High Resolution Analysis of Heparin and Heparin-like Impurities on Glycomix™ – an Anion Exchange Column

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Abstract

Analysis of heparin is challenging due to its heterogeneity, both in its size distribution and charge variance. Moreover, its impurities usually have properties that resemble heparin which makes it difficult to distinguish heparin and its impurities using analytical methods. To effectively separate heparin from its impurities, including naturally occurring impurities during manufacturing process (e.g., dermatan sulfate DS) and non-native contaminant from adulteration (e.g., oversulfated chondroitin sulfate OSCS), U.S. Pharmacopeia (USP) published a chromatographic method to identify heparin and heparin-like impurities using ion-exchange liquid chromatography. However, current commercially available ion-exchange column barely meets USP separation resolution criteria; the situation calls for a new stationary phase which can improve the resolution. Sepax has recently developed an ion-exchange column – Glycomix ™ SAX. It grafts charged and a certain level of hydrophilic functional groups to polymer-based resin. Here we present the high efficiency separation of highly charged glycans.

Introduction

Heparin has been widely used as an anticoagulant or anti-thrombotic agent. It is a complex mixture of sulfated glycosaminoglycans (GAGs), which are highly negatively charged. During its extraction process from mammalian tissue (such as pig intestine), other polyanionic GAGs can co-purify 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[2] 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

Ion-exchange columns

Glycomix™-SAX (4.6 x 250 mm, P/N 901665-4625)
Glycomix™-SAX guard column (4.6 x 50 mm, P/N 901665-4605)

Chemicals and Reagents

Heparin sodium salt and Dermatan sulfate (DS or chondroitin sulfate B) were purchased from Sigma-Aldrich. Oversulfated chondroitin sulfate (part no. 1133580) was purchased from U.S. Pharmacopeia. Monobasic sodium phosphate and sodium perchlorate were purchased from EMD. All solutions were made with Milli-Q water.

LC Method

Mobile phase:
A: 0.04% NaH_2PO_4, pH3.0;
B: 0.04% NaH_2PO_4+ 14% $NaClO_4$, pH3.0
Gradient: 20% B to 90% B in 60 min
Flow rate: 0.22 mL/min
Column temperature: 25 °C
Detection: UV 202 nm
Injection volume: 10 µL
Pressure: 10 bar

Results

Following the conditions listed on USP heparin sodium monograph, Figure. 1 demonstrates the separation of heparin from its impurities, dermatan sulfate (DS) and oversulfated chondroitin sulfate (OSCS). The three peaks, according to elution order, are identified as DS, heparin sodium salt and OSCS, the concentration of which are 0.2 mg/mL, 20 mg/mL and 0.2 mg/mL, respectively. The resolutions are 3.8 between DS and heparin and 5.8 between heparin and OSCS; both are well beyond the USP resolution requirements, which are 1.0 and 1.5, respectively. Glycomix™ column shows the separation resolution much higher than that of Competitor D column, which is 1.1 and 1.8[3]. It was also stated in competitor D's literature[3] that dermatan sulfate may be unresolved from heparin at >20 mg/mL concentrations as the heparin peak starts eluting from the column within 2 min after the dermatan peak. Heparin loading is not a concern on Glycomix™ column.

The high resolution between heparin and dermatan sulfate also enables identification of any unknown impurities that elutes between heparin and dermatan sulfate. As shown in Figure. 2, there is an impurity peak eluted after dermatan sulfate, which is marked by an arrow on the graph. Comparing it with each single standard, it is found that this unknown impurity peak, which may be chondroitin sulfate, is also present on heparin sodium salt (Sigma-Aldrich) sample. Figure 3 is another example of a real heparin injection product analysis, which shows the presence of OSCS.

Glycomix™ can be used in the quantitative analysis of heparin and its impurities. Figure 4 shows the calibration curve with different heparin and impurity loadings over concentration range from 0.5 mg/ml to 10 mg/ml for heparin and from 0.25 mg/ml to 4 mg/ml for OSCS. The R^2 values exceed 0.99 for both analytes ($R^2_{heparin}$=0.9997; R^2_{OSCS}=0.9972).

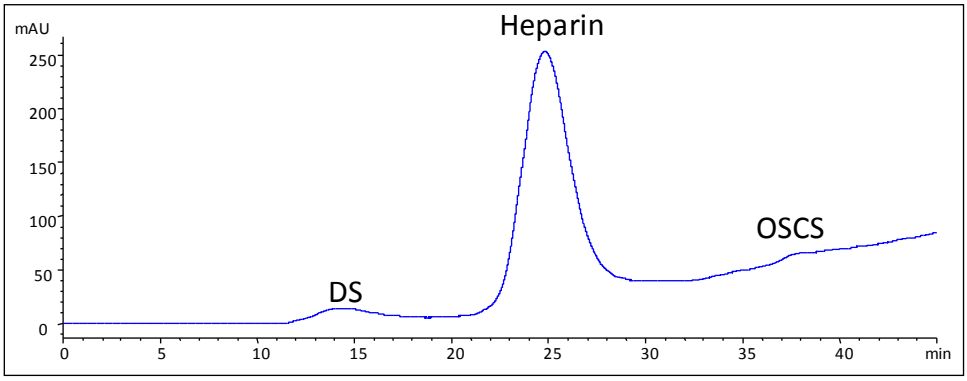

Figure 1. Elution profile of standard solution, containing 0.2 mg/mL dermatan sulfate (DS), 20 mg/mL heparin sodium and 0.2 mg/mL oversulfated chondroitin sulfate (OSCS).

