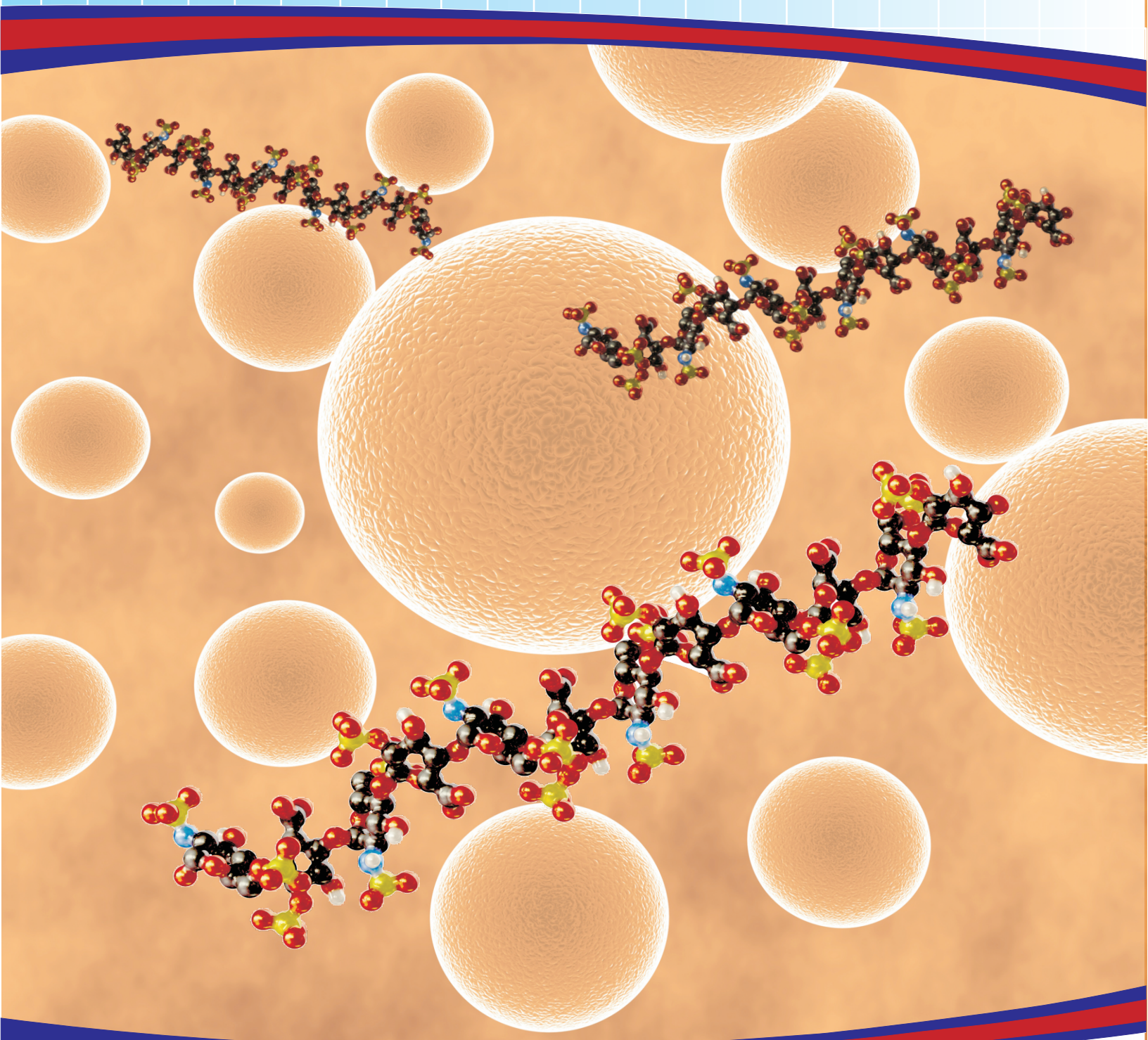


Glycomix™ (SAX)



Sepax Technologies



Better Surface Chemistry for Better Separation

Glycomix™ Strong Anion-exchange (SAX) Phase

General Description

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.

