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USER MANUAL

Glycomix™ SAX Ion-Exchange Phase

Column Information

Glycomix™ strong anion-exchange phase is made of narrow-dispersed and hydrophilic polymer particles. It is a strong anion exchanger with quaternary ammonium functional groups that are chemically bonded to the hydrophilic surface of the polymer particles.

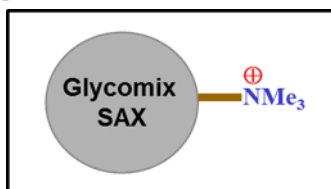
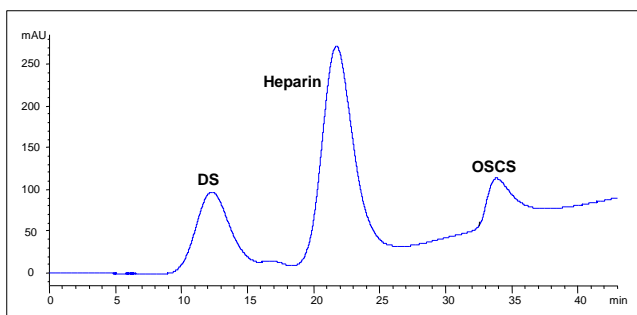


Figure 1. Chemical compositions of Glycomix™ SAX

Column Stability and Performance

With well controlled surface chemistry and resin production, Glycomix resins exhibit high reproducibility. The Glycomix SAX columns are specially designed for highly charged polysaccharides separation such as heparin sodium. Figure 2 shows a representative separation profile of heparin sodium, dermatan sulfate (DS) and oversulfated chondroitin sulfate (OSCS).

Figure 2. Separation of heparin, dermatan sulfate and oversulfated chondroitin sulfate



Column: Glycomix™ SAX, 4.6 x 250 mm

Guard column 4.6 x 50 mm

Mobile phase: A: 0.04% NaH₂PO₄, pH 3.0
B: 0.04% NaH₂PO₄+14% NaClO₄, pH 3.0

Flow rate: 0.22 mL/min

Gradient: 20% - 90% in 60 minutes

Wavelength: 202 nm

Column temp: 40 °C

Injection volume: 10 µL

Sample: 20 mg/mL Heparin Sodium, 1 mg/mL Dermatan Sulfate (DS) and 1 mg/mL Oversulfated chondroitin sulfate (OSCS)

Table 1. Gradient table for column running and re-equilibration

Time (min)	Flow (mL/min)	A%	B%
0	0.22 mL/min	80	20
60		10	90
60.1		80	20
61	1 mL/min	80	20
74		80	20
74.1	0.22 mL/min	80	20
76		80	20

A: 0.04% NaH₂PO₄, pH 3.0

B: 0.04% NaH₂PO₄+14% NaClO₄, pH 3.0

Table 2. Glycomix™ SAX Technical Specifications

Characteristics	Glycomix SAX
Functional Group	Quaternary ammonium
Surface characteristics	Hydrophilic
pH range	2-12
Typical backpressure for 4.6x250 mm (0.22 mL/min)	10 bar
Maximum backpressure (bar)	~ 100 bar
Salt concentration range	20 mM - 2.0 M
Maximum temperature (°C)	~ 80
Mobile phase compatibility	Aqueous and organic
Applications	Heparin, highly charged polysaccharides

Safety Precaution

Glycomix SAX ion-exchange columns are normally operated under low pressure. Loose connections will cause leaking of buffers and injected samples, all of which should be considered as hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When opening the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When column is shipped or not in use, it should always be capped at both ends. When installing 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 such as the removal of the inlet blockage. 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:

- (a) 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.
- (b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and finger-tighten the nut.
- (c) While continuing to press the tube firmly into the end fitting, use a 1/4" wrench to further tighten.
- (d) Repeat this coupling procedure for the other end of the column.

Column Care

Glycomix SAX columns are shipped with 20% ethanol. First-time use: During stocking and shipping, the packing could be dried out. Flush the column with HPLC water while gradually increasing the flow rate from 0.1 mL/min to your operation condition; flush the column with water for 10-20 column volumes at 1ml/min. Then equilibrate the column with the running mobile phase for another 10-20 column volumes, or until the baseline is stable. If the column backpressure and baseline fluctuate, there might be 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 4.6 x 250 mm column. If the mobile phase or pH is quite different from the storage buffer in the column, it is recommended that the column is washed first with new mobile phases for 10 column volume.

Pressure

Glycomix SAX 4.6 x 250 mm can be operated up to 1 mL/min flow rate and a back pressure of 100 bars can be tolerated. 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 maximum operating temperature is 80°C. The optimum operation temperature for longest lifetime is 10 - 50°C. Continuous use of the column at higher temperature (>80°C) can damage the column, especially under extreme pH (>12 or <2.0).

Flow rate Range

Normal operation is 0.22 mL/min for 4.6 mm I.D. columns for heparin sodium separation using the recommended USP running conditions.

Storage

When not in use for extended time, store the *Glycomix* SAX columns in deionized water with 20% ethanol. Flush the column with water and then 20% ethanol for at least 15 column volumes for each step. 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 solutions for 15-30 min. If washing does not improve the column performance, replace the filter or the guard column. The washing solutions are 150 mM potassium nitrate in 75% acetonitrile at pH 2 (adjusted by HCl) for Glycomix strong anion-exchange

columns.

- (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 the increasing backpressure and the broadening peaks. When this occurs, it is time to clean your column. The general guidelines for column cleaning are as follows.

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 or change the washing buffer as the cleaning solutions may be of different viscosities.

4. Typically, 10-15 column volumes of cleaning solution are sufficient. Some general guidelines are recommended for choosing cleaning solutions:

- A low pH salt solution will help to remove basic proteins.
- A high pH salt solution will help to remove acidic proteins.
- Organics will help to remove hydrophobic proteins.
- One cleaning solution is recommended for general cleaning: 150 mM potassium nitrate in 75% acetonitrile at pH 2 (adjusted pH by HCl).

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, 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.

Glycomix™ Products

Product	ID x Length (mm)	Part number
Glycomix SAX	4.6 x 250 mm	901665-4625
Glycomix SAX guard column	4.6 x 50 mm	901665-4605
Glycomix Kit	Column + Guard	901665-KIT
Heparin Sodium	50 mg	HP-50
Chondroitin Sulfate B	5 mg	CS-5
Oversulfated Chondroitin Sulfate	5 mg	OSCS-5
Glycomix SAX	Custom size	Inquire