2 mL/min.

If both methods fail, replace the column or contact technical support.

3. Use of Guard Columns

A preparative guard column is installed between the injector and the preparative column. Its purpose is to trap insoluble particles from samples or mobile phases, and to retain strongly adsorbed contaminants that are difficult to elute, thereby protecting the main column.

Precautions for Guard Column Use:

- Use the same packing material as the preparative column.
- When not in use, store the guard cartridge in its holder and seal it with end plugs to maintain moisture.
- If stored for extended periods and partially dried, reactivate the cartridge before reuse.
- If pressure spikes or peak shape deteriorates suddenly, remove the guard column and test with a standard sample to isolate the issue; if the guard is contaminated, replace the cartridge.
- Avoid ultrasonic cleaning, as intense sonication may damage the packing and produce fines that clog the frit and raise pressure.