

# High Resolution Analysis of Heparin and Heparin-like Impurities on Glycomix™ SAX Column



Xiaomi Xu, Aiyan Cui, Yuna Li and Haiying Chen

Sepax Technologies, Inc., 5-100 Innovation Way, Newark DE 19711

Poster #: T2085

## Introduction

Heparin has been widely used as an anticoagulant or anti-thrombotic agent. It is a complex mixture of sulfated glycosaminoglycans (GAGs), which is highly negatively charged. During its extraction process from mammalian tissue, other polyanionic GAGs can be co-purified with heparin since they share heparin-like properties[1]. One representative example of such impurities is dermatan sulfate (chondroitin sulfate B). Oversulfated chondroitin sulfate (OSCS) was identified as a non-native contaminant, which can induce severe side effects even death. The most recent heparin sodium monograph published by U.S. Pharmacopeia[1] details the analytical method that heparin manufacturers must follow to ensure the quality of their heparin products. This application note reports the separation of heparin, DS and OSCS using Glycomix™ SAX with USP recommended method. The performance of Glycomix SAX is also discussed in comparison to competitor D's column separation efficiency.

## Experimental

HPLC system:  
Agilent 1200 HPLC with binary pump  
LC Method

Mobile phase:  
A: 0.04% NaH<sub>2</sub>PO<sub>4</sub>, pH3.0;  
B: A + 14% NaClO<sub>4</sub>, pH3.0  
Flow rate: 0.22 mL/min  
Column temperature: 25 °C  
Detection: UV 202 nm  
Injection volume: 10 µL

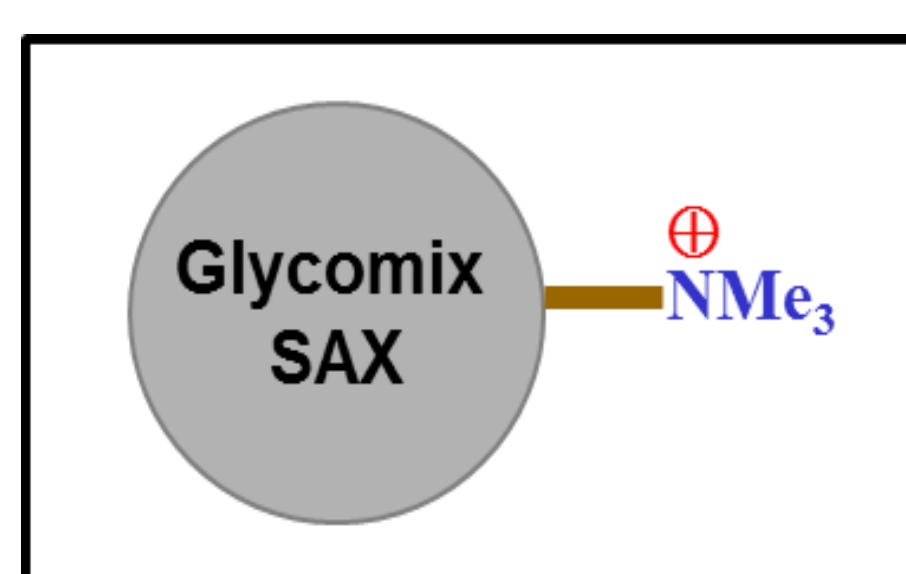
Time (min)	Flow (mL/min)	A%	B%
0	0.22	80	20
60		10	90
61		80	20
75		80	20

Sample: Heparin sodium salt, Dermatan sulfate and Oversulfated chondroitin sulfate

### Sample nomenclature

Heparin: Heparin Sodium  
DS: Dermatan Sulfate or Chondroitin Sulfate B  
OSCS: Oversulfated Chondroitin Sulfate

