

Reverse Phase Chromatographic Column (RPC)

Instruction Manual

VI.1.0

1. Application

A series of chromatographic columns with silica gel as matrix and surface bonded C18 (ODS), C8 (MOS), C4 (Butyl), C1 (SAS), C6H5 (Phenyl) functional groups as reverse phase chromatography (RPC) packing.

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.

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. Use pH range and water resistance

The pH range of conventional chromatographic column is 2.0~8.0, and that of special packing chromatographic column is wider, and the pH range of some commonly used columns is shown in the table below.

Columns	pH range	Columns	pH range
Hypersil	2-8	SinoChrom	2-8
SinoPak C18	1-12	SinoPak BEH T-C18	1-12.5
SinoPak AQ-C18	2-8	SinoPak BEH AQ-C18	2-11
SinoPak RP-C18	1-8	SinoPak BEH RP-C18	2-11
Supersil ODS2	1.5-10	SinoPak HSS AQ-C18	2-11
Supersil AQ-C18	2-9	ECoreShell C18	2-9

For specific pH ranges of other columns, please scan the QR code of the "Column Selection Guide" below.

Conventional columns are made with a special chemistry column that is not higher than 90% aqueous and has higher water resistance, as shown below.

Columns	Maximum water use ratio	Columns	Maximum water use ratio
AQ-C18 、 RP-C18	100%	SinoChrom ODS-BP	95%
Other	90%		

5. Temperature range and Operating pressure

Recommended operating temperature is not higher than 60 °C.

When using phosphate buffer solution and operating at medium or high pH value, it is recommended that the column temperature should not exceed 40 °C. Avoid sudden temperature rise during use.

The maximum working pressure of the particle size is 4 0 MPa for a column with a particle size greater than or equal to 5 μm, and the maximum working pressure of a column with a particle size of less than 5 μm is 60 MPa.

6. Column storage

Overnight or short-term storage: when using buffer solution or salt-containing mobile phase, it is necessary to replace the chromatographic

column and the mobile phase in the system with 10%-20% methanol/water or acetonitrile/water, and the concentration of organic solvent in the storage solvent of the chromatographic column is not less than 20%.

Storage for more than 1 week: pure methanol or acetonitrile can be used for storage, and the chromatographic column plug shall be tightened. The chromatographic column must be thoroughly cleaned before storage to avoid residual sample impurities. For chromatographic column using mobile phase with high salt concentration, before storage, the chromatographic column and mobile phase in the system must be replaced with 10%~20% methanol/water or acetonitrile/water to avoid salt precipitation and crystallization.

The storage temperature is preferably room temperature.

7. Column regeneration and activation

Respectively use methanol: water=90:10 (v/v), pure methanol and dichloromethane as mobile phases, and wash them in turn. Each mobile phase flows through the chromatographic column not less than 20-30 times the volume of the chromatographic column. Then use dichloromethane, pure methanol, methanol: water=90:10 (v/v) as mobile phase to wash in turn.

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.

If the column is used for the first time or has not been used for a long time, the storage solvent (pure methanol or acetonitrile) is set at rinse 20~30 times the column volume at a low flow rate of 0.2~0.5 mL/min for activation.

8. Column maintenance

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.

9. 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 solution is 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 it is judged that there is salt crystallization, wash the chromatographic column with aqueous solution of about 20% organic solvent to dissolve all the salt in the column, and then replace it with mobile phase containing high concentration methanol or other organic solvent.

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



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>