Stationary Phase Structure

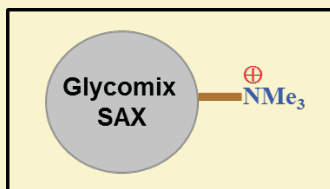


Figure 1. Chemical composition of Glycomix SAX

Featured Characteristics

- High capacity and high resolution
- High lot-to-lot reproducibility
- Wide pH range (2-12)
- From analytical to preparative scale
- Ideal for separation and analysis of heparin, glycans

Table 1. Glycomix SAX Technical Specifications

Characteristics	Glycomix SAX
Functional Group	Quaternary ammonium
Surface characteristics	Hydrophilic
pH range	2-12
Typical backpressure for 4.6x250 mm	10 bar for 0.22 mL/min 38 bar for 1 mL/min
Maximum backpressure (psi)	~ 1,500
Salt concentration range	20 mM - 2.0 M
Maximum temperature (°C)	~ 80
Mobile phase compatibility	Aqueous and organic
Applications	Heparin, highly charged polysaccharides

Sample nomenclature:

Heparin: Heparin Sodium

DS: Dermatan Sulfate or Chondroitin Sulfate B

OSCS: Oversulfated Chondroitin Sulfate

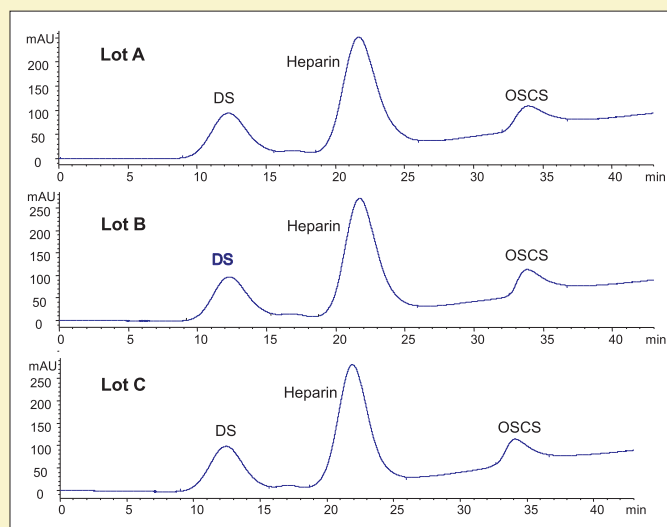
Heparin and OSCS standards were purchased from USP.

DS standard was purchased from Sigma-Aldrich.

High Lot to lot Reproducibility

With well-controlled surface chemistry and resin production, Glycomix resins exhibit high lot to lot reproducibility which leads to consistent column performance. The separation variation for Heparin from batch to batch is less than 1% for retention time and 5% for the peak area.

Figure 2. High Lot-to-lot reproducibility of three lots of Glycomix SAX resins.



Column: Glycomix SAX, 4.6 x 250 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% B in 60 minutes
 Wavelength: 202 nm
 Column temp: 25 °C
 Injection volume: 10 µL
 Pressures: 9.5 bar
 Sample: 20 mg/mL Heparin sodium
 1 mg/mL Dermatan sulfate (DS)
 1 mg/mL Oversulfated chondroitin sulfate (OSCS)
 in H₂O

