Column Care and Use Instructions
YMC CHIRAL Polysaccharide derivative Series
Cellulose-SB

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column. The YMC CHIRAL Cellulose-SB column is designed for separating optical isomers (in normal/ reversed phase mode). The immobilized chiral selector, a polysaccharide derivative, provides high compatibility with wide range of organic solvents, and superior separation and selectivity. With its advantages, YMC CHIRAL Cellulose-SB column is suitable for separating a variety of chiral compounds.

YMC CHIRAL Cellulose-SB column, which is manufactured under highly controlled conditions, must pass a series of stringent tests before being accepted for shipment. (Please refer to the column inspection report). To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

2. Specifications

Item	YMC CHIRAL Cellulose-SB
Particle size	5, 10, 20 µm
Chiral selector	Cellulose tris(3,5-dimethylphenylcarbamate)
Type	Immobilized type
Separation mode	Normal Phase/ Reversed Phase
Shipping solvent[1]	*n*-hexane/2-propanol (90/10)
Temp. range (℃)	0 – 40 ℃ (Max. 25 ℃ in pH 7 – 9)
Usable pH range	pH 2 – 9
Pressure limit[2]	30 MPa
Recommended flow rate[3]	4.6 mm I.D. : 0.5 – 1.0 mL/min（Max. flowrate: 3.0 mL/min） 10 mm I.D. : 2.5 – 5.0 mL/min（Max. flowrate: 15 mL/min）

[1]: After use in normal phase; if you intend to store the column for a long time, replace the mobile phase in the column with shipping solvent.

The initial use in reversed phase (aqueous); replace the shipping solvent with ethanol or 2-propanol before replace with the mobile phase for separation.

After use in reversed phase; the mobile phase should be replaced with ethanol before storing in the shipping solvent. For the mobile phase containing buffer salts/additives, the replacement process should be carried out with caution to prevent the precipitation of salt when replacing.

[2]: Avoid using a column repeatedly near the pressure limit or abrupt change in the pressure in order to prevent from shortening the column lifetime. The recommended operational pressure of the column is 25MPa or less.

[3]: Adjust flow rate according to the recommendation in the table above to obtain the optimum results under the application. The repeated use at or near the upper limit of flow rate can reduce the column lifetime. When using column dimensions other than listed, adjust flow rate according to the cross-section area of the column.

[2, 3]: Pressure changes depending on column length, temperature, types of organic solvent etc. If pressure exceeds the upper limit, reduce flow rate to below the lower rate of recommended range.

3. Column installation

- The column endfitting is Waters style connection.
- Tubing must have flat ends and must bottom out in the column endfitting. Tubing must be connected to the column correctly to avoid creating a void between the column frit and tubing, which can cause a leak and result in poor column performance (e.g. peak tailing, loss of theoretical plate number).
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- Do not disconnect a column from the LC system before the pressure drops to zero.

4. Mobile phase and sample solvent

- YMC CHIRAL Cellulose-SB is suitable for any mobile phase commonly used in HPLC columns, from aqueous to nonaqueous solvents. The column can be used on both normal phase and reversed phase (aqueous mobile phase). We recommend that the column is dedicated to either phase. Frequent switch of modes between phases can cause extensive damage to the column. Please refer to the recommendation of the solvents in the table below.
- Make sure of miscibility among the organic solvents. When switching the mobile phase from alkane/alcohol mobile phase to polar organic solvents (methanol, acetonitrile etc), flush the column with compatible solvent such as ethanol or 2-propanol for at least ten column volumes beforehand.
- HPLC system and PEEK tubing should have resistance to solvents when using solvent for normal phase.
- When a target compounds is ionic, addition of modifier listed below can improve peak shape and/or separation reproducibility. High concentrations of modifiers can result in reducing column lifetime. Add/reduce the modifiers according to the notes in the table.
- Whenever possible, the sample should be dissolved in the same composition as the initial mobile phase. Using a stronger solvent than mobile phase for sample dissolution may result in broad peaks and reducing the separability and reproducibility. In addition, before injection, please check the miscibility of the sample solvent and mobile phase in order to prevent the sample from precipitating on injection
- In order to avoid blockage which can cause pressure increase, the sample solution should be filtered through a membrane filter (0.2 μm or smaller porosity).

【Recommended solvents for Normal phase】

	Acid compounds	Neutral compounds	Basic compounds
Organic solvents	alkane (n-hexane or n-heptane), alcohols(methanol, ethanol, 2-propanol), acetonitrile, ethyl acetate, tetrahydrofuran (THF), dichloromethane, chloroform, methyl tert-butyl ether (MTBE)		
Modifiers	0.1% (Upper limit 0.5%) trifluoroacetic acid (TFA), acetic acid, formic acid, etc	None	0.1% (Upper limit 0.5%) diethylamine (DEA), butylamine, ethanolamine, etc
Composition ratio	Any ratio (those should be miscible)		

【Recommended solvents for Reversed phase】

	Acid compounds	Neutral compounds	Basic compounds
Organic solvents	Acetonitrile, methanol, ethanol, 2-propanol, THF, etc		
Modifiers	0.1% phosphoric acid、0.1% formic acid 50 – 100 mM phosphate buffer(pH 2.0 – 3.5), etc	Water	20 mM NH_4HCO_3-DEA buffer(pH 9.0), etc
Composition ratio	Organic solvent/ aqueous solution (10/90 – 100/0)		

5. Column cleaning (general method)

- The column needs to be replaced when the cleaning methods do not regenerate the column performance. To extend the column lifetime, especially for samples containing large amount of impurities, we recommend a sample pretreatment conducted carefully prior to introducing the sample to the column and /or a guard column to use with.

【Normal phase】

- Flush the column with the one of the organic solvents, which has the highest polarity among the composition of mobile phase with increased concentration (for example, for alkane/alcohol mobile phase, concentration of alcohol should be increased) to wash out the residual substances strongly retained on the column. For further cleaning, flush with 100% ethanol.
- If 100% ethanol does not improve the column performance, flush 10 column volumes of a (30 column volumes when mobile phase contained modifiers). There is a possibility that the column performance is restored after storing the column with ethyl acetate for several days.
- When the mobile phase is containing acid or amine, replace with solvent containing neither of them (at the same ratio as the mobile phase), then wash as above procedure. Do not store the column with solvent containing modifiers even for a short period of time.

【Reversed phase】

- Flush the column with solution containing a higher ratio of organic solvent for washing out the compounds that have a great capacity for retention in the column after using mobile phases not containing buffer salts/additives. Usable concentration of organic solvent is up to 100%.
- When using mobile phase containing buffer salts/additives, first replace with a water/organic solution containing no buffer salts/additives (A ratio of water to organic solvent should be set at the same proportions as a mobile phase). Then flush the column in accordance with the method described above.

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