Instruction Manual for NH_2-S Chromatographic Column

V1.0.2

1. Application

Silica gel matrix chromatographic packing material with an aminopropyl bonded group on the surface, suitable for the separation of common monosaccharides.

2. Performance acceptance

After receiving the column, unpacking the column for inspection and confirmation, please test the column as soon as possible according to the chromatographic conditions in the column evaluation report to confirm the column quality.

Sample:

① Fructose (1.2×10^{-3}g/mL)
② Glucose (6.0×10^{-3}g/mL)
③ Sucrose(6.0×10^{-3}g/mL)
④ Maltose (1.0×10^{-2}g/mL)
⑤ Lactose(5.0×10^{-3}g/mL)

Note: If the above conditions are inconsistent with the chromatographic column test report, the test report shall prevail。

3. Column connection

The column inlet and outlet are connected in the direction indicated by the column label arrow.

The end of the connecting tube of the chromatographic column must be flat and free of burr and cut slope.

If possible, the connecting pipes at both ends of the chromatographic column should be as short as possible, and the inner diameter of the connecting pipe should be as small as possible to prevent the sample diffusion from not reflecting the true column efficiency of the chromatographic column.

4. Column use

Item	Range
pH	3.0 - 7.0
Temperature	20 - 60 °C
Pressure	<40 MPa
Common Flow Rate	1.0 - 1.5 mL/min

5. Column storage

The storage solvent for the NH_2-S chromatographic column when it leaves the factory is pure acetonitrile (If the storage solution is inconsistent with the test report, the mobile phase in the test report shall prevail).

For overnight or short-term storage: When using a buffer solution or a salt-containing mobile phase, the mobile phase in the chromatographic column and the system needs to be replaced with methanol/water or acetonitrile/water with an organic solvent concentration of not less than 70%.

For long-term storage: Store with pure acetonitrile and tighten the column end plugs. Store at room temperature.

6. Column regeneration

Rinse the chromatographic column with 5 - 10 times the column volume of a 75% acetonitrile/water solution containing 0.5 - 1.0% ammonia water. After rinsing, rinse again with a 75% acetonitrile/water solution to remove excess ammonia.

The above chromatographic column regeneration method is not an absolute standard method. Users can select a suitable solvent that can dissolve the contaminants in the column as the mobile phase to rinse the chromatographic column according to their actual experience and purpose. However, regardless of the method used to regenerate the chromatographic column, it is impossible to restore the column efficiency or other parameters to the level of a new column. If the column efficiency decreases due to the pH value of the mobile phase exceeding the operating range or the proportion of the organic phase being lower than 70%, it cannot be regenerated and restored.

7. Column maintenance

High-performance liquid chromatographic columns are consumables, and proper operation and maintenance are crucial to prolong the life of the chromatographic column.

Mobile Phase

The types of mobile phases used for the NH2-S chromatographic column are usually acetonitrile and water.

The storage solvent for the NH2-S chromatographic column when it leaves the factory is pure acetonitrile (If the storage solution is inconsistent with the test report, the mobile phase in the test report shall prevail). When using a mobile phase containing an aqueous phase, the proportion of the organic phase shall not be lower than 70%.

When using a buffer salt as the mobile phase, the optimal concentration of the buffer salt is (0.01 - 0.02) mol/L, and the pH range of the buffer salt is 3.0 - 7.0. Before and after using the buffer salt mobile phase, it must be replaced with an aqueous phase in the same proportion as the buffer salt, with a volume of not less than 20 times the column volume.

The organic solvents used in the mobile phase must be chromatographically pure, and the water used in the mobile phase is preferably ultrapure water or double-distilled water in all-glassware. The buffer salt must be filtered through a solvent filter (0.45μm filter membrane) before use. The mobile phase should be prepared freshly, and salt-containing solutions left for a long time may breed bacteria or form precipitates.

Sample

Impurities in the sample are one of the main reasons for chromatographic column contamination and column efficiency decline. Complex samples can be pretreated by appropriate methods to remove impurities. The sample must be filtered

through a 0.22μm or 0.45μm filter membrane before injection. If the sample is inconvenient to handle, it is recommended to use a guard column. Sometimes, the mismatch between the sample solvent and the mobile phase (immiscibility, polarity difference, etc.) may lead to poor chromatographic peak shape and the appearance of ghost peaks. It is recommended to dissolve the sample in the mobile phase as much as possible.

8. Problems and solutions in use

The problems that are easy to occur in the use of high-performance liquid chromatographic columns are the decline of column efficiency and the sudden rise of column pressure. The possible reasons are as follows:

(1) Filtration sieve plate contamination in column head;

(2) Contamination of column head packing;

(3) The salt in the buffer solution in the chromatographic column forms crystals and precipitates;

(4) The pH value of the mobile phase exceeds the range of use of the chromatographic column, causing the structure of the stationary phase to be destroyed or dissolved.

The solutions are as follows:

When judging that the sieve plate or filler at the column head is contaminated, the mobile phase that can dissolve pollutants can be used. Flush the chromatographic column (about 20 to 30 times the column volume, or depending on the specific situation; at this time, it is better not to connect the detector) in the opposite direction of the chromatographic column, flush the pollutants out of the chromatographic column, and then use it according to the arrow direction (flow direction) marked on the chromatographic column.

When the above method cannot solve the problem, it is recommended to replace a new chromatographic column

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Suzhou Elite Science & Technology Co., Ltd.
Address: 701#, Building 10, Liando U Valley, No. 179 Zhujiawan Street, Gusu District, Suzhou, Jiangsu, China.
Tel: +86-512-67997535
Fax: +86-512-67997535

Elite (Dalian) Analytical Instruments Co., Ltd.
Address: No. 2-2 Xuezi Street, Qixianling Subdistrict, Dalian High-Tech Industrial Zone, Liaoning, China.
Tel: +86-0411-84753333
Fax: +86-0411-84753333

Customer service email : info@eliteHPLC.com
http://www.eliteHPLC.com