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Exploring Ion Mobility Data File Conversions to Leverage Existing Tools and Enable New Workflows

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Introduction

Adding Ion Mobility to LC-MS experiments provides an additional dimension of separation for complex samples. While IM can uncover new information about a sample, downstream data analysis workflows are often not equipped to properly mine the additional dimension of data. To enable traditional LC-MS workflows to process LC-IM-MS data files, new data transformations have been added to the PNNL PreProcessor, including mapping the IM axis to the LC axis and mapping All Ions IM/MS drift aligned fragmentation spectra into individual spectra creating a LC-MS DDA file. Enhanced resolution results from a newly automated High-Resolution Demultiplexing workflow is also evaluated for the new conversions. Additionally, converting 2D-LC MS data to LC-IM-MS data is explored as both formats share the same four-dimensionality.

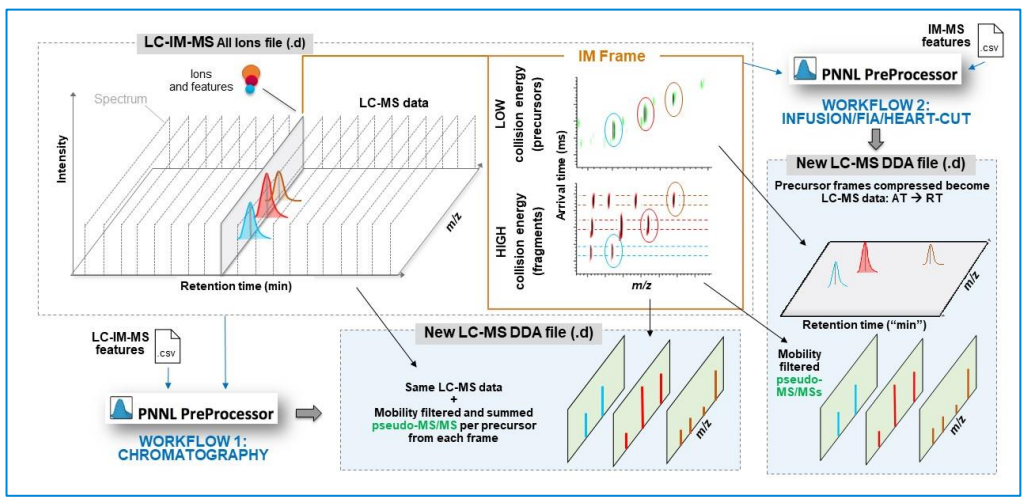

Figure 1. Diagram for IM-to-DDA workflows in the PNNL PreProcessor. Two workflows are available, one where the chromatographic separation is to be maintained in the output data file and one where the IM separation becomes the chromatographic separation in the output data file.

The complexity of the sample should be considered when determining if the conversion to DDA format will benefit data analysis as well as determining which workflow best captures the information in the data file. For samples where IM isomers are not present (i.e. all features have unique *m/z*, RT values) then Workflow 1 is best. For samples where IM isomers are present (i.e., same *m/z*, RT value and only separated by DT) or for data collected with direct infusion or FIA then Workflow 2 is best. For very complex samples where multiple peaks are present at drift times within the same RT elution period then traditional four-dimensional data analysis methods or implementing some form of deconvolution prior to the IM-to-DDA conversion is recommended.

Experimental

Experiments were performed on a 6560 IM-QTOF (Agilent Technologies, Santa Clara, CA) paired with a commercial LC (1290 Infinity II series, Agilent Technologies) used for LC and 2D-LC separations. Standard samples across different applications including lipidomics (NIST SRM 1950 extract) and PFAS (ITA-70, Agilent Technologies) were evaluated. The PNNL PreProcessor[1] was used for converting LC-IM-MS data to LC-MS DDA format and for converting 2D-LC MS data to LC-IM-MS format. IM-MS Browser was used to perform Ion Mobility Feature Extraction (IMFE) for the DDA conversion workflow and for evaluating converted 2D-LC data files. Downstream software applications including MassHunter Qual, Lipid Annotator, and Mass Profile software as well as MS-DIAL[2] were used to evaluate converted data files.

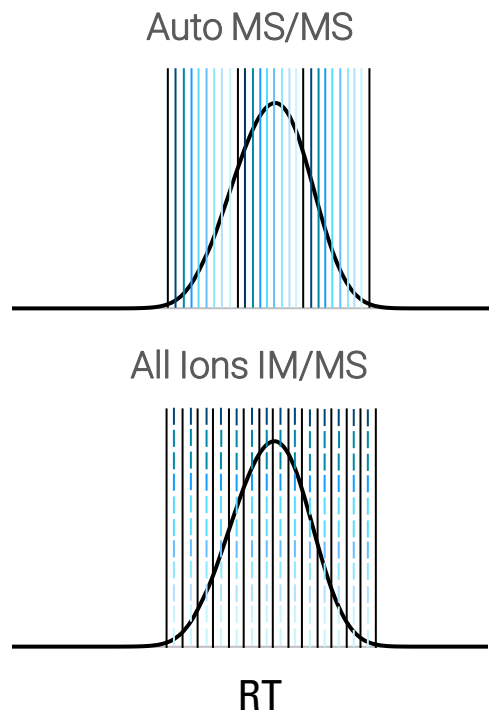

Figure 2. Diagrams for Auto MS/MS and All Ions IM/MS are shown across a chromatographic peak. The black vertical line indicates a MS1 frame, and blue lines indicate fragmentation frames. With Auto MS/MS the instrument must cycle through the precursor ions whereas with All Ions IM/MS fragmentation spectra are acquired every other frame for all precursors.