## Stationary Phase Structure

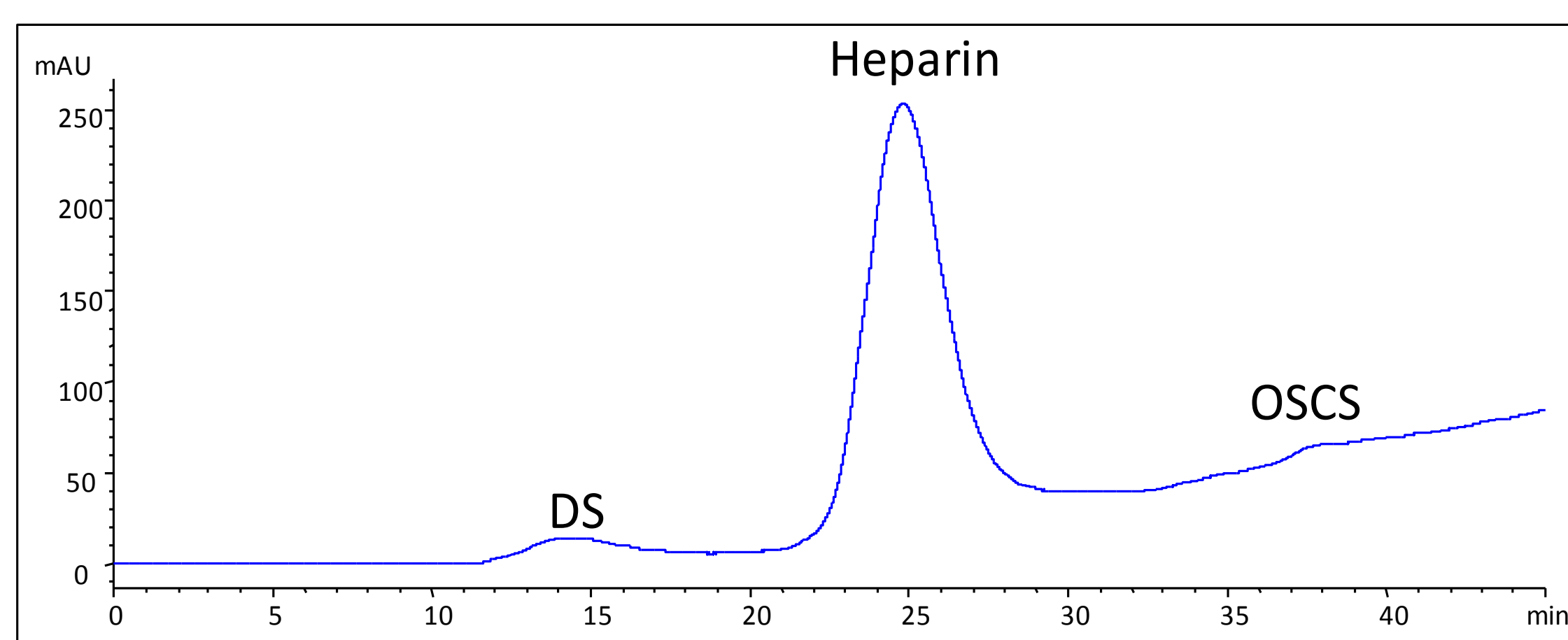


### Featured Characteristics

- ✓ 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 and other highly charged polysaccharides

