PATTERN TARGETING SOFTWARE FOR THE DETECTION OF POLYMERIC COMPOUNDS IN DATA ACQUIRED USING LIQUID CHROMATOGRAPHY-ION MOBILITY-MASS SPECTROMETRY

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INTRODUCTION

Liquid chromatography-ion mobility-mass spectrometry (LC-IM-MS) is used for the non-targeted acquisition of samples containing polymeric compounds. The analysis of the resulting data is challenging due to the presence of a range of charge-state distributions, multiple adducts and isomeric oligomers. It is common for such samples to contain many individual ions related to each polymeric species and this can make the assignment of the individual polymers difficult.

We present a dedicated software application that can target user specified patterns within complex data and enables the assignment of polymers, determines the individual oligomeric contributions, and measures the overall abundance of each polymer in a sample.

EXPERIMENTAL

Polymeric standards were chromatographically separated using a Waters™ ACQUITY™ I-Class inlet with an ACQUITY UPLC® BEH C18 column (2.1 mm × 100 mm, 1.7 µm), and analysed using electrospray on a Waters Synapt™ XS mass spectrometer. The mass spectrometer was mass and ion-mobility calibrated using Major Mix, and HDMS™ data were acquired.

METHODS

Find A Common Mass Difference

The acquired data were peak detected and the resulting ions were imported into the Pattern Targeting Application (PTA). The application performs isotope clustering to yield a collection of components. The components can be displayed as a component spectrum together with the raw *m/z* spectral data (Figure 1). Several ion series are clearly visible in the component spectrum and focussing on one of the series reveals a repeating mass difference of 44 Da. Examining the isotope cluster of one of the ions in the series indicates that the ion is singly charged. Therefore the data contains a polymer with a repeat unit of 44 Da which is indicative of polyethylene glycol (PEG).

Define Polymer

Target polymers are specified in the PTA via a dialog in which the repeat unit and two end groups can be entered, either as chemical formulae or as mass values. A definition for PEG is shown in Figure 2. Adducts and the maximum charge expected are also specified in the dialog together with a mass tolerance used to screen the measured data for the generated targets. Once a polymer has been defined, a list of potential target oligomers are generated as shown in Figure 3. The measured data is then screened against the targets generated for all selected polymers.

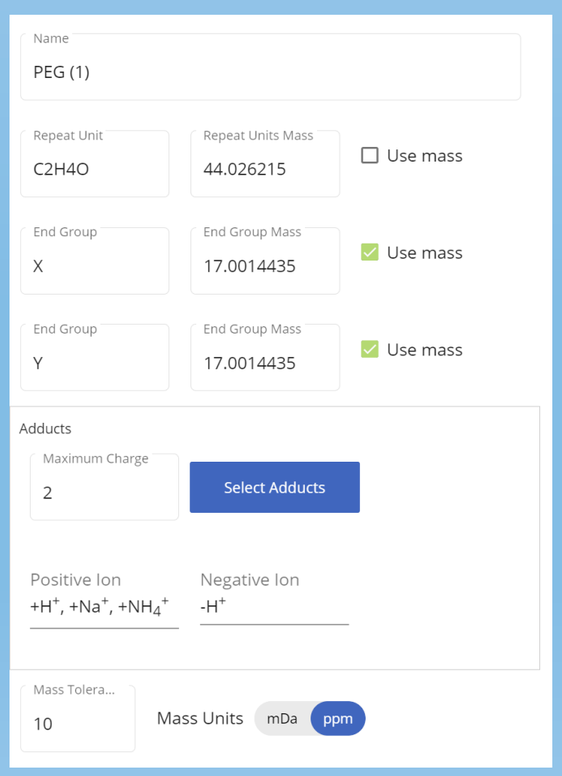


Figure 2. Polymer definition dialog.

	m/z	Charge	Adduct	Neutral Mass	Repeats	Formula
49	299.1675	1	$+H^+$	298.1602	6	$X(C_2H_4O)_6Y$
50	299.1926	2	$+2NH_4^+$	562.3175	12	$X(C_2H_4O)_{12}Y$
51	304.1480	2	$+2Na^+$	562.3175	12	$X(C_2H_4O)_{12}Y$

Figure 3. Example polymer targets.

