Normal Phase Chromatographic Column (NPC) Instruction Manual

V1.1.0

1. Application

Silica gel (SiO_2) chromatographic packing, or a series of chromatographic columns with silica gel as the matrix and NH2 (APS), CN (CPS), Diol, amide (HILIC mode) and other functional groups bonded on the surface.

2. Performance acceptance

After receiving the column and checking it out of the box, please test according to the chromatographic conditions in the column evaluation report as soon as possible to confirm the column quality.

3. Column connection

Column inlets and outlets are connected in the direction indicated by the column label arrows.

The port part of the column connection tube must be flat, without burrs and incision bevels.

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. Use the pH range

The pH range is 2.0~8.0.

5. Temperature range

Recommended operating temperature is not higher than 60 ℃.

Avoid sudden temperature increases during use.

6. NPC and RPC system transformation

Amino (NH2), cyano(CN), diol (Diol) and amide (HILIC) columns are evaluated under normal-phase conditions, if reversed-phase mobile phase conditions are actually used, the mobile phase in the column must be completely replaced with an intermediate mobile phase (such as isopropanol), followed by flushing at a low flow rate of 0.2–0.3 mL/min for 20–30 column volumes, and then use a reversed-phase mobile phase, and the proportion of organic phase in the mobile phase is not less than 50-65%. Frequent switching of mobile phase modes for this type of column is not recommended, as it may shorten column lifetime. Silica gel columns are not recommended for use under reversed-phase conditions.

7. Column storage

Reversed-phase conditions：

Overnight or short-term storage: When using buffer solutions or salt-containing mobile phases, Mobile phase of chromatographic column and system need to be replaced with a concentration of at least 65% methanol/water or 50% acetonitrile/water.

Prolonged storage: It can be stored in pure methanol or pure acetonitrile, and the column must be thoroughly cleaned before storage to avoid residual sample impurities and tighten the column plug.

Normal phase conditions：

Overnight or short-term storage, experimental mobile phase can be used, long-term storage can use n-hexane.

(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 storage temperature is preferably room temperature.

8. Column regeneration

Hexane, isopropanol, dichloromethane, methanol and other solvents are used as mobile phases, and rinsed in turn, and each mobile phase flows through the column at least 20~30 times the column volume (isopropanol and other solvents have a large viscosity, and the flow rate can be reduced during the flushing process to avoid excessive pressure). Note that the order of solvent use cannot be reversed; After rinsing with methanol, rinse to n-hexane in reverse order. It should be noted that the solvent used must be strictly dehydrated.

The regeneration method of the above chromatographic column is not an absolute standard method. Users can choose a suitable solvent that can dissolve the pollutants in the column to wash the mobile phase in the positive or reverse direction according to their actual experience and purpose. However, no matter what method is used to regenerate the chromatographic column, it is impossible to restore the column efficiency or other parameters to the new column level.

9. Column maintenanace

High performance liquid chromatographic column is a consumable, and normal operation and maintenance are essential to prolong the life of chromatographic column.

(1) Precautions before use of chromatographic column Without special instructions, the chromatographic column storage liquid produced by our company is the mobile phase shown in the evaluation report. Before use, users must pay attention to the compatibility between the evaluation mobile phase of the chromatographic column and the actual use of the analytical sample.

(2) Mobile phase

The organic solvent used in the mobile phase must be chromatographically pure, and the water used in the mobile phase should preferably be ultra-pure water or double-distilled water for all glassware. The buffer salt must be filtered using a solvent filter (0.45μm filter) before use. The mobile phase should be prepared on the spot, the saline solution will breed bacteria or precipitate if it is placed for a long time.

(3) Samples

Impurities in the sample are one of the main reasons for column pollution and column efficiency decline. For complex samples, appropriate methods can be taken to pretreat and remove impurities. A 0.22μm or 0.45μm filter must be used before injection. If the sample is inconvenient to handle, it is recommended to use a protective column. Sometimes the mismatch between the sample solvent and the mobile phase (incompatibility, polarity difference, etc.) will lead to poor chromatographic peak shape, ghost peak and other phenomena. It is recommended to use the mobile phase to dissolve the sample as much as possible.

10. 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;

The solution is as follows:

When judging that the sieve plate or packing material 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 it is judged that there is salt crystallization precipitation: the column is flushed with an aqueous solution of about 50~65% organic solvent, so that all the salts in the column are dissolved and flushed out, and then replaced with a mobile phase containing high concentration methanol or other organic solvents.

If the pH value is used improperly, it is usually difficult to restore the chromatographic column.

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

More product information

Column Selection Guide

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