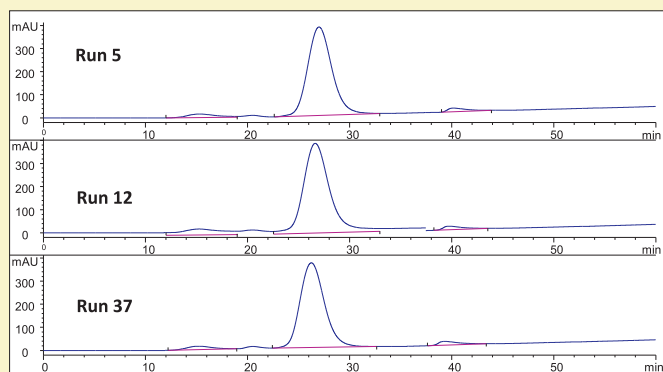
Table 2. Lot-to-Lot peak retention time relative standard deviation

Peak Name	Lot A	Lot B	Lot C	RSD%
Chondroitin Sulfate B	12.286	12.300	12.434	0.41%
Heparin	21.71	21.723	21.919	0.44%
OSCS	33.936	33.853	34.114	0.32%

Long column life time

Glycomix SAX has long column life time due to its resin surface chemistry and stable manufacturing process. Figure 3 exhibits one column's performance for duplicated injections. The column shows consistent separation efficiency.

Figure 3. Chromatogram overlays of duplicated injections for column S/N 27197



Column: Glycomix SAX, 4.6 x 250 mm
 Guard column: Glycomix SAX, 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% B in 60 minutes
 Wavelength: 202 nm
 Column temp: 25 °C
 Injection volume: 10 µL
 Pressures: 9.5 bar
 Sample: 20 mg/mL Heparin sodium
 0.2 mg/mL Dermatan sulfate (DS)
 0.2 mg/mL Oversulfated chondroitin sulfate (OSCS) in H₂O

Sensitive separation of different Heparin loading

Glycomix SAX is able to separate components from commercially available standards. In Figure 4, Panel A shows the standard run with 20 mg/mL Heparin, 1 mg/mL DS and 1 mg/mL OSCS. Panel B is the OSCS chromatogram with 1 mg/mL concentration. In comparison to Panel B, the small peak between DS and Heparin comes from the standard OSCS sample. Column running conditions are the same as for figure 3. Glycomix SAX can be used in the quantitative analysis of Heparin and its impurities. Figure 5 shows the different heparin loadings over concentration range from 0.5 mg/ml to 10 mg/ml, 0.125 mg/ml to 4 mg/ml for OSCS and 0.25 mg/ml to 4 mg/ml for DS. The R² values for both standards exceed 0.99 in the tested concentration range.

