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Preparative Column Care and Use Manual

Thank you for choosing Welch preparative column. Our preparative columns are packed with fully porous, spherical silica gel matrices, available in a variety of bonded phases. From the production of the packing material to the column packing process, we employ rigorous quality management to ensure batch-to-batch reproducibility. Each preparative column undergoes stringent quality testing before leaving the factory. To fully utilize the performance of the column and extend its lifespan to the maximum extent, please read these instructions carefully.

Column Identification

Each column undergoes strict quality testing before leaving the factory and comes with a CoA report. This report includes parameters such as specifications, bonded phase, particle size, particle shape, matrix type, pore size, factory testing conditions, and test results (e.g., column efficiency, column pressure). Before using the column, you can test parameters such as column efficiency and column pressure according to the factory testing conditions. Please note that different testing instruments may result in variations from the factory test results.

To ensure that the column you purchased is a genuine product from Welch, please carefully check the serial number on the label of each column. This number allows you to access our after-sales service and other value-added services. Please pay attention to the following after receiving the product:

- Ensure that the packaging box is intact and that the labels match the preparative column.
- Verify that the box contains a CoA report with the inspector's signature.
- Inspect the column surface for any collision marks and check that the protective plugs on both ends of the column are intact.
- Confirm that the preparative column has a Welch identification label. Carefully compare the model and serial numbers on the packaging box with those on the column label to ensure they match.

Installation of Praparative Column

1. Avoid dropping the column. Do not subject it to strong impacts with hard objects, as this can cause irreversible damage to the column bed.
2. Use 1/16 inch or 1/8 inch stainless steel or PEEK tubing to connect the preparative column to HPLC system to minimize dead volume. Our columns with an inner diameter of 50mm or larger use 1/8 inch fittings and come with 1/16 inch adapters.
3. Correctly connect and tighten the 1/16 inch or 1/8 inch stainless steel or PEEK tubing to the fittings.
4. If leakage occurs at the tubing and fitting connections, re-fit the 1/16 inch or 1/8 inch stainless steel or PEEK tubing to the fittings and reconnect.
5. If leakage occurs between the fitting and the column tube, use a wrench to hold the column tube in place and manually tighten the fitting clockwise with a wrench. Be careful not to over-tighten, especially with stainless steel fittings, to prevent seizing.
6. Ensure that the flow direction of the mobile phase matches the direction indicated on the column tube.

Column Equilibration

Unless otherwise specified, the mobile phase used for our preparative columns is the same as that used in CoA report. Therefore, before conducting experiments, please ensure that the new mobile phase you use is miscible with the mobile phase used during the factory testing. Reversed Phases including ODS(C18), Octyl(C8), Phenyl, Butyl(C4) and Cyano(CN), shall use organic solvents that are miscible with water. Normal Phases shall use solvents like n-hexane, dichloromethane, trichloromethane and isooctane etc. Balance the preparative column with at least 10 times the column volume of the corresponding mobile phase.

Prep column volume calculating formula: $V=\pi r^2 L$
(V=column volume; π=3.1415926; r=column radius/cm; L=column length/cm)

Table1: The volume of mobile phase required for equilibrium

Column Dimension	Column Volume	Minimum Equilibration Volume
10×250mm	19.6ml	196ml
21.2×250mm	88.2ml	882ml
30×250mm	176.7ml	1767ml
50×250mm	490.8ml	4908ml

Note: The volume of mobile phase required for equilibrium is 10 times the volume of the preparative column.

1. After purging, connect the inlet end of the preparative column to the outlet end of the injection valve correctly.
2. Once the mobile phase flows evenly from the outlet end of the preparative column, connect the outlet end of the column to the inlet end of the detector. This prevents air bubbles from entering the detector and reduces the equilibration time.
3. When changing the mobile phase, increase the flow rate slowly to avoid sudden pressure changes.
4. Wait until the column pressure and baseline are stable, indicating that the preparative column is fully equilibrated and ready for the experiment.

Note: If the mobile phase contains low concentrations of additives (e.g., 5-10 mmol/L ion-pairing reagents), equilibrate with 100-200 column volumes of the mobile phase.

Sample Preparation

1. It is suggested to dissolve the sample in the mobile phase or in a solvent that is weaker than the mobile phase.
2. If the sample is not soluble in the mobile phase, ensure that the sample, mobile phase, and solvent used to dissolve the sample are mutually soluble to avoid precipitation.
3. After dissolving the sample, filter it using a 0.22 μm filter membrane.