RESULTS

Screen Against The Data

On screening the measured data against the targets generated with the initial PEG definition, it was evident that other PEG series were present in the data. Through an iterative process, five additional PEG series were defined and screened against the measured data. The components that match any of the defined targets are highlighted in the component spectrum (Figure 4a), and a spectrum showing the remaining unassigned components is also generated (Figure 4b). The unassigned components spectrum shows that the dominant PEG ion series within the data have been assigned to the generated targets.

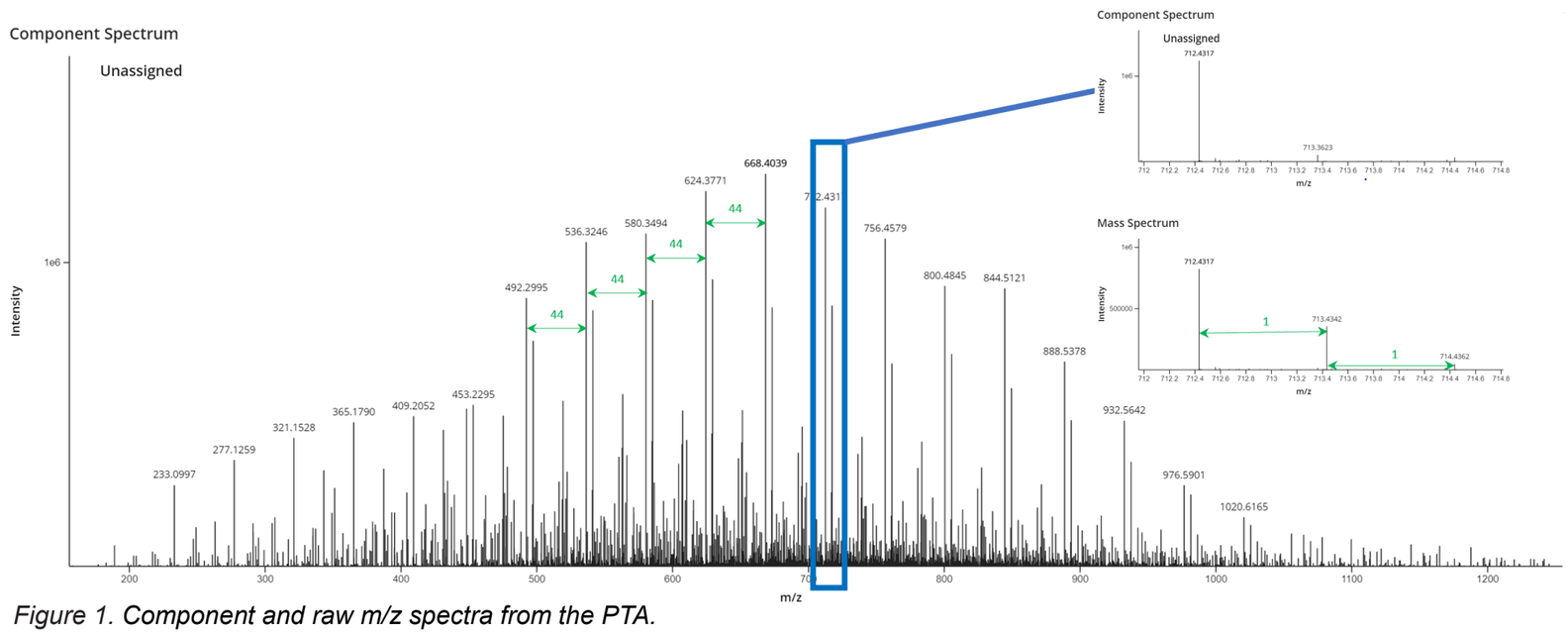
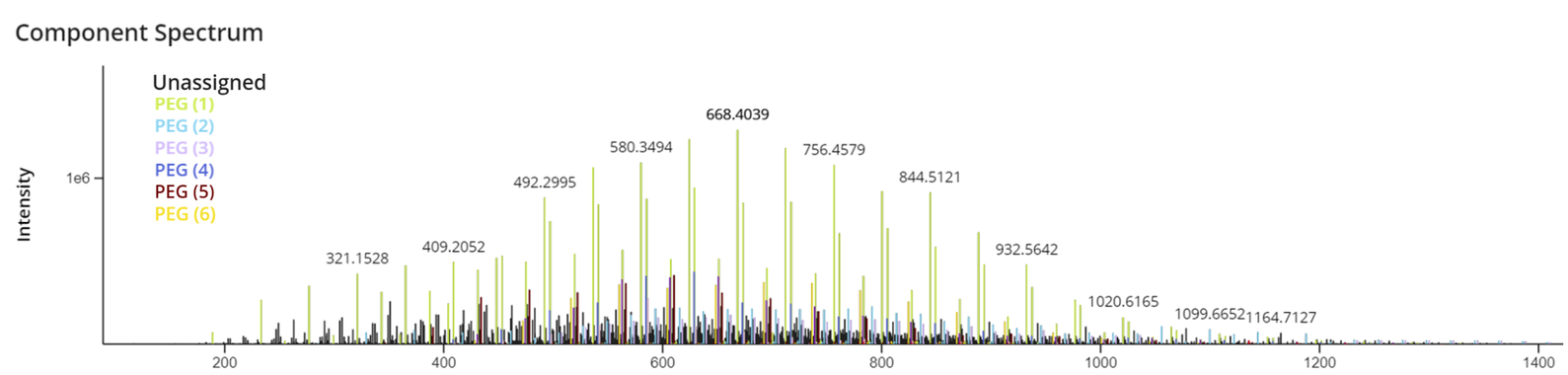