## Heparin System Suitability Standard

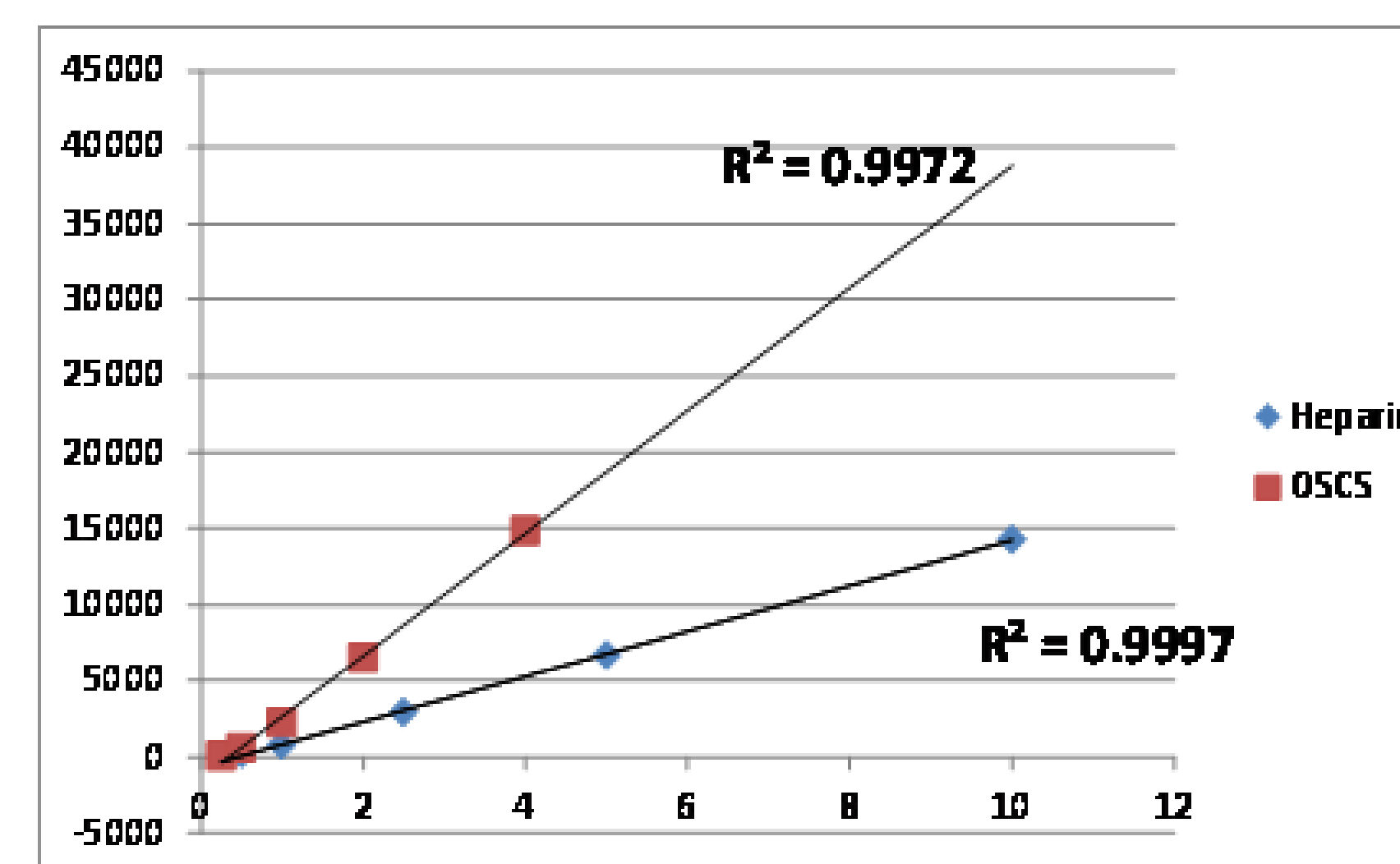
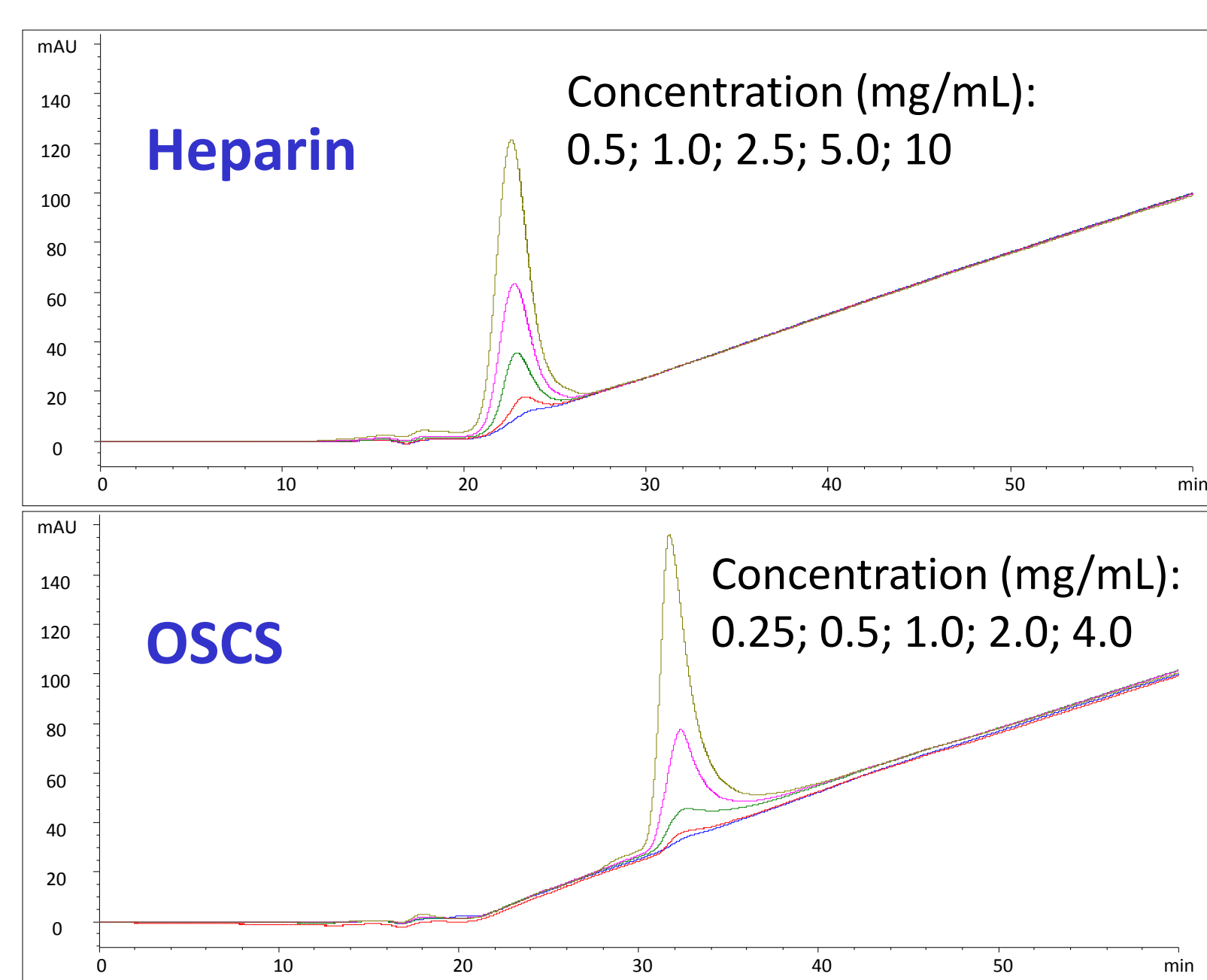
**System suitability solution:** Heparin sodium, 20 mg/mL with 1% DS and 1% OSCS



