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C013-1403-4

COSMOSIL® HILIC PACKED COLUMN

1. INTRODUCTION

Thank you for purchasing our COSMOSIL HILIC Packed Column. We recommend you to read this manual carefully to ensure maximal efficiency and long life of the column.

2. TYPES OF STATIONARY PHASES AND THEIR CHARACTERISTICS

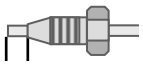
COSMOSIL HILIC Packed Columns are made of stainless steel and packed with triazole bonded high purity spherical porous silica. The COSMOSIL HILIC packed columns are for hydrophilic interaction chromatography (HILIC) of polar compounds, which are not retained by reversed phase chromatography.

Table 1 Product Specification and recommend conditions

Packing material	2.5HILIC	HILIC
Silica gel	High purity porous spherical silica	
Average particle size	2.5µm	5µm
Average pore size	13nm	12nm
Ligand	Triazole	
Column size	I.D. 1.0 mm - 3.0 mm, Length 50 mm - 150 mm	I.D. 1.0 mm - 28 mm, Length 10 mm - 250 mm
Flow rate	I.D. 2.0 mm: 0.4ml/min, I.D. 3.0 mm: 1.0ml/min	I.D. 4.6 mm: 1.0ml/min, I.D. 10 mm: 5.0ml/min
Pressure	Under 30 MPa	Under 20 MPa (under 15 MPa for 10 mm I.D. or wider columns).
Response time of detector	Under 0.1sec	1.0sec

3. CARE AND USE

1. Avoid mechanical shocks to the column.
2. The connection type of COSMOSIL Column is the same type (HPLC type) as the former Waters compatible type. In the case of using systems having UHPLC type fittings, please use suitable or PEEK fittings to connect COSMOSIL Column. [Fig.1]

	HPLC type	UHPLC type
The shapes of the joint	 about 3.3mm (0.13 in.)	 about 2.3mm (0.09 in.)

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 1. Difference between HPLC type and UHPLC type fitting.

3. Connect the column according to the flow direction indicated on the label.
4. Avoid injecting air, changing flow rate rapidly and changing mobile phase at high flow rate.
5. Filter the sample before injection. Avoid precipitation at injection.
6. In order to maximize the column performance, minimize the dead volume in the equipment by shortening and/or narrowing the width of tubing.
7. Maintain constant column and tubing temperature.
8. Do not tighten nuts more firmly than necessary.
9. After analysis, wash the column with solvents that do not contain acid or buffer, and then fill the column with approx. 90% acetonitrile. Store the column tightly plugged at room temperature.

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4. SELECTION GUIDE OF MOBILE PHASE

COMOSIL HILIC column generates retention and separation by hydrophilic interaction (mainly hydrogen bond) and anion-exchange. Refer to following recommendations to select an appropriate mobile phase condition.

(1) The effect of organic solvent type and content
- In general, acetonitrile/water is used as mobile phase.
- Retention increases as water content in the mobile phase decreased. (Fig.2)
- Use acetonitrile content in the mobile phase within the range of 0-95% (in general 50-95%).
- Methanol/water generates shorter retention than acetonitrile/water. (Fig.3)
- Use only HPLC grade solvents.

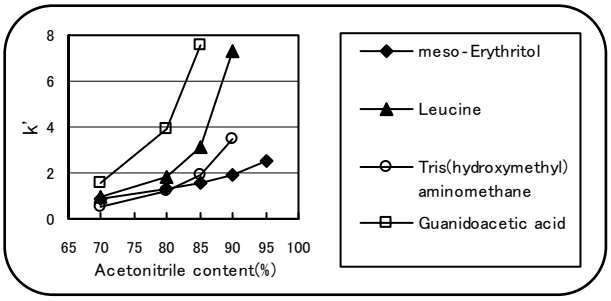

Fig.2 The effect of acetonitrile content on retention
Column; COSMOSIL HILIC
Mobile phase; Acetonitrile/ 10mmol/l CH_3COONH_4

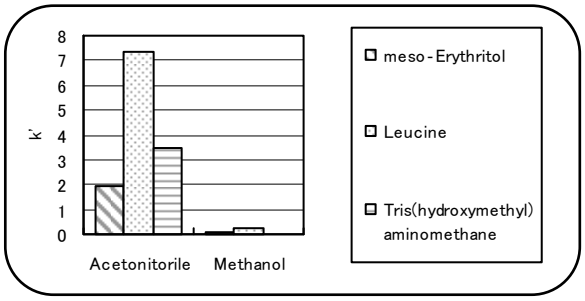

Fig.3 Difference of acetonitrile and methanol on retention
Column; COSMOSIL HILIC
Mobile phase; Organic solvent/ 10mmol/l CH_3COONH_4 = 90/10

(2) The effect of buffer pH
- Keep pH of the mobile phase within the range of 2-7.5.
- The buffer around neutrality generates larger retention. (Fig.4)

