



Heparin Analysis on SEC and SAX

Highlighted FACTS:

- For Heparin applications, Sepax offer solutions for SEC separations (under USP and EP conditions) as well as impurity analyses by IEX using Sepax's Glycomix SAX (also under USP conditions).
- Sepax's SEC columns come in a range of pore sizes to give the best separation of the MW range for these types of samples.
- We have shown that running two Zenix SEC-100 7.8x300 mm columns can further increase the separation between MW species since resolution is correlated to column length.
- Sepax's Glycomix SAX was specifically developed to identify any impurities in Heparin Samples, as shown by our separation between Dermatan Sulfate, an unknown impurity, Oversulfated Chondroitin Sulfate and Heparin.

Order Information

215300-7830	SRT SEC-300, 5µm, 300 Å 7.8 x 300 mm
215500-7830	SRT SEC-500, 5µm, 500 Å 7.8 x 300 mm
215300-7805	SRT SEC-300, 5µm, 300 Å 7.8 x 50 mm
213100-7830	Zenix SEC-100, 3µm, 100 Å 7.8 x 300 mm
213150-7830	Zenix SEC-150, 3µm, 150 Å 7.8 x 300 mm
213300-7830	Zenix SEC-300, 3µm, 300 Å 7.8 x 300 mm
213100-7805	Zenix SEC-100, 3µm, 100 Å 7.8 x 50 mm
901665-4625	Glycomix SAX, 4.6 x 250 mm
901665-4605	Glycomix SAX, 4.6 x 50 mm
901665-2125	Glycomix SAX, 2.1 x 250 mm

Heparin Analysis on SEC

Column: SRT SEC-300 (5 µm, 300 Å, 7.8 x 300 mm)

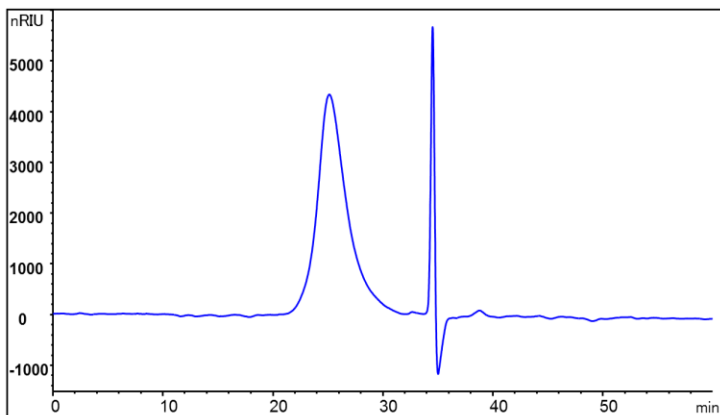
SRT SEC-500 (5 µm, 500 Å, 7.8 x 300 mm)

Guard column: SRT SEC-300 (5 µm, 300 Å, 7.8 x 50 mm)

Column temperature: 30 °C, Flow rate: 0.6 mL/min, Detection: RI,

Mobile phase: 0.1 M ammonium acetate, 0.02% sodium azide in water,

Injection Volume: 20 µL of 5 mg/mL Heparin



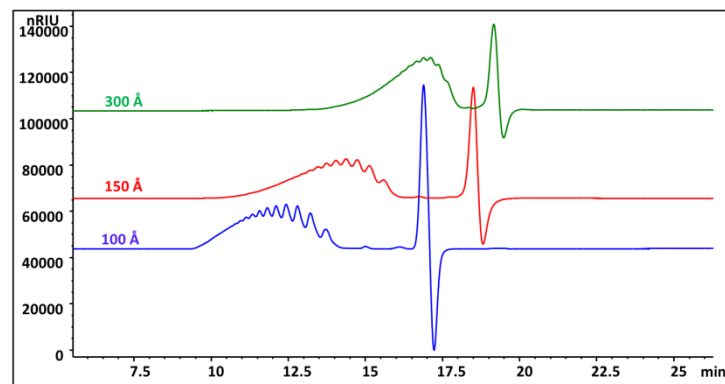
Enoxaparin Sodium Analysis on Zenix SEC

Column: Zenix SEC (3 µm, 100 Å, 150 Å, 300 Å, 7.8 x 300 mm each)