Resolution	Glycomix SAX	Dionex IonPak <sup>[2]</sup>	USP <sup>[1]</sup>
DS/heparin	3.8	1.1	1.0
Heparin/OSCS	5.8	1.8	1.5

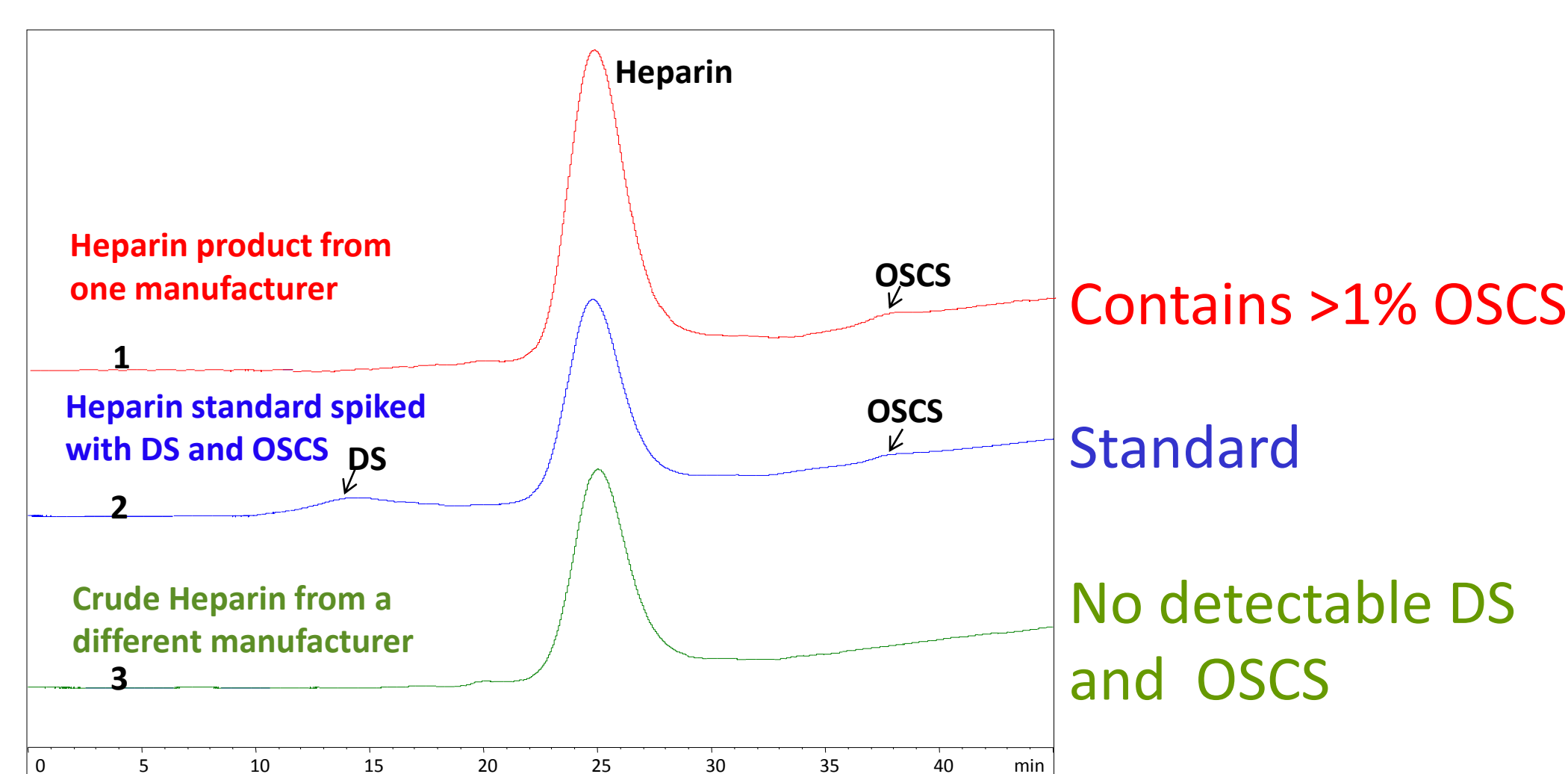
\*Glycomix™ offers much higher resolution than Dionex IonPak column!

## Quantitative Analysis – Calibration Curve



Good linearity over a wide concentration range ensures quantitative analysis.