Prexautions for Use

To ensure the performance and longevity of preparative columns, please follow these guidelines:

1. Guard Column

Impurities in the sample can easily reduce the lifespan and separation efficiency of the preparative column. Here are two solutions:
A: Use appropriate Solid Phase Extraction (SPE) pre-treatment.
B: Solid particles that cannot be completely filtered from the sample and mobile phase, as well as particles generated by pump wear, seal, and tubing aging, can enter the preparative column and clog the frit, leading to increased column pressure and decreased column efficiency. Guard columns or inline filters can prevent these particles from reaching the preparative column. Increased column pressure is a major issue among related column failures, so it is recommended to use an inline filter or guard column at the front end of the preparative column. When adding a guard column before the preparative column, ensure that the physicochemical properties of the guard column match those of the preparative column to avoid affecting separation performance.performance.

2. pH Range

Each preparative column has a specific allowable pH usage range. Please carefully check the pH usage range of your preparative column (Table 2). Using it outside of this range may cause the dissolution of the silica gel matrix or hydrolysis of the bonded phase, leading to irreversible damage to the preparative column. When using near the critical pH value, use a mobile phase containing more than 10% organic solvent. Additionally, using near the critical pH value may shorten the column's lifespan due to factors such as temperature and mobile phase conditions. After experiments near the critical pH value, immediately replace the mobile phase with a suitable washing solution that is compatible with both the preparative column and the current mobile phase for storage.

Table2: pH Tolerance Range for Each Series of Preparative Columns

Column	C4, C8, C18, Phenyl	SiO_2, Amide, NH_2	CN
pH Range	1.5-10.0	2.0-8.0	1.5-9.0
Column	LP-C18, LP-C8	AQ-C18	Xtimate Series
pH Range	0.5-8.0	1.5-10.0	1.0-12.5

3. Reagents

To achieve better chromatographic separation, use high-grade chromatographic reagents. Filter the reagents thoroughly before use to prevent suspended particles from entering the inlet of the preparative column, which can increase column pressure and reduce chromatographic performance. Degas the mobile phase reagents before use to prevent bubbles from entering the pump and detector.

4. Pressure

Column back pressure is concerned with:
A. Particle size and distribution;
B. Column dimension(ID and length);
C. Reagents viscosity, flow rate and temperature.
When using liquid chromatography, the flow rate should be changed slowly to prevent sudden pressure fluctuations that could damage the column. To extend the lifespan of the preparative column, try to operate within the following pressure range.

Particle Size	5μm	10μm	15μm	20μm	20-40μm	40-70μm
Max. Pressure	40Mpa	25Mpa	15Mpa	10Mpa	5Mpa	4Mpa

Note: For other models of preparative columns, please contact Welch.

5. Column Temperature

Suggested column temperature range is 30-50℃. Suitable temperature will decease reagent viscosity, and increase column selectivity and reproducibility.

Storage

Do not use mobile phases containing buffer salts, acids, or bases to store the preparative column. Before storing a preparative column that has been used with buffer salts or saline mobile phases, please refer to the preparative column cleaning methods. Then, replace the mobile phase with the one used during factory testing and store the column. When storing, make sure to tighten the plugs to prevent liquid evaporation, which can cause the column packing material to dry out.
Note: Preparative columns come equipped with appropriate plugs from the factory.

Flow Rate & Sample Loading

The flow rate and sample loading volume of the preparative column are related to the specifications of the column. Please follow the parameters in the table below or use the following formulas for calculation:
A. The flow rate scales linearly and is proportional to the square of the radius of the preparative column: $F2=F1(r2/r1)^2$
B. Retention time unchanged: $F2=F1(L2/L1)(r2/r1)^2$
C. Sample Loading: $W2=W1(L2/L1)(r2/r1)^2$
D. Scaling Factor=$(L2/L1)(r2/r1)^2$
(L=column length(mm); r=column radius(mm); F=flow rate(ml/min);

Table3: Partial Preparative Column Scaling Parameters

Column Dimension	4.6×250 mm	10×250 mm	21.2×250 mm	30×250 mm	50×250 mm
Packing Media(g)	2.5	11.8	53.1	106.3	295.4
Scaling Factor	1	4.73	21.2	42.5	118
Sample(mg)	0.25-25	1.18-118	5.31-531	10.63-1063	29.54-2954
Flow Rate(ml/min)	0.5-2	3-5	10-20	20-45	70-130

Maintenance

As a consumable, the performance of a preparative column will gradually decline with increased usage. Issues such as peak broadening, decreased resolution, and increased column pressure may occur. When these symptoms appear, it is time to consider replacing the preparative column. Following proper operating procedures and conducting regular maintenance can extend the lifespan of the preparative column, whereas improper use can shorten its lifespan. Below are some common maintenance issues encountered during the use of preparative columns.