Figure 4. Chromatogram overlays of OSCS standard and Heparin with DS and OSCS

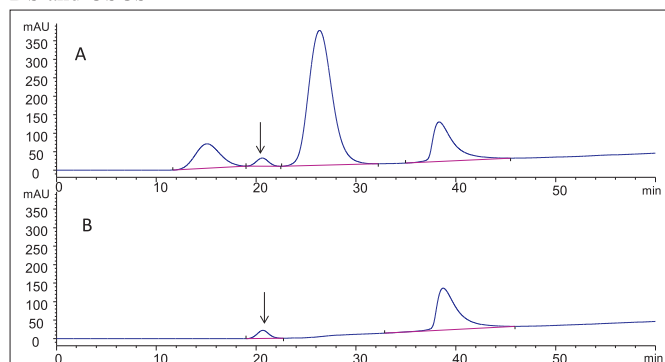
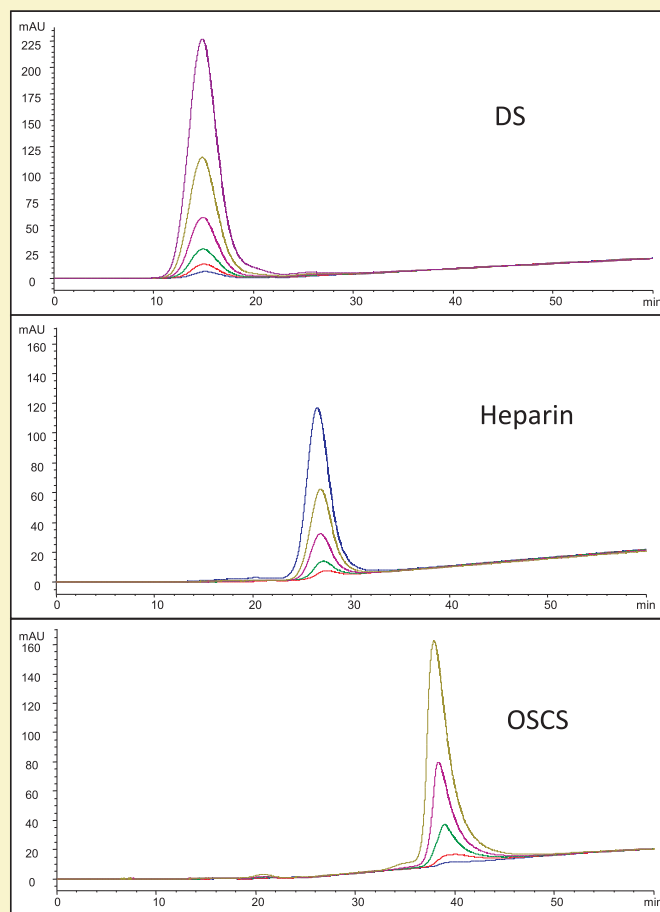
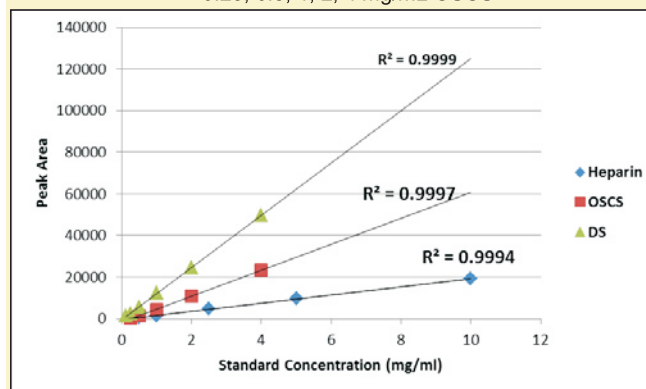


Figure 5. Chromatogram overlays of different heparin injections from 0.5 mg/mL to 10 mg/mL concentration, DS from 0.125 mg/mL to 4.0 mg/mL and OSCS from 0.25 mg/mL to 4.0 mg/mL



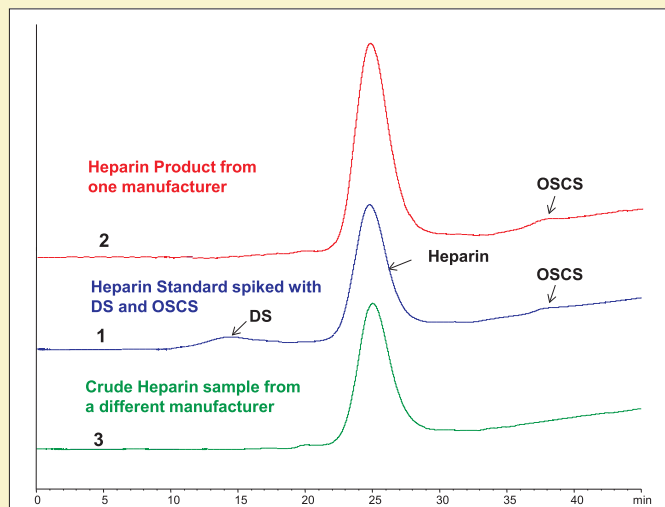
Column: Glycomix SAX, 4.6 x 250 mm
 Guard column: Glycomix SAX, 4.6 x 50 mm
 Mobile phase: A: 0.04% NaH₂PO₄, pH 3.0
 B: 0.04% NaH₂PO₄+14% NaClO₄, pH 3.0
 Flow rate: 0.22 mL/min
 Gradient: 20% - 90% B in 60 minutes
 Wavelength: 202 nm
 Column temp: 25 °C
 Injection volume: 10 µL
 Pressures: 9.5 bar
 Sample: 0.5, 1.0, 2.5, 5.0 and 10 mg/mL heparin sodium
 0.125, 0.25, 0.5, 1, 2, 4 mg/mL DS
 0.25, 0.5, 1, 2, 4 mg/mL OSCS



