The work covers the development of two analytical methods for the analysis of heparin and heparin derived oligosaccharides.

An ion-pair reversed-phase ultra-performance liquid chromatography method was developed for rapid analysis of heparin oligosaccharides coupled with electrospray ionization time-of-flight mass spectrometry (IPRP-UPLC ESI/TOF-MS).

An anion exchange chromatography (AXC) method was developed to separate and quantify intact heparin and oversulfated chondroitin sulfate (OSCS).

The IPRP-UPLC ESI/TOF-MS method combines a high-resolution LC separation with the accurate mass measurement of Q-ToF mass spectrometry to yield in-depth physicochemical characterization of heparin oligosaccharides.

The anion exchange chromatography method achieves complete resolution between heparin and OSCS, and less than 1% of OSCS in overall content is successfully quantified by UV absorbance.

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**OVERVIEW**

**EXPERIMENTAL METHODS**

- **Samples:**
  - dp6/dp10 oligosaccharides: U-Lails, Inc., LA, USA
  - Heparin std & heparin system test std U.S. Pharmacopeia
- **IPRP-UPLC Conditions:**
  - LC System: Waters ACQUITY UPLC System
  - Column: ACQUITY UPLC™ BEH C18 5µm SAX, 4.0X250mm
- **MS Conditions:**
  - Mass Spectrometer: Waters Q-ToF Premier™
  - Acquisition Mode: Negative Ion Mode
  - Capillary Voltage: 2.5 KV
  - Source Temperature: 120 °C
  - Extractor Voltage: 1 V
  - MZ Range: 250 – 1500
- **Anion Exchange Chromatography Conditions:**
  - LC System: Waters Alliance® 2690 Separation System (Alliance2690)
  - Column: Waters Spherisorb® 5µm SAX, 4.0X250mm
  - Column Temperature: 40 °C
  - Mobile Phases:
    - A: 50 mM NaH2PO4 (pH 2.5)
    - B: 10 mM NaH2PO4 + 2.0 M NaCl (pH 2.5)
  - LC Gradient: 90% B in 10 minutes

**Structures of Heparin Oligosaccharides Derived from Heparinase Digestion of Bovine Heparin**

<table>
<thead>
<tr>
<th>Sulfates</th>
<th>dp6 isomers</th>
<th>dp8 isomers</th>
<th>dp10 isomers</th>
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**Chromatographic Separations of Heparin Oligosaccharide Isomers/Mixture**

**Accurate Mass Measurement to Determine the Molecular Entities of Heparin Isomers**

**CONCLUSIONS**

- A simple, rapid and sensitive IPRP-UPLC/TOF MS method was developed for analysis of heparin derived oligosaccharides. Structural information such as molecular weight, number of sulfate groups, and composition of disaccharide blocks of heparin can be reliably obtained using the method.

- An anion exchange chromatography method was developed for separation and quantification of intact heparin and oversulfated chondroitin sulfate. The results demonstrate the method not only generates highly reproducible and fast separations (10 minutes) but also quantifies OSCS with abundance less than 1% of overall contents.

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