Mobile phase: 0.5 M LiNO₃, Flow rate: 0.6 mL/min,

Detector: RI (35 °C), Column temperature: 30 °C, Injection volume: 25 µL,

Sample: 10.0 mg/mL Enoxaparin sodium in mobile phase, MW 3,000 – 8,000 Da



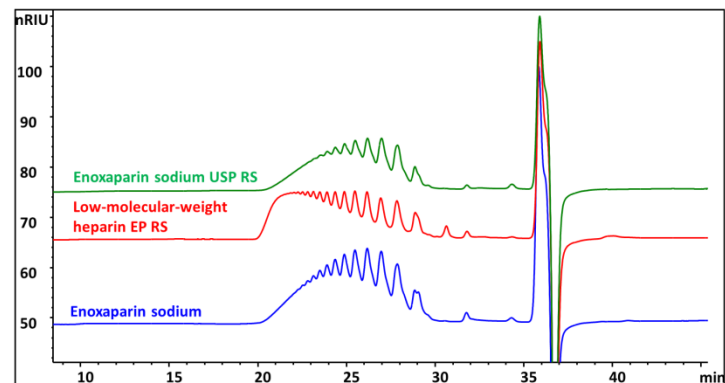
LMW Heparin Analysis on Zenix SEC-100

Column: 2x Zenix SEC-100 (3 µm, 100 Å, 7.8 x 300 mm)

Guard column: Zenix SEC-100 (3 µm, 100 Å, 7.8 x 50 mm)

Mobile phase: 0.5 M LiNO₃, Flow rate: 0.6 mL/min, Detector: RI (35 °C),

Column temperature: 30 °C, Injection volume: 25 µL, Sample: 10.0 mg/mL in mobile phase



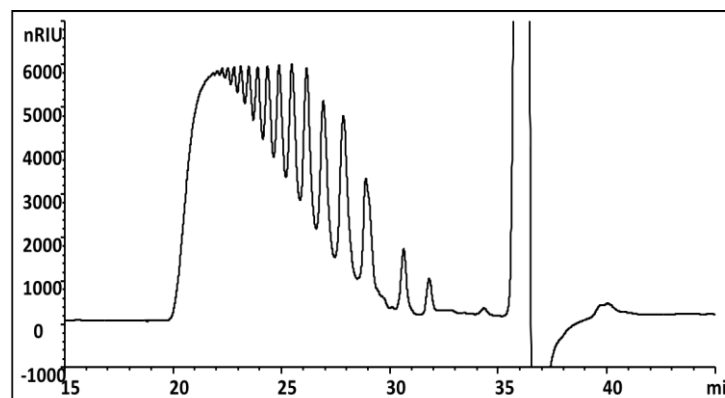
Low Molecular Weight Heparin-two Zenix SEC-100 in tandem

Column: 2x Zenix SEC-100 (3 µm, 100 Å, 7.8 x 300 mm)

Guard column: Zenix SEC-100 (3 µm, 100 Å, 7.8 x 50 mm)

Mobile phase: 0.5 M LiNO₃, Flow rate: 0.6 mL/min, Detector: RI (35 °C)

Column temperature: 25 °C, Injection volume: 20 µL, Sample: EP RS

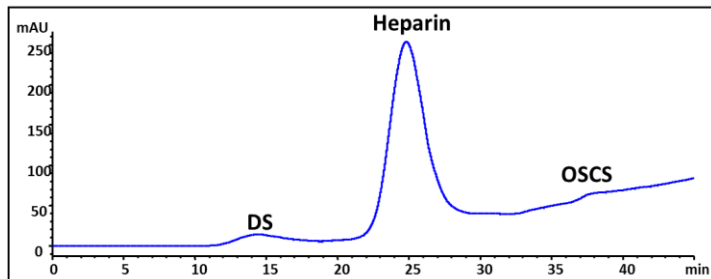




Heparin Analysis on SEC and SAX

High Resolution Heparin Analysis - Glycomix SAX

Column: Glycomix 4.6 x 250 mm with Guard 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; Detector: UV 202 nm; Column temperature: 25 °C
 Injection volume: 10 µL Heparin 20 mg/mL, 0.2 mg/mL DS and OSCS

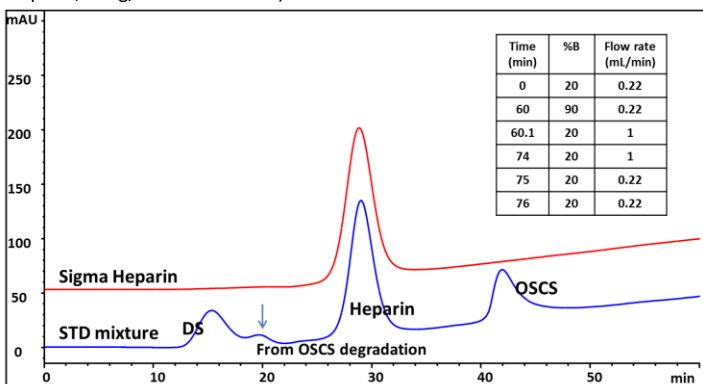


Resolution	Glycomix	US Pharmacopeia ^[1]
DS / Heparin	3.8	1.0
Heparin / OSCS	5.8	1.5

