Universal open-source software for detecting metabolites in complex mixtures by scanning precursors with predetermined neutral losses from MS/MS

Komal Kedia, Aivett Bilbao, Mowei Zhou, Samuel H. Payne, John R. Cort
Biological Science Division, Pacific Northwest National Laboratory (PNNL), Richland WA

Pacific Northwest NATIONAL LABORATORY

Overview

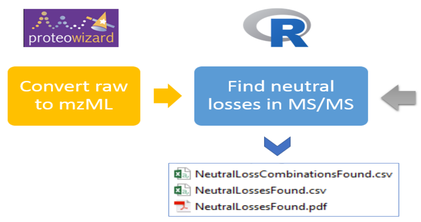
- We developed a tool, called AurkituMS, for rapid screening of neutral losses in MS/MS. AurkituMS is a universal open-source tool.
- Two modes are available: targeted screening using a list of predetermined neutral loss masses and untargeted screening to find frequent mass offsets
- Proof of concept: we identified precursors showing signature neutral losses from a heterogeneous sulfated polysaccharide drug, pentosan polysulfate sodium (PPS, brand name Elmiron). We then used the tool to search for evidence of this drug or its metabolites in urine from patients taking PPS.

Introduction

- There are few easy-to-use tools that can scan for user-defined neutral losses from molecular ion peaks as well as between products ions in MS/MS spectra.
- Existing methods are typically limited to finding neutral losses from the precursor ion or across MS1 data [1-3].
- To address this need, we developed AurkituMS, which is an open-source software written in R with few dependencies to facilitate easier application and customization.
- The user provides a list of masses and gets an output including a broad range of information related to precursor presenting those neutral losses.
- AurkituMS successfully identified precursors in LC-MS data of PPS, showing signature neutral losses specific. These were used it to compare the molecular profile of PPS from Elmiron vs. an Indian generic version.
- AurkituMS was used to search GC-MS data for evidence of metabolites in urine of patients taking Elmiron.

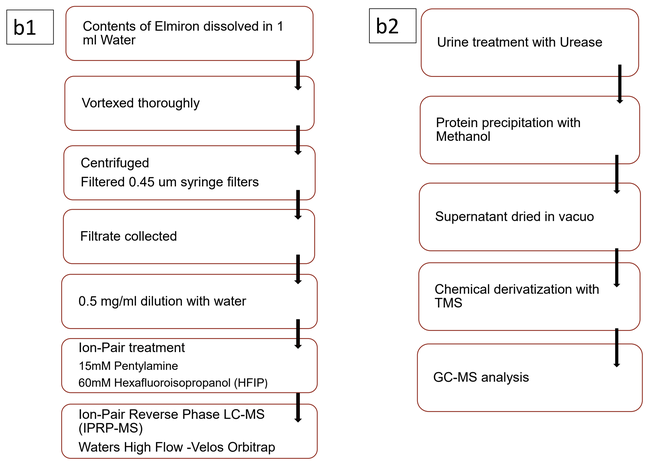
Methods

a) Workflow to determine neutral losses from MS/MS spectra



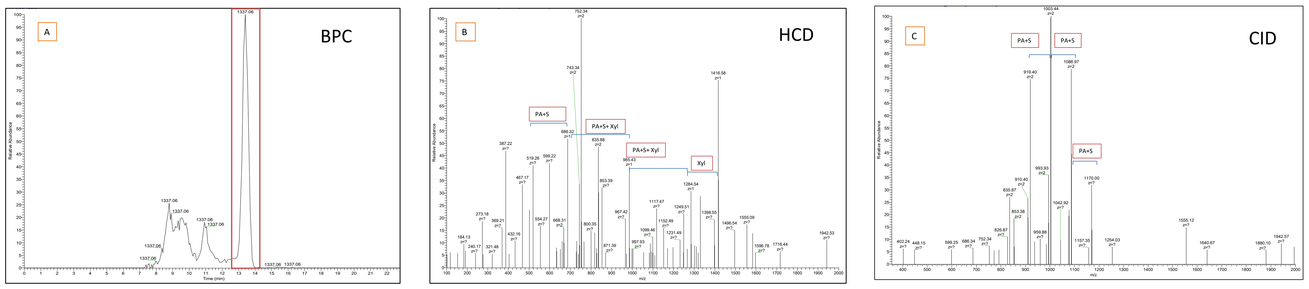
Name	Mass
H2O	18.01057
Sodium/H	21.98195
Acetyl	42.01056
PEG	44.02621
Pyridinium	78.03437
Sulfate	79.95682
Pentylamine	87.10479
SO3Na-H	101.9388
NaHSO4	119.9493
Xylose	132.0423
Na2SO4	141.9313
Pentylamine+Sulfate	167.0616
des-Ome_GlcA	176.0321
4-OMe GlcA	190.0477
Xylose+1Sulfate	211.9991
Xylose+2Sulfate	291.9559
Pentylamine+Sulfate+Xylose	299.1039
4-OMe_GlcA+2Sulfate	347.9613