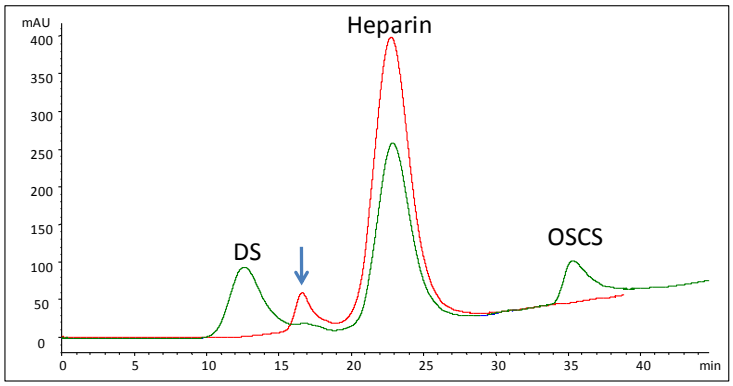

Figure 2. Comparison of standard solution (1 mg/mL DS, 20 mg/mL heparin and 1 mg/mL OSCS) and one heparin injection product provided by an undisclosed drug company.

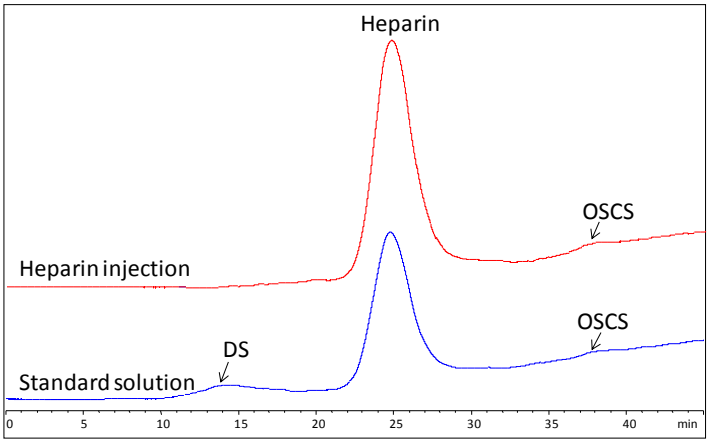

Figure 3. Comparison of standard solution (0.2 mg/mL DS, 20 mg/mL heparin and 0.2 mg/mL OSCS) and another heparin injection product provided by an undisclosed drug company, a different source than that on Figure 2.

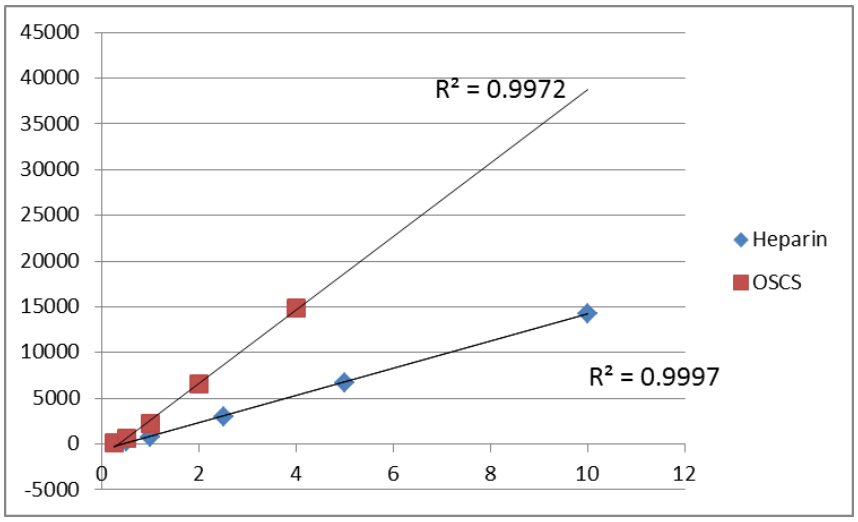

Figure 4. Heparin calibration curve over the concentration range from 0.5 mg/ml to 10 mg/ml, OSCS from 0.25 mg/ml to 4 mg/ml.

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™ column. Glycomix™ column is well suitable for quality control for heparin products, as well as quantitative analysis due to its high sensitivity.

Reference

1. Advances in the separation, sensitive detection, and characterization of heparin and heparin sulfate. Anal Bioanal Chem (2009) 393: 155-169
2. Heparin Sodium, *Pharmacopeia Forum* **2009**, 35 (5), 1-4.
3. Determination of oversulfated chondroitin sulfate and dermatan sulfate in heparin sodium using anion-exchange chromatography with UV detection. *Dionex Application Note* 235.

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
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

For more information on Glycomix™ products, please visit Sepax website: http://www.sepax-tech.com or contact us at 1-877-SEPAX-US