1. Increasing Column Pressure

A gradual increase in column pressure over long-term use is a normal phenomenon because the use of a preparative column inherently involves contamination. A sudden increase in column pressure over a short period is usually caused by abnormal conditions. After ruling out equipment malfunctions, solutions can be sought from the following points:
A. Contamination of the column head filter frits
Solution: If the sample is complex and contains small particulate impurities, it is recommended to add an inline filter or guard column at the front end of the column. If insoluble substances cause clogging, use an appropriate solvent or filter the sample through amembrane before injection. If the column head is contaminated,

reverse flush the preparative column at a low flow rate with 20-30 column volumes. If reverse flushing does not solve the issue, it is recommended to replace the inlet frit or contact our after-sales service.

B. Contamination of the column head packing material

Prolonged use of the preparative column leads to the accumulation of contaminants at the column head, eventually causing an increase in column pressure.

Solution: Reverse flush the preparative column at a low flow rate with 20-30 column volumes of a solvent that can dissolve the contaminants. If reverse flushing does not resolve the issue, it is recommended to contact our after-sales service or replace the chromatography column.

C. Damage due to improper pH usage

Solution: If improper pH usage has caused damage, it is recommended to replace the chromatography column with a new one.

2. Buffer Salts Precautions

Buffer salts are usually easily soluble in water but poorly soluble in organic solvents. An excessively high proportion of organic solvents or improper use can lead to salting out. The precipitated salt particles can accelerate the wear of the pump plunger, seals, and flow valves. If these salt particles enter the chromatography column with the mobile phase, they can clog the inlet frit of the preparative column. Some salt particles entering the column can block the micropores on the packing materials or even the gaps between the packing materials, hindering the free extension of the bonded phase carbon chains on the matrix. This can cause the packing material to compact, increasing column pressure and leading to a decrease in retention capacity, column efficiency, and resolution, significantly reducing the column's lifespan. Once buffer salts precipitate, they are difficult to remove. Therefore, correct use of buffer salts is crucial for extending the lifespan of the preparative column. The specific methods are as follows:

A. Isocratic: Before and after the experiment, flush the column with a transition mobile phase for no less than 20-30 column volumes, or after the experiment, flush the column with the transition mobile phase overnight at a flow rate of 2 mL/min (adjust according to the column specifications).

B. Gradient: Before the experiment, flush the column with a mobile phase that has the same composition as the initial mobile phase at the analytical flow rate for no less than 20-30 column volumes. After the experiment, flush the column with the transition mobile phase for no less than 20-30 column volumes. Ensure that the gradient is as gentle as possible to avoid the precipitation of buffer salts.

Transition Mobile Phase: The proportion of the organic phase and aqueous phase should match that of the analytical mobile phase or have a higher proportion of the aqueous phase. It should absolutely not contain buffer salts.

C. Precipitation of buffer salts: Avoid the precipitation of buffer salts during the experiment. Once precipitation occurs, it can cause irreversible damage to the preparative column, leading to a rapid increase in column pressure. The following two methods can be used to address this issue:

Method 1: Reverse flush the preparative column with a methanol/water mixture (10/90) at the analytical flow rate and 35°C for 20-30 column volumes.

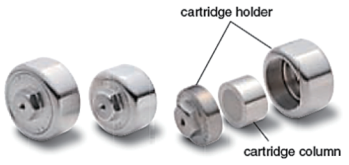
Method 2: Reverse flush the preparative column with a methanol/water mixture (10/90) at a flow rate of 2 mL/min overnight.

3. Guard column

Guard column is installed before the preparative column and after the injector. Its primary function is to intercept insoluble particles in the mobile phase or sample, preventing them from entering the preparative column. Additionally, guard column can capture substances that would otherwise be strongly adsorbed by the preparative column and cannot be washed away by the mobile phase, thus extending the lifespan of the preparative column.

Notes on using guard column:

1. The packing material of the guard column cartridge should be consistent with that of the preparative column.
2. When not in use, it is best to place the guard column cartridge in the guard column holder and seal it with plugs to ensure it remains moist. If the guard column cartridge is left semi-dry for an extended period, it should be reactivated before use.
3. If there is a sudden increase in pressure or a significant deterioration in peak shape, immediately remove the guard column and inject a standard sample for troubleshooting. If contamination of the guard column cartridge is confirmed, replace the guard column cartridge.
4. Ultrasonic cleaning of the guard column cartridge is not recommended, as intense ultrasonic waves can break the packing material, and the resulting fragments can clog the frit, causing increased pressure.



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