b) Sample preparation scheme: (b1) Water spiked PPS, (b2)Healthy and patient urine



Results

A) Base peak chromatogram (BPC) B) MS/MS acquired in HCD mode C) MS/MS acquired in CID mode for m/z 1337.06 (z=+2)

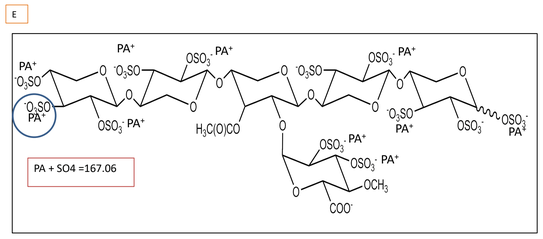


D) Section of output generated from AurkituMS tool, highlighting the neutral losses observed in HCD and CID spectrum of 1337.06

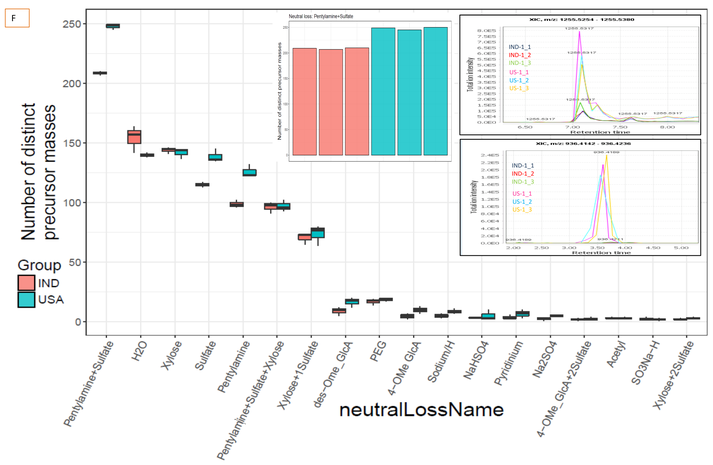
D

mz1	mz2	NeutralLossName	NeutralLossCharge	PeakType	Intensity1	Intensity2	Scan Number	FragmentationType	Retnetion time	PrecMz	PrecCharge	BasePeakIntensity
519.2590332	686.3195801	Pentylamine+Sulfate	1	fragment-fragment	4543.461426	5725.010742	1226	HCD	13.31986833	1337.063232	2	11088.88086
387.2157288	599.2167969	Xylose+1Sulfate	1	fragment-fragment	5194.336914	4634.794434	1226	HCD	13.31986833	1337.063232	2	11088.88086
985.427063	1284.536011	Pentylamine+Sulfate+Xylose	1	fragment-fragment	4469.621582	3388.287598	1226	HCD	13.31986833	1337.063232	2	11088.88086
686.3195801	985.427063	Pentylamine+Sulfate+Xylose	1	fragment-fragment	5725.010742	4469.621582	1226	HCD	13.31986833	1337.063232	2	11088.88086
387.2157288	686.3195801	Pentylamine+Sulfate+Xylose	1	fragment-fragment	5194.336914	5725.010742	1226	HCD	13.31986833	1337.063232	2	11088.88086
1002.937134	1169.995972	Pentylamine+Sulfate	1	fragment-fragment	10534.7207	3377.000488	1227	CID	13.32759	1337.063232	2	10619.48828
1086.466064	1169.995972	Pentylamine+Sulfate	2	fragment-fragment	6067.399414	3377.000488	1227	CID	13.32759	1337.063232	2	10619.48828
1003.937866	1087.472534	Pentylamine+Sulfate	2	fragment-fragment	4357.439941	4692.562988	1227	CID	13.32759	1337.063232	2	10619.48828
919.9030762	1086.965698	Pentylamine+Sulfate	1	fragment-fragment	4362.950195	8353.928711	1227	CID	13.32759	1337.063232	2	10619.48828
1003.43866	1086.965698	Pentylamine+Sulfate	2	fragment-fragment	10619.48828	8353.928711	1227	CID	13.32759	1337.063232	2	10619.48828
919.4024658	1086.466064	Pentylamine+Sulfate	1	fragment-fragment	7905.692383	6067.399414	1227	CID	13.32759	1337.063232	2	10619.48828
919.9030762	1003.43866	Pentylamine+Sulfate	2	fragment-fragment	4362.950195	10619.48828	1227	CID	13.32759	1337.063232	2	10619.48828

