DETECTION OF HEPARIN CONTAMINATION USING ANION EXCHANGE CHROMATOGRAPHY

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INTRODUCTION

Heparin is a blood thinning drug that is primarily used to prevent the development of blood clots. Heparin and its derivative, low-molecular-weight heparin (LMWH), have been widely used as anticoagulant drugs for decades during surgery and kidney dialysis. Heparin belongs to the group of linear polysaccharides called gly-cosaminoglycan (GAG), and consists of alternating glucosamine and hexuronic acid residues. Raw heparin material is extracted from mammalian tissues, such as pig intestines. The heparin material requires many treatment and purification steps before it can be used in a drug formula. Stringent quality control in the purification steps is essential to ensure the quality of heparin as a final active pharmaceutical ingredient (API).

Recent incidents, including severe allergic reactions and several deaths have been attributed to heparin adulteration, resulting in a massive recall of heparin drugs by the manufacturer [1]. Oversulfated chondroitin sulfate (OSCS) is the heparin contaminant responsible for the adverse clinical events [2]. Because heparin is a drug commonly used in clinics, these adverse events have created a worldwide crisis and a call for an analytical method that can readily monitor the purity of heparin API before formulation of the drug.

Here we present a simple method to separate and quantify OSCS in the presence of heparin. The method uses anion exchange chromatography to achieve complete resolution between heparin and OSCS, and UV absorption to quantify the concentrations of heparin and OSCS. The results demonstrate that the method not only generates reproducible, fast separations (10 minutes) but also can detect OSCS at a concentration of less than 1% of overall content. The ability to quickly and unambiguously analyze the purity of heparin drugs can improve and accelerate the quality control of raw API materials in pharmaceutical industry. This sensitive method can be used for monitoring heparin quality and OSCS adulteration in order to protect patient health.

METHODS

LC System: Waters Alliance® Bioseparation (AllianceBIO) System

Column: Waters Spherisorb® 5µm SAX 4.0 x 250mm

Column Temp: 40°C
Flow Rate: 0.5 mL/min

Mobile Phases

Eluent A: 50 mM NaH2PO4 (pH 2.5)
Eluent B: 50 mM NaH2PO4+ 2.0 M NaClO4 (pH 2.5)

Gradient: 10% - 90% B in 10 minutes
Sample Vol: 25µL

UV Detector: Waters 2998 Photodiode Array (PDA) Detector
Wavelength: 190 nm - 400nm
Sampling Rate: 2pts/s
Resolution: 1.2nm

Sample Preparation
Heparin Sodium Identification RS (Catalog #: 1304038) and Heparin Sodium System Suitability RS (Catalog #: 1304049) were purchased from U.S. Pharmacopeia. The Heparin Sodium System Suitability RS is a mixture that contains approximately 80% of Heparin and 20% of OSCS.
Stock solutions (10 mg/ml) of heparin sodium standard or heparin sodium system suitability standard were prepared by re-constituting the samples in Milli-Q water. Samples with diluted concentrations were prepared by diluting the stock solutions to the desired concentration using Milli-Q water.

Figure 1. Waters Alliance® Bioseparation System.

RESULTS

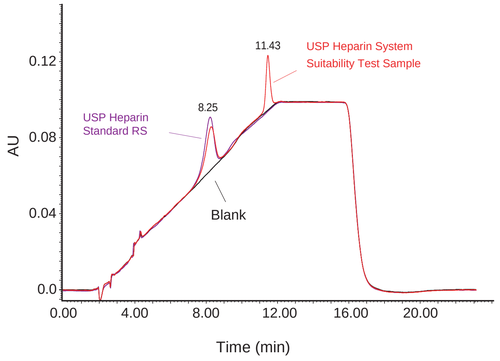

Figure 2. UV chromatograms (202 nm) of USP heparin standard RS (purple) and USP heparin system suitability RS (red). 25 µg of sample were injected onto the column for each analysis.

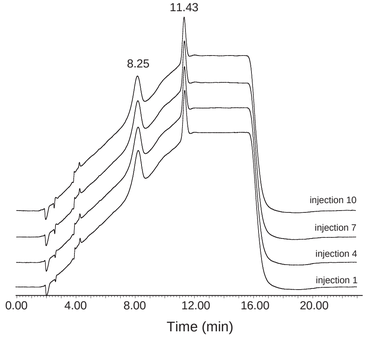

Figure 3. Reproducibility of the chromatographic separation is illustrated by 10 repetitive injections of USP heparin system suitability test sample.

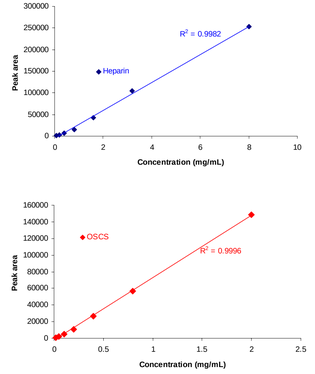

Figure 4. Calibration curves of heparin (top) and oversulfated chondroitin sulfate (OSCS, bottom).

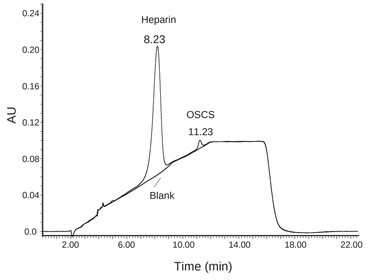

Figure 5. UV chromatograms showing the separation between heparin and oversulfated chondroitin sulfate (OSCS) where the concentration of OSCS is approximately 1% of heparin concentration.

CONCLUSIONS

- The combination of the Alliance Bioseparation (AllianceBIO) System with the Spherisorb SAX 5 µm column is an ideal solution for the separation and quantification of heparin and OSCS.
- The method yields rapid, sensitive, and high-resolution separations, and generates quality data for the evaluation and determination of heparin purity.
- The linear dynamic range of the assay spans over 3 order of magnitude, making the method well-suited for quantitative analysis of heparin impurities: OSCS at approximately 1% of heparin concentration was readily detected by the system.
- The calculated lower limits of quantification (LOQ) were 0.03 mg/mL for heparin and 0.015 mg/mL for OSCS.
- This sensitive method can be used for monitoring heparin quality and OSCS adulteration in order to protect patient health.

References:

1. *Chem. & Engineering News*, **2008**, 86, p46.
2. *Nature Biotechnology*, **2008**, 26, p669.

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