Figure 1. Component and raw m/z spectra from the PTA.



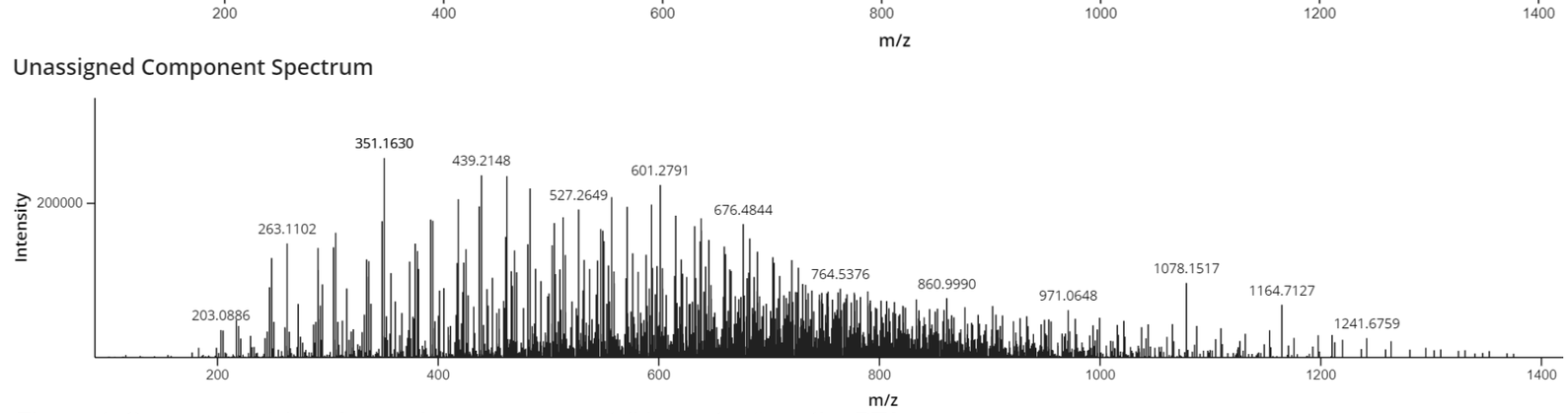


Figure 4. Component (a) and unassigned component (b) spectra from the PTA.

