Mass spectrometry profiling of pentosan polysulfate sodium (PPS)

Komal Kedia, Xueyun Zheng, Mowei Zhou, Charles Ansong, Erin S. Baker, John R. Cort
Biological Science Division, Pacific Northwest National Laboratory (PNNL), Richland WA

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

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

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

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

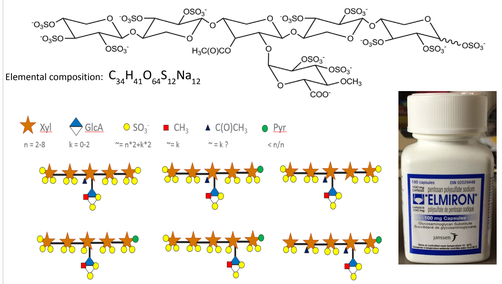

Figure 1. General Structure of PPS. PPS samples may have different combination of sulfation and other modifications.

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)

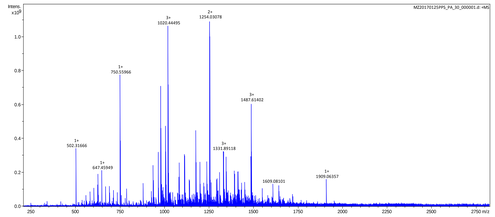

Figure 2. FTCIR mass spectra of PPS paired with pentylamine

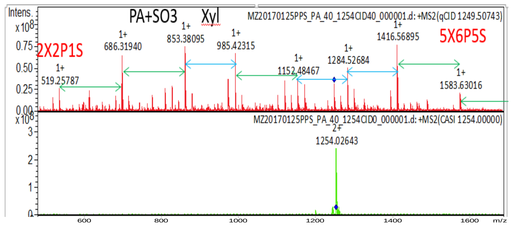

Figure 3. Collision induced dissociation of m/z 1254 (z=+2)

- 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)

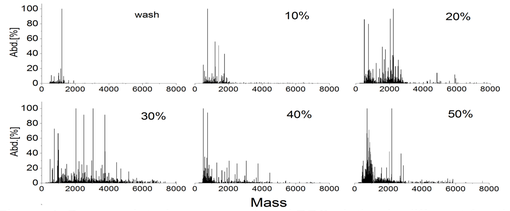

Figure 4. Deconvoluted mass spectra of PPS eluting in different fractions of ACN

- 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).

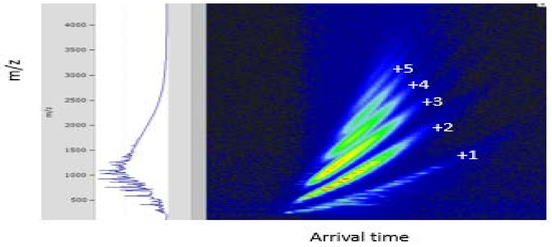

Figure 5. 2D IM-MS map for PPS without treatment in the +ve mode

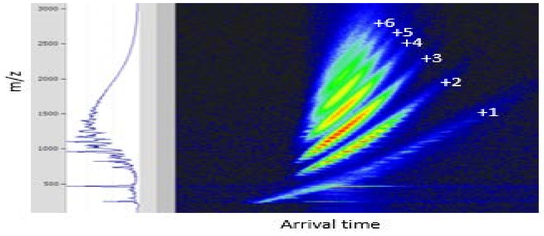

Figure 6. 2D IM-MS map for PPS with Dowex Resin and ion-pair treatment in the +ve mode

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

Acknowledgements

Disclaimer: This article reflects the views of the authors and should not be construed to represent FDA's views or policies.

Funding Acknowledgement: Financial support was provided by the U.S. Food and Drug Administration (contract HHSF223201610114C and grant U01FD005288).

Research was performed in the Environmental Molecular Sciences Laboratory, a DOE national scientific user facility on the PNNL campus. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under contract DE-AC05-76RL01830.

References

1. Doneanu CE, Chen W, & Gebler JC (2009). Analysis of oligosaccharides derived from heparin by ion-pair reversed-phase chromatography/mass spectrometry. *Analytical Chemistry, 81*(9), 3485-3499. doi:10.1021/ac802770r
2. Erickson DR, Sheykhnazari M, & Bhavanandan VP (2006). Molecular size affects urine excretion of pentosan polysulfate. *The Journal of Urology, 175*(3 Pt 1), 1143-1147. doi:10.1016/S0022-5347(05)00319-8

Career Opportunities: http://omics.pnl.gov/careers

CONTACT: John Cort, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: John.Cort@pnnl.gov

omics.pnl.gov