Mass spectrometry profiling of pentosan polysulfate sodium (PPS)

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Overview
- Our goal is to develop an approach using MS to profile pentosan polysulfate (PPS) at the molecular level.
- PPS is a semisynthetic heterogeneous sulfated polysaccharide mixture believed to interact with the interior lining of the bladder to alleviate pain associated with interstitial cystitis.
- Compositional profiling of PPS is important to understand mechanism of action as well as pharmacokinetics/pharmacodynamics of this drug.
- Our goal is to develop an approach using mass spectrometry profiling of pentosan polysulfate sodium (PPS) at the molecular level.

Introduction
- PPS is a complex sulfated polysaccharide (mass range of 4000-6000 Da) derived from the US.
- PPS is used to treat interstitial cystitis (IC), a condition of the epithelial lining of the bladder that manifests as bladder or pelvic pain and discomfort. Yet the mechanism of action is not fully understood.
- PPS is a semisynthetic heterogeneous sulfated polysaccharide mixture believed to interact with the interior lining of the bladder to alleviate pain associated with interstitial cystitis.

Methods
- Contents of Elmiron capsule dissolved in 1 ml HPLC grade water
- Soluble PPS separated from insoluble excipients by centrifugation and filtration
- PPS spiked at different concentrations in HPLC water and spiked solutions treated with Dowex ion exchange resin to convert sodiated esters of sulfate to protonated form
- Pentylamine with hexafluoroisopropanol (HFIP) added as an ion-pairing reagent
- Treated PPS solution loaded on conditioned C18 SPE cartridge
- Elution performed using a linear acetonitrile gradient
- Individual fractions injected in FTICR and IMS-MS

Results
- Characterization of PPS with ion pearing reagents
  - In MS1 spectra obtained from FTICR for PPS spiked water, a mass difference of 185.06 Da was observed between most abundant peaks. This difference corresponds to -SO3NH3(CH2)4CH3 + 18.01 Da (Figure 2)
  - In our opinion this mass difference arises from different degree of sulfation and not due to loss of sulfate as a result of in-source fragmentation
  - Collision induced dissociation (CID) on PPS associated peaks showed abundant product ions resulting from a neutral loss of SO3NH3(CH2)4CH3 (187.06 Da) and xylose (132.04 Da) (Figure 3)
  - The IM-MS profiles for a PPS sample shows different trend lines for different charge states (+1, +2, +3, +4, +5), which provides additional dimension of separation of species that cannot be resolved by m/z alone.
  - The treatments of ion exchange resin and ion-pair reagent to PPS sample enhanced the signal (Figure 5 and 6).

Conclusions
- We used ion-pair reverse phase chromatography to enable future separations of PPS from complex matrices.
- Presence of alkylammonium counterions improves ionization efficiency, reduces complexity from multiple sodium adducts and reduces loss of sulfate group by in-source fragmentation.
- Using IM-MS we were able to investigate high charge state species as well, coming from high molecular weight components of PPS.
- Addition of alkylammonium counterions enhanced the signal in 2D IM-MS performed in positive mode.

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References
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