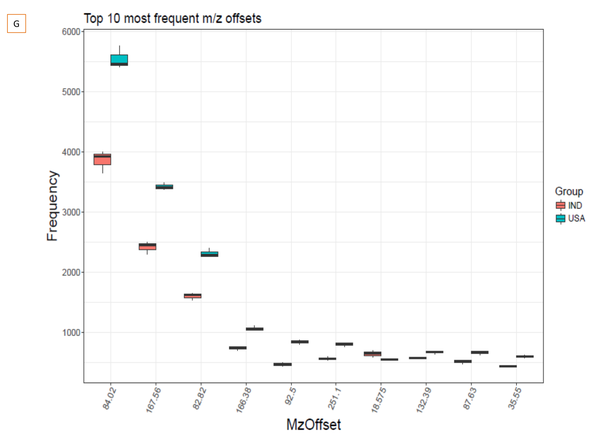
E) Partial structure of PPS with an example of predicted cleavage site during fragmentation



F) Comparison of Elmiron: US PPS and Indian generic PPS based on their neutral loss profile found by AurkituMS



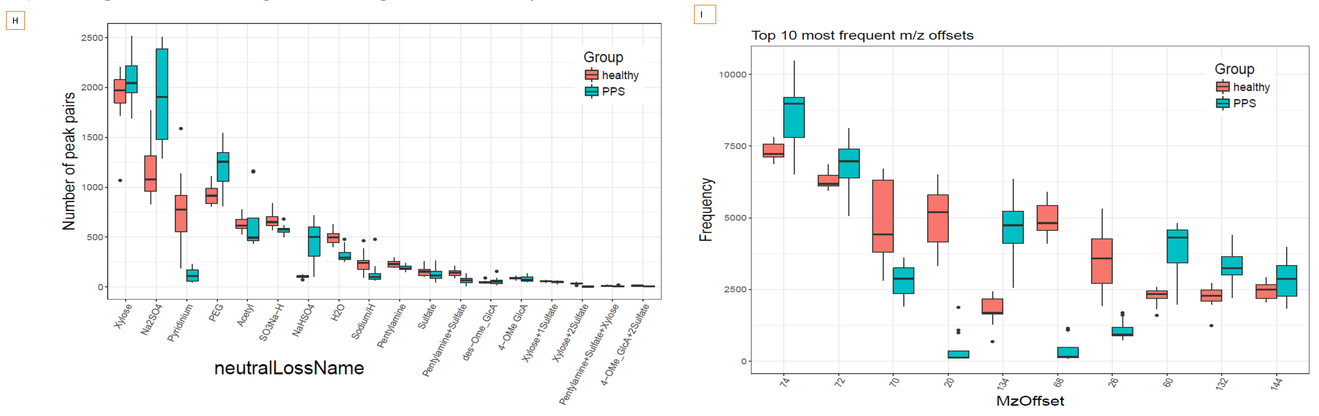
G) Untargeted screening to investigate most frequent neutral losses from Indian and US PPS



Comparison of neutral losses in MS/MS of metabolites in urine from healthy subjects vs. those taking Elmiron to treat interstitial cystitis, acquired on GC-MS and analyzed using AurkituMS

H) Targeted screening using defined neutral losses

I) Untargeted screening to investigate most frequent neutral losses



Conclusions

- Our tool, AurkituMS facilitated data interpretation of a complex and heterogeneous drug mixture that traditional metabolomics software was unable to process.
- Our tool is compatible with any source data and can extract precursor information from different analytical techniques: LC-MS and GC-MS.
- AurkituMS is applicable in a plethora of MS-based study areas: metabolomics, lipidomics, proteomics as well as drug metabolism of xenobiotics and natural products

Acknowledgments

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CONTACT:

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

Komal Kedia, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: komal.kedia@pnnl.gov

Aivett Bilbao, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: aivett.bilbao@pnnl.gov

www.pnnl.gov

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