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Chiralomix SA-5 and SB-5 Phases

Column Information

Chiralomix SA-5 and SB-5 columns are specially designed for high resolution, high efficiency and high recovery separation of chiral compounds. The packing support consists of amylose tris(3,5-dimethylphenylcarbamate) (SA) or cellulose tris(3,5-dimethylphenylcarbamate) (SB) coated on 5 μ m spherical silica gel.

Column Stability and Performance

Chiralomix columns are based on rigid, spherical silica particles with coatings physically attached on the surface. Even trace amounts of solvent incompatible with the column (such as ethyl acetate, halogenated solvents, acetone, DMF, DMSO, THF, etc.) can destroy the chiral stationary phase. Therefore the entire HPLC system (including the injector, injection loop, and auto-sampler (if in use)) must be flushed with a solvent compatible with the column and its storage solvent prior to connecting the column to the instrument.

Technical Specifications

Phases	Silica based
Packing	Amylose tris(3,5-dimethylphenylcarbamate) or cellulose tris(3,5-dimethylphenylcarbamate) coated onto 5 μ m silica
Particle size	5 μ m
pH stability	Avoid strong acidic and basic conditions that may damage the chiral phase
Operating temperature limit	0 – 40 °C
Operating pressure limit	Below 700 psi
Mobile phase compatibility	Compatible with alkanes, methanol, ethanol, propanol, methyl- <i>tert</i> -butyl ether, and their mixtures. The column will be transferred to a polar mode when 100% methanol (and acetonitrile for SA-5 column) is used. It cannot go back to the previous mode by changing to a less polar solvent and should be dedicated to specific applications using 100% methanol as the mobile phase.
Flow rate	Flow rate depends on the column dimensions and the solvent viscosity. It should be adjusted to maintain the pressure below the operating limit. For 4.6 x 250 mm column, the ideal flow rate is 1 mL/ min.

Safety Precaution

Chiralomix columns are normally operated under high pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper protections should be used to avoid inhalation of the organic solvent.

Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

- Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.
- Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and finger-tighten the nut.
- While continuing to press the tube firmly into the end fitting, use a 1/4" wrench to further tighten.
- Repeat this coupling procedure for the other end of the column.

Samples and Mobile Phases

To avoid clogging the column, all samples and solvents should be filtered through a membrane filter of approximately 0.5 μ m porosity before use. It is also strongly recommended to use a pre-column filter (0.5 μ m frit) or a guard column to protect the column. Chiralomix columns are compatible with organic mobile phases consists of alkanes, alcohols, methyl-*tert*-butyl ether, and their mixtures. Typical HPLC mobile phases other than those listed above (such as ethyl acetate, halogenated solvents, acetone, DMF, DMSO, THF, etc.) MUST BE avoided as they damage the stationary phase. Always use an inline degasser or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

For SA-5 column, avoid hexane/MeOH and hexane/EtOH ratio from 85/15 to 40-60 (v/v). At this composition, the baseline stability will be affected adversely due to the leakage of the amylose carbamate from the column. Acetonitrile/IPA mixture (0-100%) can be used as the mobile phase for Chiralomix SA-5 columns. When other alcohol/acetonitrile mixtures are used as the

mobile phase, the alcohol concentration should be limited below 15%.

Column Care

Shipping Solvent Chiralomix SA and SB columns are shipped in *n*-hexane/2-propanol mixture (90:10 v/v).

First-time use During stocking and shipping, the packing could be partially dried out. It is recommended that 10-20 column volume of the running solvent be purged to activate the column. Flush the column with your mobile phase with gradual increasing the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable. If the running solvent is incompatible with the shipping solvent, use another solvent that is compatible with both shipping and running solvents and gradually change to the running solvent. If the column backpressure and baseline fluctuate, this might be due to the air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 1.0 mL/min for a 5 µm, 4.6x250 mm column. The backpressure should be calculated by taking the difference between the total pressure (LC system and column together) and the pressure of the LC system without the column.

pH The optimum performance and operation for longest lifetime are at neutral pH. However, if the sample is basic or acidic, it is recommended to add a modifier to the mobile phase to get the separation. Modifiers that can be used with basic samples are diethylamine, butylamine (if the sample is a primary amine), and ethanol amine (for primary amino alcohols). For acidic samples, acetic acid and trifluoroacetic acids can be used. In both cases the modifier concentration should be below 0.5%, typically 0.1%. The column should be washed with the storage solvent after using the modifier.

Pressure The operating pressure should be maintained ≤450 psi for maximum column life. In any case do not exceed the pressure 700 psi. The backpressure should be calculated by taking the difference between the total pressure (LC system and column together) and the pressure of the LC system without the column. Continuous use at high pressure may eventually damage the column. Since the pressure is generated by the flow rate, the maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in backpressure suggests that the column inlet frit might be plugged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent.

Temperature The operating temperature should be 0 - 40°C. Continuous use of the column at higher temperature (>40°C) can damage the column, especially when using modifiers.

Flow rate Range Normal operation is 0.1-1.0 mL/min for 4.6 mm I.D. columns.

Storage When not in use for extended time, store the columns in hexane/2-propanol mixture (9:1) (for columns used with alkane and alkane / alcohol mixture as the mobile phase) or the regular mobile phase without the modifier (for columns dedicated to polar

solvents). Flush the column with the storage solvent for at least 15 column volumes. Then seal both ends with the removable end plugs provided with the column, to prevent the drying of the column bed.

Column clean-up (1) If a pre-column filter or a guard column is used before the separation column, clean the pre-column filter or the guard column first by flushing the filter or the guard column in reverse flow direction using washing solvent for 15-30 min, or replace the filter or the guard column if washing does not improve the column performance. The washing solvent should be pure ethanol (for columns used with alkane and alkane / alcohol mixture as the mobile phase).

(2) From time to time, some samples could get adsorbed onto the inlet frit or the packing material. When the adsorption accumulates to a certain level, it is usually indicated by an increase in the backpressure and the peak becomes broader. When this occurs, it is time to clean your column. The general guidelines for column cleaning are the followings.

1. Disconnect the column from the detector.
2. Clean your column in the reverse flow direction.
3. Run the column at less than 50% of the maximum recommended flow rate. Monitor the backpressure. If you see the pressure is much higher than the normal operating conditions, you need to lower the flow rate.
4. Typically, 10-15 column volumes of cleaning solvent are sufficient.

Column Protection

In addition to filtering the sample and the mobile phase, the best way to protect the separation column is to install a guard column or a pre-column filter in front of it. In most cases, a pre-column filter helps to remove the residual particulates in the sample or the mobile phase, or leached from HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it will more effectively trap highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.

Chiralomix SA-5 Product

ID x Length (mm x mm)	Particle size	Pore size	P/N
4.6x250	5 µm	120 Å	701105-4625
4.6x150	5 µm	120 Å	701105-4615

Chiralomix SB-5 Product

ID x Length (mm x mm)	Particle size	Pore size	P/N
4.6x250	5 µm	120 Å	702105-4625
4.6x150	5 µm	120 Å	702105-4615