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 glycosaminoglycans (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 formulation. 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. Oversulfated chondroitin sulfate (OSCS) is the heparin contaminant responsible for the adverse clinical events. Because heparin is a drug commonly used in clinics, these adverse events have created a worldwide crisis for the adverse clinical events.

We present a simple method to separate and quantify OSCS in the presence of heparin. This 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 sensitivity of the method 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.

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.

References: