Amber GPC chromatography column Instruction Manual

V1.0.2

1. Introduction

The Amber GPC column is specifically designed for high-performance separation of macromolecular compounds. This novel packing material utilizes highly cross-linked polystyrene/diethylbenzene (PS/DVB) particles with extremely narrow particle size and pore size distribution as the matrix. The homogeneous pore size distribution ensures an accurate linear relationship between retention time and molecular weight during analysis. The highly cross-linked porous particles exhibit chemical and physical stability, allowing the shape of calibration curves and column efficiency to remain nearly unchanged when organic solvents are replaced. The Amber GPC packing material possesses a large pore volume, ensuring high resolution in polymer separation. The packing material can be either single-pore or a mixture with varying pore size distributions.

2. Column Inspection and First Use

Upon receiving the chromatographic column, please confirm that the received column matches the information you ordered. Verify that the direction of the mobile phase is printed on the column label and that the column remains undamaged during transportation.

The preservation solvent in the new Amber GPC column is tetrahydrofuran (THF). During storage and transportation, polymer packing material may become desiccated. It is recommended to flush the column with 10-20 times the column volume of pure organic solvent (e.g., THF) to activate the chromatographic column. Subsequently, the column can be flushed with a user-selected mobile phase, gradually increasing the flow rate from 0.1 mL/min to the desired operating conditions until baseline stabilization is achieved. Significant column pressure fluctuations and baseline instability may indicate bubble ingress into the column. Column flushing at high flow rates (e.g., 3.0 mL/min for a 300 mm ID7.8 column) can be performed for 2-5 minutes.

3. Chromatographic column connection

The inlet and outlet of the chromatographic column were connected in the direction indicated by the arrow on the column label.

The port section of the chromatographic column connecting tube must be smooth, with no burrs or beveled cuts.

Where possible, the connecting tubing at both ends of the chromatographic column should be as short as possible, and the inner diameter of the connecting tubing should be minimized to prevent sample diffusion from causing situations where the true column efficiency of the chromatographic column cannot be accurately reflected.

4. Temperature and pH range for use

The maximum operating temperature is 80°C. Prolonged operation at high temperatures (>145°C) may damage the chromatographic column, which is particularly prominent when using pure organic solvents.

The mobile phase can be used within a pH range of 0 to 14. When not in use, avoid storing the chromatographic column in solutions with a pH below 1 or above 12. Long-term storage of the column under extreme pH conditions may damage the filter and stainless column tube.

5. Chromatographic column storage

When not in use for an extended period, store in pure THF solvent.

6. Column Maintenance

Prolonged operation under high pressure can damage chromatographic columns and infusion pumps. Since pressure originates from flow rate, the maximum flow rate is limited by the system's pressure tolerance. Generally, column pressure increases gradually with extended column usage time. Sudden pressure escalation indicates blockage of the sieve plate at the column inlet. In such cases, it is recommended to reverse the column orientation and flush it with an appropriate solvent.

If significant changes in the calibration curve are observed after prolonged use, it may be necessary to replace the sieve plate, particularly the sieve plate at the column inlet end. This is because blockage of the inlet sieve plate could potentially lead to retention of high molecular weight polymers.

7. Chromatographic column maintenance

High-performance liquid chromatography (HPLC) columns are consumables, and proper operation and maintenance are crucial for extending their lifespan.

(1) Precautions before using the chromatographic column

Unless otherwise specified, the chromatographic column storage solutions produced by our company are all the mobile phases indicated in the evaluation reports. Before use, users must ensure that the mobile phase specified for chromatographic column evaluation is compatible with the actual mobile phase used for sample analysis.

(2) Mobile phase

To prevent column clogging, all solvents—including buffer solutions—must be filtered through 0.45 μm or 0.22 μm filter membranes prior to use. Amber GPC bonded stationary phases demonstrate temperature resistance up to 80°C in nearly all organic solvents. Typical solvent systems include tetrahydrofuran (THF), chloroform, dimethylacetamide (DMAC), dimethylformamide (DMF), trichlorobenzene (TCB), N-methylpyrrolidine (NMP), hexafluoroisopropanol (HFIP), and toluene. Solvent switching between systems should not compromise column integrity. Prior to solvent transition, confirm compatibility between the new mobile phase and existing column solvent. Always flush the column with 20-column-volume aliquots of the new mobile phase at 0.2 mL/min flow rate.

(3) Samples

Impurities in samples are one of the primary causes of column contamination and reduced column efficiency in chromatography. For complex samples, appropriate pretreatment methods should be employed to remove impurities, followed by filtration using 0.22μm or 0.45μm filter membranes prior to injection. If sample processing is impractical, the use of protective columns is recommended. In some cases, incompatibility between sample solvents and mobile phases (e.g., immiscibility or polarity differences) may lead to poor chromatographic peak resolution or the appearance of ghost peaks. It is advisable to dissolve samples

using the mobile phase whenever possible.

8. Common Issues and Solutions During Use

The Amber GPC column is fabricated from polystyrene/diethylbenzene materials containing abundant aromatic rings, which exhibits specific interactions with samples containing aromatic rings or lone electron pairs such as oxygen and nitrogen atoms. When the mobile phase lacks electron-rich solvents, these samples may demonstrate pronounced retention and tailing on the GPC column. To achieve sharp peak shapes and high resolution, electron-rich competitive solvents like acetonitrile or mobile phase additives such as triethylamine (TEA) or n-butylamine can be employed to "modulate" the surface chemistry of the packing material. These additives bind to aromatic rings on the packing surface, thereby reducing electron cloud density on the stationary phase. For specific separation requirements, sodium acetate may also be utilized to adjust peak morphology and retention intensity. Similarly, small proportions of glycerol, isopropanol, or other hydrophilic hydroxylating reagents can mitigate the impact of surface hydrophobicity. Recommended mobile phase compositions include 0.5–2.0% TEA or ethylene glycol, or 0.01 M sodium acetate. Organic solvent concentrations such as acetonitrile, methanol, and isopropanol may range from 2.0% to 100%.

When the aforementioned methods fail to resolve the issue, it is recommended to replace the chromatographic column with a new one.

Scan the QR code for more product information.

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