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C015-2403-3

# **Ultra High Performance Liquid Chromatography**

# COSMOSIL® PACKED COLUMN

#### 1. INTRODUCTION

nacalai tesque

The quality for certainty.

Thank you for purchasing our COSMOSIL Packed Column. The products are made of stainless steel and packed with 1.8 or  $2.5 \mu m$  totally porous spherical silica-based materials. Please read this manual carefully to ensure maximum separation efficiency and long lifetime of the columns.

#### 2. CARE AND USE

- 1. Use the UHPLC equipments suitable for rapid separation.
- 2. When using normal HPLC equipments, set the response time of detector under 0.05 second. [Fig.1]
- 3. When using 2 mm I.D. column, use the detector cell and injector for semi-micro and the 0.1 mm I.D. pipe. [Fig.2]

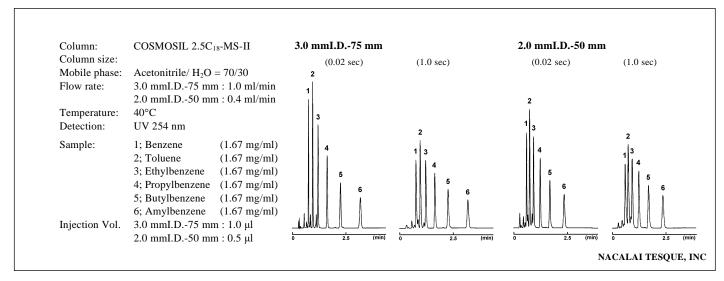


Figure 1. Effect of response time of detector

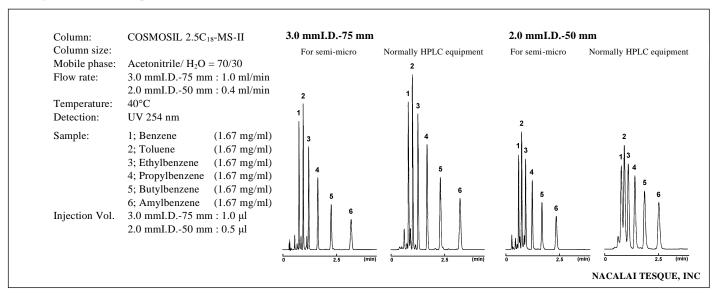


Figure 2. Effect of HPLC equipment

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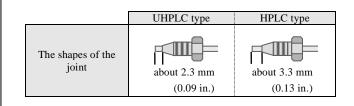
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4. The connection type of 1.8 μm particles packed column is the former Waters' UPLC compatible type (UHPLC type), and of 2.5 μm particles packed one is the Waters type (HPLC type). please use suitable or movable fittings to connect each column. [Fig.3]



HPLC type ; former Waters type connection, having long nib.

UHPLC type ; Waters' UPLC® compatible type connection, having

short nib.

The length of seating depth of the nib is different.

UPLC® is a registered trademark of Waters Corp.

Figure 3. Difference between HPLC type and UHPLC type fitting.

- 5. Keep pressure under 80 MPa (1.8  $\mu m$  particles packed column) or 30 MPa (2.5  $\mu m$  particles packed column).
- 6. We recommend using 0.8 mL/min and 0.4 mL/min flow rate for 3 mm I.D. and 2 mm I.D. columns, respectively.
- 7. Avoid mechanical shocks to the column.
- 8. Connect the column according to the flow direction indicated on the label.
- 9. Wash the column with 20 to 30 mL mobile phase before connecting to the detector.
- 10. Use scrupulously degassed mobile phase. Air bubbles generate detection noise and accelerate column deterioration.
- 11. Use only HPLC grade solvents.
- 12. Avoid precipitation in the column. Check the solvent constitution at shipment on the enclosed inspection record.
- 13. Keep the pH of the mobile phase within the range of 2 to 7.5. Buffer concentration is usually sufficient within the range of 0.005-0.020 mol/L. Filter the mobile phase using membrane filter with 0.45 μm or smaller pore size prior to use. When using trifluoroacetic acid, keep the concentration under 0.1%.
- 14. After performing reversed phase chromatography, wash the column with acid-and/or salt-free solvent first, then with acetonitrile/water=70/30 or methanol/water=70/30. Store the column with caps tightly plugged.
- 15. Filter the sample before injection. Avoid precipitation at injection.
- 16. Removal of the end filters or change of the end-fittings will result in low performance of the column.
- 17. Do not tighten nuts more firmly than necessary.
- 18. In order to maximize the column performance, minimize the dead volume in the equipment by shortening the length and/or narrowing the width of tubing.
- 19. Maintain column and tubing temperature constantly.
- 20. Avoid following on use: injecting air, changing flow rate rapidly and changing mobile phase at high flow rate.
- 21. Insoluble matters from the pumping system, mobile phase, or samples trapped in the filter at the inlet of the column may increase the pressure.
- 22. We recommend keeping the chromatography conditions constant, since frequent change of mobile phases will shorten the column lifetime.

## 3. TROUBLESHOOTING

Trouble	Cause	Solution
Increase of pressure	Clogging of the end filter Clogging of the packing material Precipitation in the column	(1) (2) (2)
Poor resolution	Contamination of packing material Disorder of packing material	(2) Not regenerable
Split peak	Void in the column	Not regenerable
Unstable baseline	Contamination of packing material Contamination of mobile phase	(2) (3)

- (1) Disconnect column from the detector. Wash with mobile phase through the column in reverse direction at half flow rate for 30 minutes.
- (2) Wash the column with a solvent capable dissolving the contaminants. The column can be washed, if necessary, with water, acetonitrile, methanol and/or tetrahydrofuran.
- (3) Use the new deionized water or HPLC grade solvents.



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### 4. WARRANTY

Nacalai Tesque will change defective columns reported within 2 weeks of receipt. 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 apparatus with large dead volume, injecting big amount of sample, or dissolving the sample with solvents which are foreign to the mobile phase.)

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