## Real Heparin Sample Analysis – Case Studies

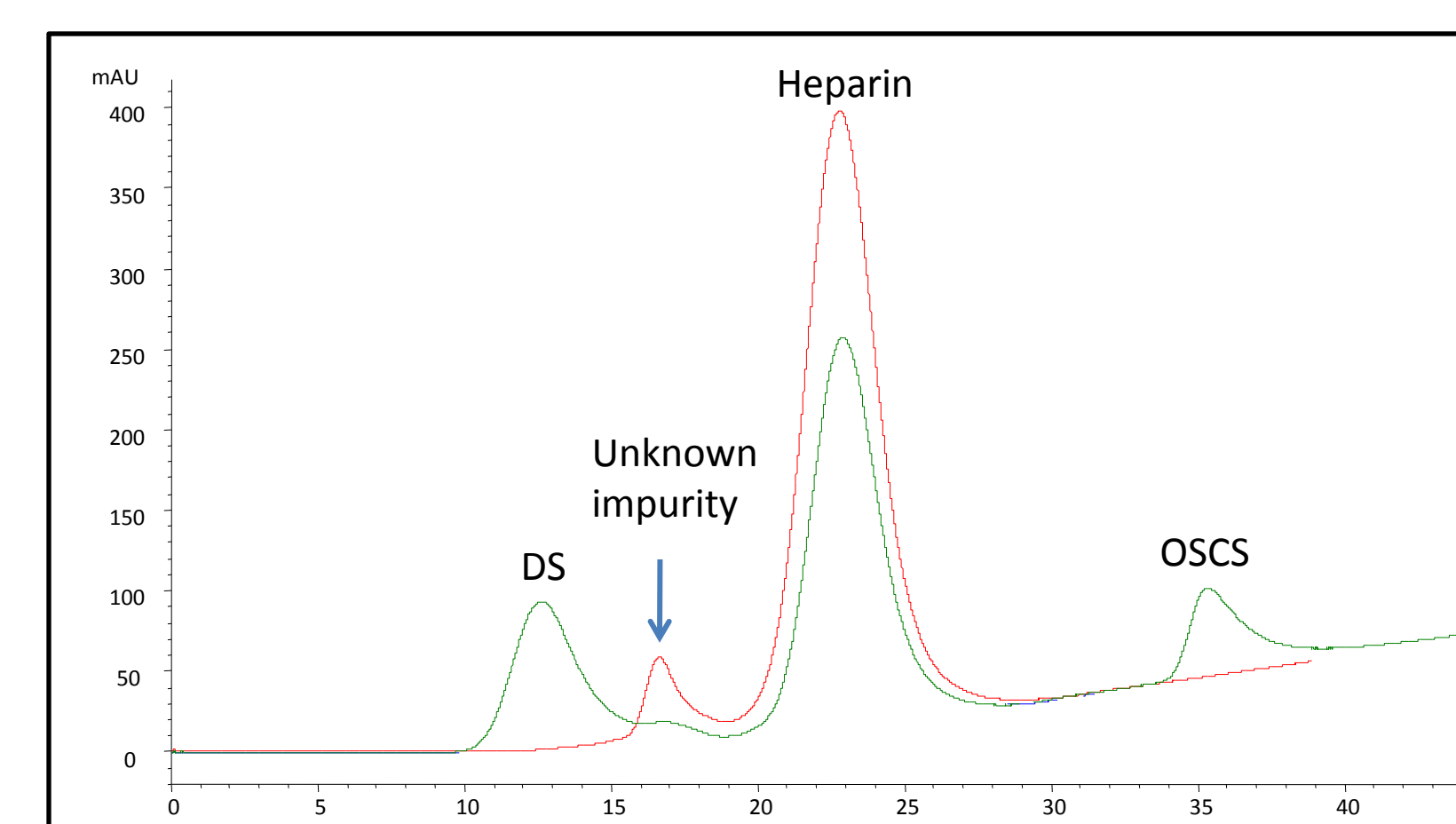


### Case Study #2

The high resolution between heparin and dermatan sulfate ( $R_s = 3.8$ ) enables identification of any unknown impurities that elutes between heparin and dermatan sulfate. Another manufacturer's heparin sample is shown to be contaminated with an impurity (in red). The right chromatogram overlays indicate that the contaminant elutes between DS and Heparin.

