

For High Performance Liquid Chromatography

COSMOSIL[®] CHiRAL PACKED COLUMNS

1. INTRODUCTION

Thank you for purchasing COSMOSIL Packed Column. To ensure maximum efficiency and long column life, we ask you to read this manual carefully.

COSMOSIL Packed Columns are made of stainless steel and packed with specially bonded high purity spherical porous silica. The COSMOSIL CHiRAL packed columns are for chiral separation of optical isomers.

2. CARE AND USE

1. Avoid mechanical shocks to the column.
2. Connect the column according to the flow direction indicated on the label.
3. Though the columns are made for using under 30 MPa, we recommend keeping pressure under 25 MPa.
4. We recommend keeping flow rate under 1.0 mL/min for 4.6 mmI.D. columns.
5. Keep the temperature 0 - 40°C. When mobile phase pH is over 7, keep the temperature under 25°C.
6. Wash the column with 20 - 30 ml mobile phase before connecting to the detector.
7. The product can be used in normal phase or reverse phase mode. The column is shipped in normal phase solvent. If you want to use the column in reversed phase mode, replace the shipping solvent to ethanol or 2-propanol first as an intermediate step (they are compatible to both normal phase and reversed phase solvents), before replacing it with reversed phase solvents.

	Normal phase mode	Reversed phase mode
Organic solvent	Alkane (<i>n</i> -Hexane, etc.), Alcohol (2-Propanol, Ethanol, etc.), Tetrahydrofuran, <i>tert</i> -Butylmethylethel, Chloroform, Ethyl acetate, etc.	Methanol, Acetonitrile, Ethanol, 2-Propanol, Tetrahydrofuran, etc.
Additives (for acidic compounds)	Trifluoroacetic acid, Formic acid, Acetic acid, etc. normally; 0.1%, maximum; 0.5%	0.1%-Phosphoric acid, 0.1%-Formic acid, 20mmol/L-Phosphate buffer (pH2.5), etc.
Additives (for basic compounds)	Diethylamine, Triethylamine, Ethylenediamine, 2-Aminoethanol, etc. normally; 0.1%, maximum; 0.5%	20mmol/L-Phosphate buffer (pH7.0), 20mmol/L-Ammonium hydrogen carbonate buffer (pH9.0 with diethylamine), etc.
pH of mobile phase	2.0 - 9.0	2.0 - 9.0
Ratio	Organic solvent / Organic solvent = any ratio	Organic solvent / Aqueous solution = 10 / 90 - 100 / 0

8. If you use a mixture of aqueous and organic solvents in reversed phase mode, be careful not to cause salt precipitation. Use HPLC grade solvent.
9. Frequent changes of the separation mode will destroy the column. One column should be used in one mode consistently. Also we recommended keeping the chromatography conditions constant, since frequent changes of mobile phase shorten column life.
10. Pass mobile phase through membrane filter (less than 0.45 μm in pore size) before use.
11. Filter samples before injection. Avoid precipitation at injection. Using very different sample solvent from the mobile phase may cause peak broadening, decrease resolution and reproducibility.
12. Insoluble debris from the pumping system, mobile phase, or samples trapped in the filter (2 μm) at the inlet of the column may increase the pressure.
13. Avoid injecting air, changing flow rate rapidly. Change mobile phase at less than 1/2 of usual flow rate.
14. To maximize the column performance and to minimize the dead volume, use shorter and narrower tubing.
15. Maintain column and tubing temperature constant for good reproducibility.
16. After analysis, wash the column with shipping solvent (*n*-hexane / 2-propanol = 90 / 10) in normal phase use. In reversed phase use, wash with deionized mobile phase, then replace with ethanol. Store the column tightly plugged, on vibration-free place.
17. Do not tighten the plugs and tubing too much. The ferrules could be damaged.

3. TROUBLESHOOTING

Trouble	Cause	Solution
Increase of pressure	Clogging of the end filter Clogging of the packing material Salt precipitation in the column	(1) (2) (1) (3) (4)
Poor resolution	Contamination of packing material Disorder of packing material Change of higher-order structure of chiral selector	(3) (4) Unregenerable (5)
Split peak	Void in the column	Unregenerable
Unstable baseline	Contamination of packing material Contamination of mobile phase	(3)(4) (6)

- (1) Disconnect column from the detector. Run mobile phase through the column in reverse direction at 1/2 of usual flow rate for 30 min.
- (2) Wash the end filter or replace it with a new one. If opening the column and replacing end filter on your own, the column performance may decrease.
- (3) Wash the column with strong solvent (ex. ethanol) to wash out the long retention compound.
- (4) Wash the column with ethanol / deionized water = 10 / 90.
- (5) (i) CHiRAL A Column; Wash the column with ethanol at 1/2 of usual flow rate for 30 min, then run *N,N*-dimethylformamide at 1/3 of usual flow rate for 3 hours. After that, run ethanol at same flow rate for 1 hour, then replace with shipping solvent (*n*-hexane / 2-propanol = 90 / 10) at usual flow rate for 1 hour to equilibrate the column. Test the column and compare result to the column inspection sheet.
(ii) CHiRAL B and C Column; Wash the column with ethanol at usual flow rate for 30 min, then run ethyl acetate at usual flow rate for 30min. After that, let the column sit tightly plugged for 2 days. Test the column and compare the result to the column inspection sheet.
- (6) Use the deionized water or HPLC grade solvents.

4. WARRANTY

Nacalai Tesque will replace defective columns reported within 2 weeks of delivery. Nacalai Tesque approves return in case of:

- (1) Damage during the transportation caused by our incomplete packing.
- (2) Theoretical plate number measured according to the test method specified in the Inspection Report is significantly lower than guaranteed.
(Please note that the plate number decreases when using an apparatus with large dead volume or injecting a big amount of sample.)

We cannot accept claims for deterioration of column performance caused by taking off the end filters or end-fittings, or natural column degradation. Return shipment is unacceptable unless we have given prior permission and shipping instructions.