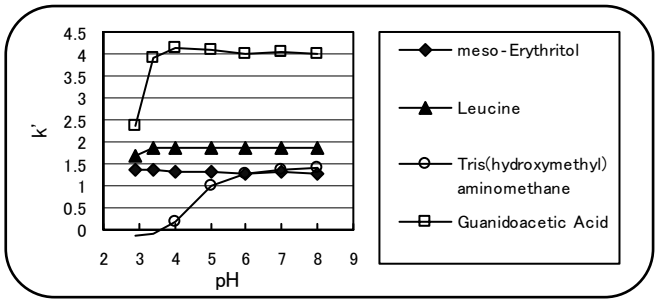

Fig.4 The effect of buffer pH on retention
Column; COSMOSIL HILIC
Mobile phase; Acetonitrile / 10mmol/l buffer = 90/10

(3) The effect of salt type and concentration
- When analyze ionic compounds, add salts or buffers in the mobile phase.
- When mobile phase has high acetonitrile content, note dissolubility of the salt. The dissolubility of phosphate buffers used widely in reversed phase chromatography is low in acetonitrile. Therefore use of phosphate buffers is not recommended. Keep the concentration of acetonitrile under 70% if use phosphate buffer. Check that the salt does not precipitate when mixed with acetonitril before use.
- Ammonium acetate or formic acid ammonium buffers are recommended because they are soluble in high acetonitrile content.
- Use the buffer concentration within the range of 5 - 100mmol/l. Moreover, check that the salt does not precipitate after mixing buffer and acetonitrile.
- High salt concentration inhibits ion exchange and decreases retention. (Fig.5)
- Run mobile phase through membrane filter (less than 0.45μm in pore size) before use.

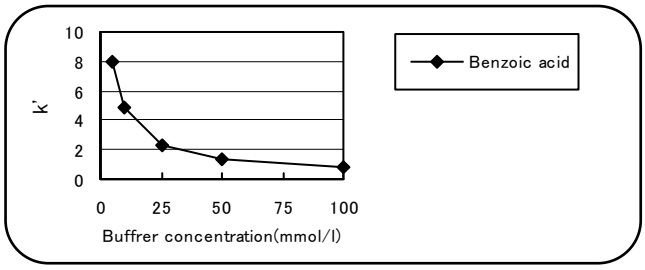

Fig.5 The effect of salt concentration on retention
Column: COSMOSIL HILIC
Mobile phase; Acetonitrile / x mmol/l CH_3COONH_4 = 50/50

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(4) Selection of mobile phase

Following are the recommended mobile phases for different compound types. Adjust retention time by acetonitrile content mainly.

Neutral compounds	→	Acetonitrile / Water = 90/10
Basic compounds	→	Acetonitrile / 10mmol/l CH_3COONH_4 = 90/10
Amphoteric compounds	→	Acetonitrile / 10mmol/l CH_3COONH_4 = 70/30
Acidic compounds	→	Acetonitrile / 10mmol/l CH_3COONH_4 = 50/50
		↓ not eluted
		Acetonitrile / 10mmol/l Phosphate buffer (pH7.0)= 50/50

(5) Improvement of peak shape

Try following if peak shape is tailing. The peak shape might improve. (Fig.6)

- Add 5mmol/l EDTA to mobile phase.
- Change to citrate buffer. (i. e. 10 mmol/l citrate buffer (pH7.0))

80%Acetonitrile/ 10mmol/l CH_3COONH_4

80%Acetonitrile/ 10mmol/l CH_3COONH_4+ 5mmol/l EDTA

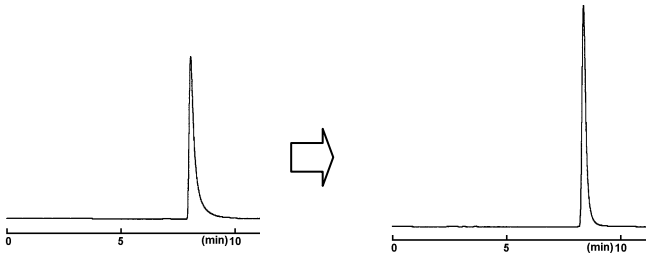

Fig.6 Improvement of peak shape
Column: COSMOSIL HILIC(4.6mmI.D.-250mm)
Sample: Tryptophan(1ng), Flow rate: 1.0ml/min
Detection: UV254nm, Temperature: 30°C

(6) Others

- Use scrupulously degassed mobile phase. Air bubbles generate detection noise and accelerate column deterioration.
- We recommend keeping the chromatography conditions constant, since frequent changes of mobile phase shorten column life.

5. TROUBLESHOOTING

Trouble	Cause	Solution
Increase of pressure	Clogging of the end filter	(1)
	Clogging of the packing material	(1)
	Precipitation in the column	(2)
Poor resolution	Contamination of packing material	(3)
	Disorder of packing material	Unregenerable
Split peak	Void in the column	Unregenerable
Unstable baseline	Contamination of packing material	(3)
	Contamination of mobile phase	(4)

(1) Disconnect column from the detector. Run mobile phase through the column in reverse direction at half flow rate for 30 min.
(2) Wash the column with deionized water.
(3) Wash the column with 50%acetonitrile for 30 min.
(4) Use deionized water or HPLC grade solvents.

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6. 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 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 long shelf life. Return shipment is unacceptable unless we have given prior permission and shipping instructions.

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