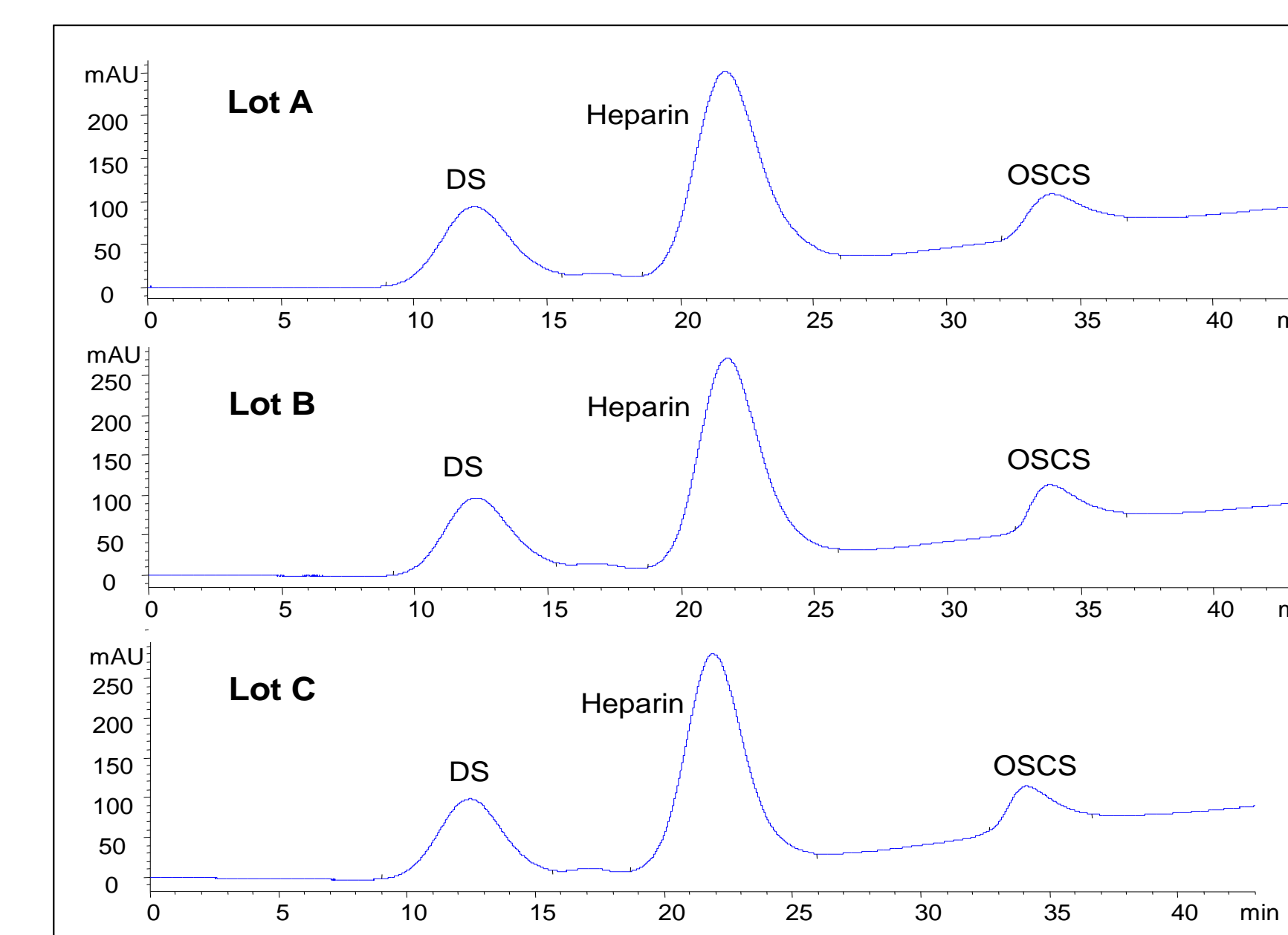
### Case Study #1

The left figure 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.



## Lot-to-Lot Consistency

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.



Peak	Lot A	Lot B	Lot C	RSD %
DS	12.286	12.300	12.434	0.41
Heparin	21.710	21.723	21.919	0.44
OSCS	33.936	33.853	34.114	0.32

## Conclusion

It has been demonstrated that with careful control of surface chemistry, high resolution separation of heparin and its impurities is successfully achieved on Sepax Glycomix™ SAX column. Glycomix™ column is well suitable for quality control for heparin products, as well as quantitative analysis due to its high sensitivity.

Analytical & Preparative

Quality control & Quantization

## Ordering Information

Product	Description	Part Number
Glycomix™ SAX	4.6 x 250 mm	901665-4625
Glycomix™ SAX guard column	4.6 x 50 mm	901665-4605
Glycomix™ SAX kit	Column + Guard	901665-KIT

## Reference

- [1] Heparin Sodium, *Pharmacopeia Forum* **2009**, 35 (5), 1-4.  
[2] Dionex Application Note 235