

# Column Care and Use Instructions

## CHIRAL ART Amylose-C/Amylose-C Neo/Cellulose-C

### Coated type for normal phase mode

#### 1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column. The CHIRAL ART Amylose-C/Amylose-C Neo/Cellulose-C column is designed for separating optical isomers (in normal phase mode). The chiral selector, a polysaccharide derivative, is coated onto silica gel. With its superior separation and selectivity, a CHIRAL ART Amylose-C/Amylose-C Neo/Cellulose-C column is suitable for separating a wide range of chiral compounds.

CHIRAL ART Amylose-C/Amylose-C Neo/Cellulose-C columns, which are 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	CHIRAL ART Amylose-C CHIRAL ART Amylose-C Neo	CHIRAL ART Cellulose-C
Particle size	3, 5, 10, 20 $\mu\text{m}$	
Chiral selector	Amylose tris (3,5-dimethylphenylcarbamate)	Cellulose tris (3,5-dimethylphenylcarbamate)
Type	Coated type	
Separation mode	Normal phase	
Shipping solvent <sup>1</sup>	<i>n</i> -hexane/2-propanol (90/10)	
Temp. range	0 – 40°C	
Pressure limit <sup>2</sup>	30 MPa	
Recommended flow rate <sup>3</sup>	4.6 mmI.D. : 0.5 – 1.0 mL/min 10 mmI.D. : 2.5 – 5.0 mL/min	(Max. flow rate: 3.0 mL/min) (Max. flow rate: 15.0 mL/min)

<sup>1</sup>: If you intend to store the column for a long time, replace the mobile phase in the column with ethanol (in the case of CHIRAL ART Amylose-C/Amylose-C Neo) or 2-propanol (in the case of CHIRAL ART Cellulose-C).

<sup>2</sup>: Avoid using a column repeatedly near the pressure limit or abruptly changing the column pressure in order to prevent shortening column lifetime. It is recommended not to exceed a column pressure of 25 MPa (30 MPa maximum).

<sup>3</sup>: Adjust flow rate as recommended in the table seen above. When repeatedly using a flow rate at or near the upper limit, the column durability will shorten. When using column dimensions other than listed, adjust flow rate according to the cross-section area of the column.

<sup>2,3</sup>: Column pressure depends on column length, temperature, kinds of organic solvent and so on. If pressure exceeds the upper limit of pressure, adjust flow rate lower than recommended range.

#### 3. Column installation

- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- The entire HPLC system must be sufficiently flushed with the mobile phase to be used prior to connecting the column. The entire HPLC system must be free from solvents that might potentially dissolve the polysaccharide coating.
- 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 column frit and tubing, which can cause a leak and/or result in poor column performance (e.g. peak tailing, loss of theoretical plate number).
- Do not disconnect a column from the LC system before the pressure drops to zero.

#### 4. Mobile phase and sample solvent

CHIRAL ART (coated type) is coated with the polysaccharide derivative. Therefore trace quantities of a solvent that might potentially dissolve the polysaccharide derivative (e.g. THF, acetone, ethyl acetate, chloroform, dichloromethane, DMSO, DMF, etc.) should be eliminated. These solvents must be avoided for a mobile phase or sample solvent.

(THF: Tetrahydrofuran, DMSO: Dimethylsulfoxide, DMF: Dimethylformamide)

- Usable solvents as mobile phase and composition (volume/volume) are shown in the table below.

alkane/2-propanol <sup>1</sup>	alkane/ethanol <sup>1</sup>	methanol/ethanol	methanol/acetonitrile <sup>2</sup>
100/0 – 0/100	100/0 – 0/100	100/0 – 0/100	100/0 – 85/15 15/85 – 0/100

<sup>1</sup>: Alkane commonly used is *n*-hexane or *n*-heptane. Alcohols other than above (methanol, 1-propanol, 1-butanol, 2-butanol, etc.) can be used; however, methanol and alkane have low miscibility. When adding more than 5% methanol, the same amount of ethanol also needs to be added.

<sup>2</sup>: 100% methanol and 100% acetonitrile can be used; however, when switching between mobile phase containing methanol and acetonitrile, flush the column with at least ten column volumes of ethanol (in the case of CHIRAL ART Amylose-C/Amylose-C Neo) or 2-propanol (in the case of CHIRAL ART Cellulose-C) as transition solvent.

- Make sure of miscibility among the organic solvents. When switching from alkane/alcohol solvents to polar organic solvents (methanol, acetonitrile, etc.), run a transition wash with at least ten column volumes of ethanol (in the case of CHIRAL ART Amylose-C/Amylose-C Neo) or 2-propanol (in the case of CHIRAL ART Cellulose-C). In addition, a column used with polar organic solvents (such as methanol/ethanol, methanol/acetonitrile) as a mobile phase should be dedicated to this specific application.
- When a target compound is ionic, it may be necessary to use an appropriate mobile phase modifier in order to improve peak shape or separation reproducibility. High concentrations of some modifiers may shorten column lifetime. When using modifiers the following guidelines are recommended:
  - Basic compounds: 0.1% (upper limit 0.5%) diethylamine (DEA), butylamine, ethanolamine, etc.
  - Acidic compounds: 0.1% (upper limit 0.5%) trifluoroacetic acid (TFA), acetic acid, formic acid, etc.
- When possible, the sample should be dissolved in the same composition as the initial mobile phase. Using a stronger solvent than the initial mobile phase for sample dissolution may result in distortion of peak symmetry and degradation of resolution. 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 prevent exposure of the column to excessive pressure, the sample solution should be filtered through a 0.2 µm membrane filter.

#### 5. Column cleaning (general method)

- When purging the column of compounds that have a great capacity to retain on the column, flush the column with a solution containing a higher ratio of the stronger solvent. In the case of an alkane/alcohol solvent, the concentration of alcohol should be increased. When further cleaning is required, flushing with ethanol (in the case of CHIRAL ART Amylose-C/Amylose-C Neo) or 2-propanol (in the case of CHIRAL ART Cellulose-C) is effective.
- When a mobile phase containing acid or amine is used, wash as above after replacing the mobile phase with solvent containing neither acid nor amine (at the same ratio as the mobile phase). Storing a column with a mobile phase containing modifier is not recommended even for a short period of time.
- To extend the column lifetime and to avoid contamination of the column, conduct sample pretreatment carefully prior to introducing the sample to the column and /or employ the use of a guard column.
- A new column is needed when the above cleaning methods do not recover column performance.