Application

Quality control of Heparin products. Figure 6 shows chromatogram overlays of heparin control, real product from one manufacturer and a crude heparin sample from a different manufacturer. Middle sample 2 is the Heparin standard spiked with DS and OSCS. Sample 1 (a Heparin product) chromatogram shows that it is contaminated with OSCS, while sample 3 (a crude Heparin from another manufacturer) is free of both impurities.

Figure 6. Chromatogram overlays of heparin control, real on the market heparin sample and crude heparin sample



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% B in 60 minutes

Wavelength: 202 nm

Column temp: 25 °C

Injection volume: 10 µL

Pressure: 10 bar

Sample:

- 20 mg/mL Heparin Sodium, 0.2 mg/mL Dermatan sulfate (DS) and 0.2 mg/mL Oversulfated chondroitin sulfate (OSCS)
- Heparin product from one manufacturer. The chromatogram shows that the product is contaminated with OSCS
- Crude Heparin sample from a different manufacturer. The chromatogram indicates the crude heparin is free of impurities.

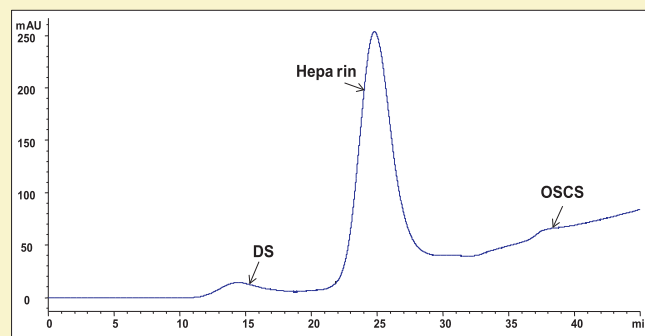
Reference

1. Sepax application note KC1001. High Resolution Analysis of Heparin and Heparin-like Impurities on Glycomix – an anion Exchange column.
2. Dionex application note 235. Determination of Oversulfated Chondroitin Sulfate and Dermatan Sulfate in Heparin Sodium Using Anion-Exchange Chromatography with UV Detection.
3. Heparin Sodium, Pharmacopeia Forum **2009**, 35 (5), 1-4.

Column Performance Comparison

In comparison, Glycomix SAX performs superior to Dionex's column for heparin separation.^{1,2} Figure 7 shows the chromatogram of Heparin separation from Glycomix SAX. The resolutions are 3.8 between DS and heparin, 5.8 between heparin and OSCS. The U.S. Pharmacopeia (USP) requirements for the separation for DS/heparin are 1.0 and 1.5 for OSCS/heparin³. Glycomix SAX has a much better separation resolution than USPs requirement. In Dionex's application note, Dionex IonPac AG11 guard with AS11 analytical column gives a 1.1 and 1.8 resolution for DS/heparin and heparin/OSCS respectively.

Figure 7. Heparin Sodium, Chondroitin Sulfate B, and Oversulfated Chondroitin Sulfate separation on Glycomix SAX



Column: Glycomix SAX , 4.6 x 250 mm

Guard column: Glycomix, 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: 25 °C

Injection volume: 10 µL

Pressure: 10 bar

Sample: 20 mg/mL Heparin Sodium, 0.2 mg/mL Dermatan Sulfate (DS) and 0.2 mg/mL Oversulfated Chondroitin Sulfate (OSCS)

Ordering Information

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
Glycomix SAX	Custom size	Inquire



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