1. Heparin Sodium, Pharmacopeia Forum 2009, 35 (5), 1-4

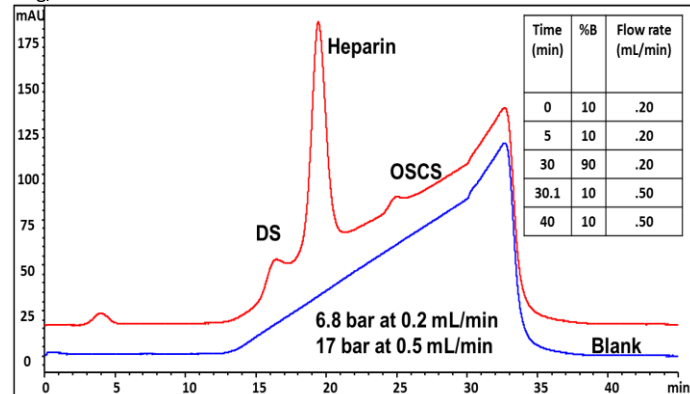
Heparin analysis -Glycomix SAX separation

Column: Glycomix 4.6 x 250 mm with Guard 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; Detector: UV 202 nm; Column temperature: 40 °C
 Injection volume: 10 µL Sigma Heparin 20 mg/mL, standard mixture (20 mg/mL heparin, 1 mg/mL DS and OSCS)

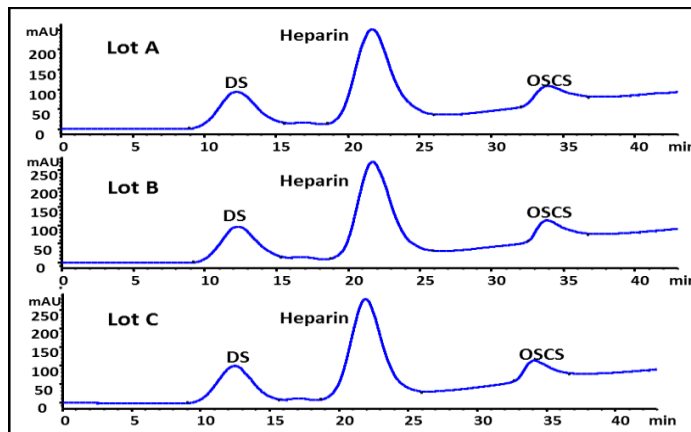


Glycomix SAX-Heparin impurity study

Column: Glycomix, 2.1 x 250 mm
 Mobile phase: A: 0.04% NaH₂PO₄, pH 3.0,
 B: 0.04% NaH₂PO₄+14% NaClO₄, pH 3.0
 Flow rate: 0.20 mL/min; Detector: UV 202 nm; Column temperature: 40 °C
 Injection volume: 0, 2 µL; Blank, Standard mixture Sigma Heparin 20 mg/mL, 1mg/mL DS and OSCS

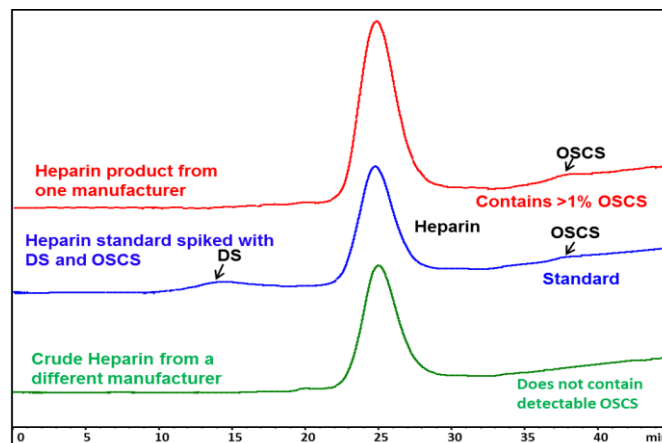


Glycomix SAX - Lot-to-Lot Consistency



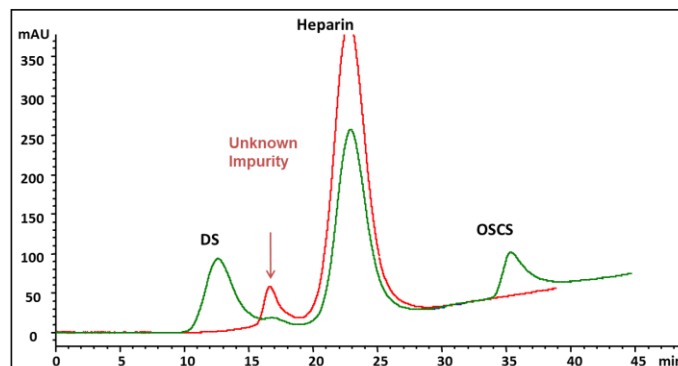
CASE STUDY

Sample Analysis: Case Study #1



Quality Control Tool for Heparin Products: Sample Red from one manufacturer is contaminated with OSCS while Sample Green (a crude heparin from a different manufacturer is free of both impurities.

Sample Analysis: Case Study #2



The high resolution between heparin and dermatan sulfate (Rs = 3.8) enables identification of any unknown impurities that elutes between heparin and dermatan sulfate.