# 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
- We used ion-pair reverse phase (IP-RP) extraction using C-18 SPE cartridges to extract PPS spiked in water

## Introduction

- PPS is a complex sulfated polysaccharide (mass range of 4000-6000 Da) derived from xylan, known by the brand name Elmiron in 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.
- Variations in the degree of sulfation, length of the oligosaccharide and various modifications add complexity to create mixtures that contain hundreds or more different species of PPS (Figure 1)
- The principal challenge in the proposed work is to profile PPS according to its molecular composition
- This would be invaluable for understanding biological activity, bioavailability, and pharmacokinetics, as well as for quality control.

## Methods

Contents of Elmiron capsule dissolved in 1 mL HPLC grade water

↓

Soluble PPS separated from insoluble excipients by centrifugation and filtration

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PPS spiked at different concentrations in HPLC water

↓

Spiked solutions treated with Dowex ion exchange resin to convert sodiated esters of sulfate to protonated form

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Pentylamine with hexafluoroisopropanol (HFIP) added as an ion-pairing reagent.

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Treated PPS solution loaded on conditioned C18 SPE cartridge.

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Elution performed using a linear acetonitrile gradient

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Individual fractions injected in FTICR and IMS-MS

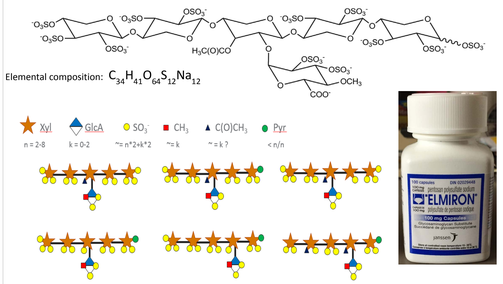


Figure 1. General Structure of PPS. PPS samples may have different combination of sulfation and other modifications.

## Results

Characterization of PPS with ion paring 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 -$SO_3NH_3(CH_2)_4CH_3$ + 18.01 (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 $SO_3NH_3(CH_2)_4CH_3$ (167.06 Da) and xylose (132.04 Da) (Figure 3)

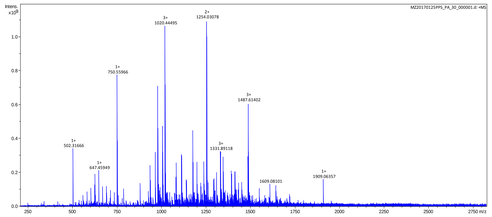

Figure 2. FTCIR mass specta of PPS paired with pentylamine

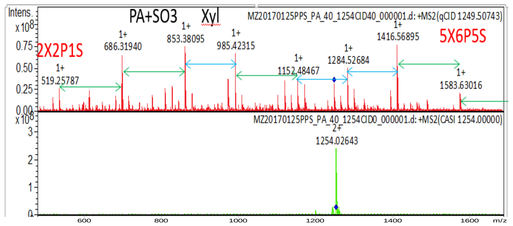


Figure 3. Collision induced dissociation of m/z 1254 (z=+2)

- Peak with m/z 1254 (z =+2) contains 5 xylose units, 6 pentylamine units and 5 sulfate groups, which corresponds to is $C_{55}H_{118}O_{35}N_6S_5$
- Most PPS elute within 15%-40% acetonitrile fraction (Figure 4)

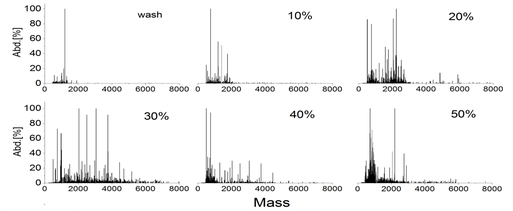


Figure 4. Deconvoluted mass spectra of PPS eluting in different fractions of ACN

- 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 resolve by *m/z* alone.
- The treatments of ion exchange resin and ion-pair reagent to PPS sample enhanced the signal (Figure 5 and 6).

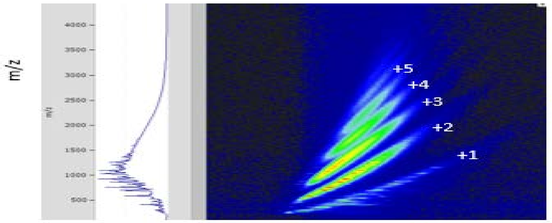


Figure 5. 2D IM-MS map for PPS without treatment in the +ve mode

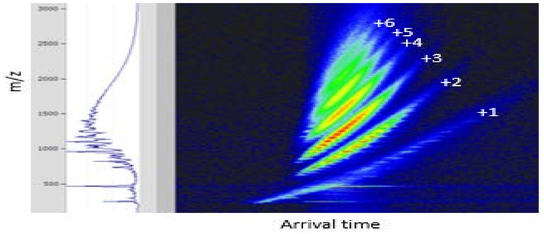


Figure 6. 2D IM-MS map for PPS with Dowex Resin and ion-pair treatment in the +ve mode

## 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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