DISCUSSION

Ion mobility mass spectrometry enables the drift time of ions to be plotted against their *m/z* and this can reveal trends within polymeric species including adducts and charge states. Figure 5 displays the singly-charged protonated and doubly-charged sodiated and ammoniated assignments in a drift plot generated in the PTA for the PEG (4) species.

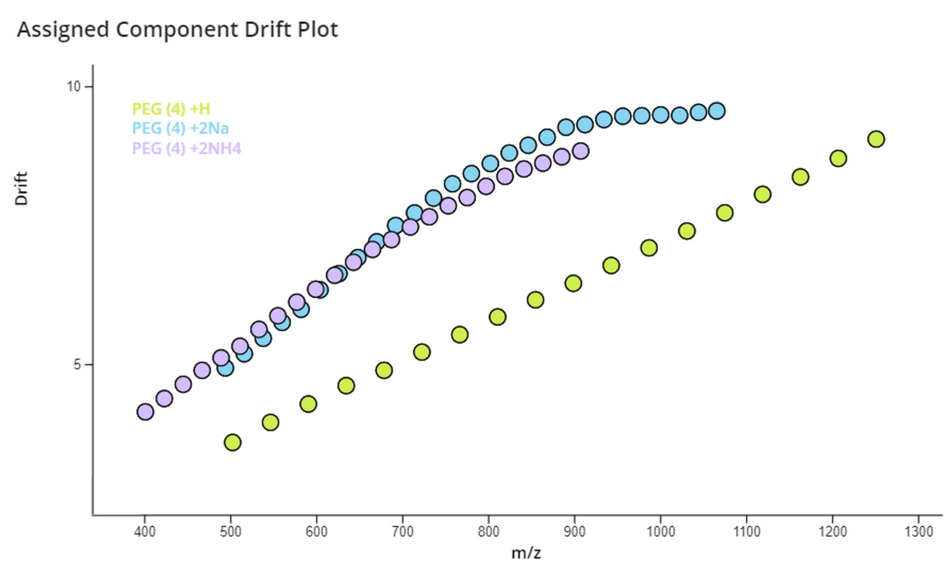


Figure 5. Component drift-plot for the PEG (4) series.

$$M_n = \frac{\sum_i I_i m_i}{\sum_i I_i}, \quad M_w = \frac{\sum_i I_i m_i^2}{\sum_i I_i m_i}, \quad M_z = \frac{\sum_i I_i m_i^3}{\sum_i I_i m_i^2},$$

$$M_{z+1} = \frac{\sum_i I_i m_i^4}{\sum_i I_i m_i^3}, \qquad PDI = \frac{M_w}{M_n}$$

Figure 6. Polymeric calculation performed by the PTA

Polymer Calculations:

Name:	PEG (4)
Mn:	1,030
Mw:	1,165
Mz:	1,322
Mz+1:	1,496
Polydispersity:	1.13
Total Response:	2600115

Figure 7. Polymeric calculations for the PEG (4) series.

Since all oligomers from a given polymer are known, standard polymeric quantities including the number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) can be determined from each mass, intensity pair (m_i, I_i). The complete list of quantities determined in the PTA are shown in Figure 6 and the values of these quantities for one of the PEG series in the example data and shown in Figure 7.

CONCLUSIONS

- **The PTA targets compounds consisting of repeated mass differences.**
- **Polymers are defined using a simple dialog encompassing repeat units, end groups, adducts**
- **Peak list data are isotope clustered into components.**
- **Targets are screened against the list of components.**

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