Figure 3. The PNNL PreProcessor has become a critical tool for most 6560 IM-QTOF workflows

All Ions IM/MS-to-DDA Conversion Evaluated for Lipidomics Workflow

Results for processing the lipid extract in MS DIAL[2] are shown below. In 4A. mirror plot and spectrum similarity results are shown for PC 16:0_18:1 for Quad Isolation DDA, All Ions IM/MS-to-DDA, and All Ions IM/MS data files. Inserts highlight coverage of smaller PC lipid fragments. The converted All Ions IM/MS-to-DDA dot product similarity score is lower than the quad isolated spectra which is expected. In 4B. Total number of lipids found in at least 2 of the triplicate data files processed with the three methods. The converted IM-to-DDA file has the most lipid identifications benefitting from the improved duty cycle with All Ions data acquisition. A Venn diagram in 4C. shows the overlap in number of lipid IDs across the three methods. Of the 680 lipids 15% were found across all three methods and 38% found in at least two.

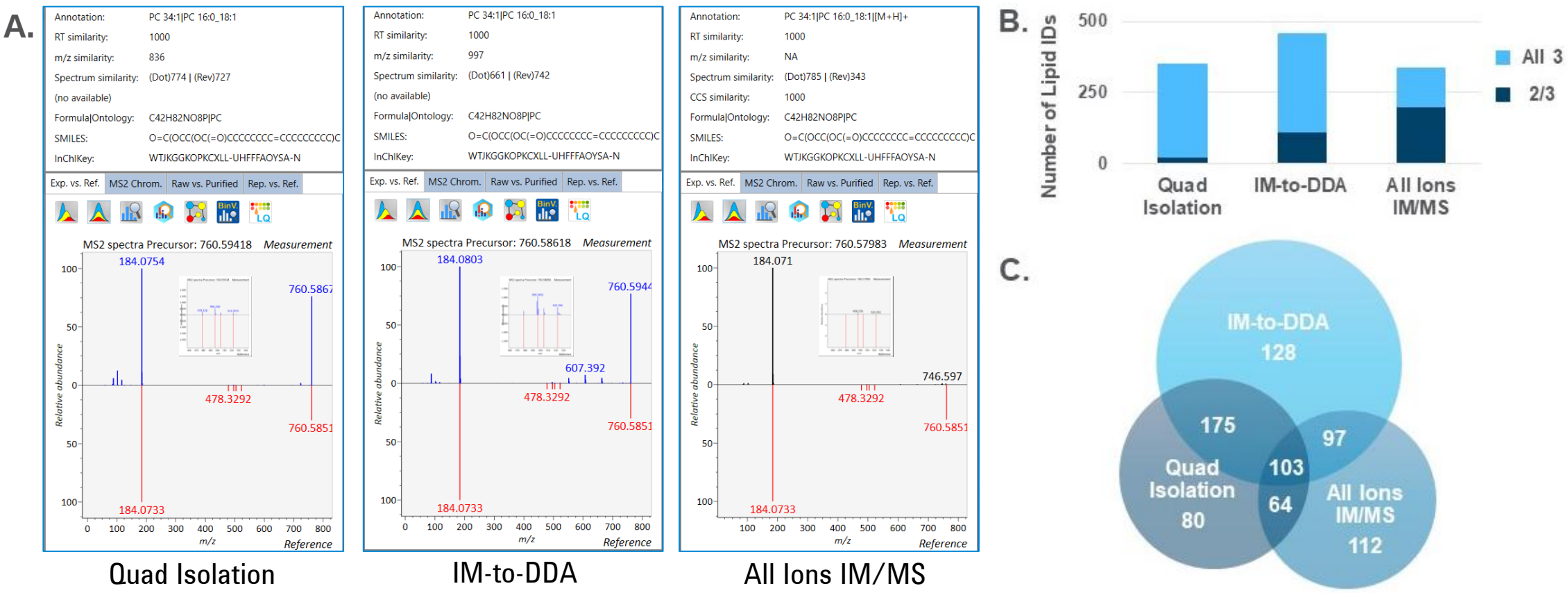

Figure 4. Results for processing the NIST SRM 1950 lipid extract in MS DIAL with three different data file formats.

Isomer Treatment with Workflow 1 and 2

The lipid sample run in the previous example was also run as a HILIC separation which results in more IM isomers. For LPC 20:3 two isomers are present. In Workflow 1, individual scans for the two isomers are written to the IM-to-DDA file as indicated in 5A. when the chromatogram is walked spectrum by spectrum. In 5B. when Find by Auto MS/MS is run on the data files, two fragmentation spectra are written for the same compound in Workflow 1 since the ramped CE function was used resulting in unique CE for each isomer. Workflow 2 results in two separate spectra. In 5C. for MS DIAL only one result for LPC 20:3 is returned, and the fragmentation spectra is a combination of the two isomers. In Workflow 2, two results are returned for LPC 20:3 with two unique fragmentation spectra.

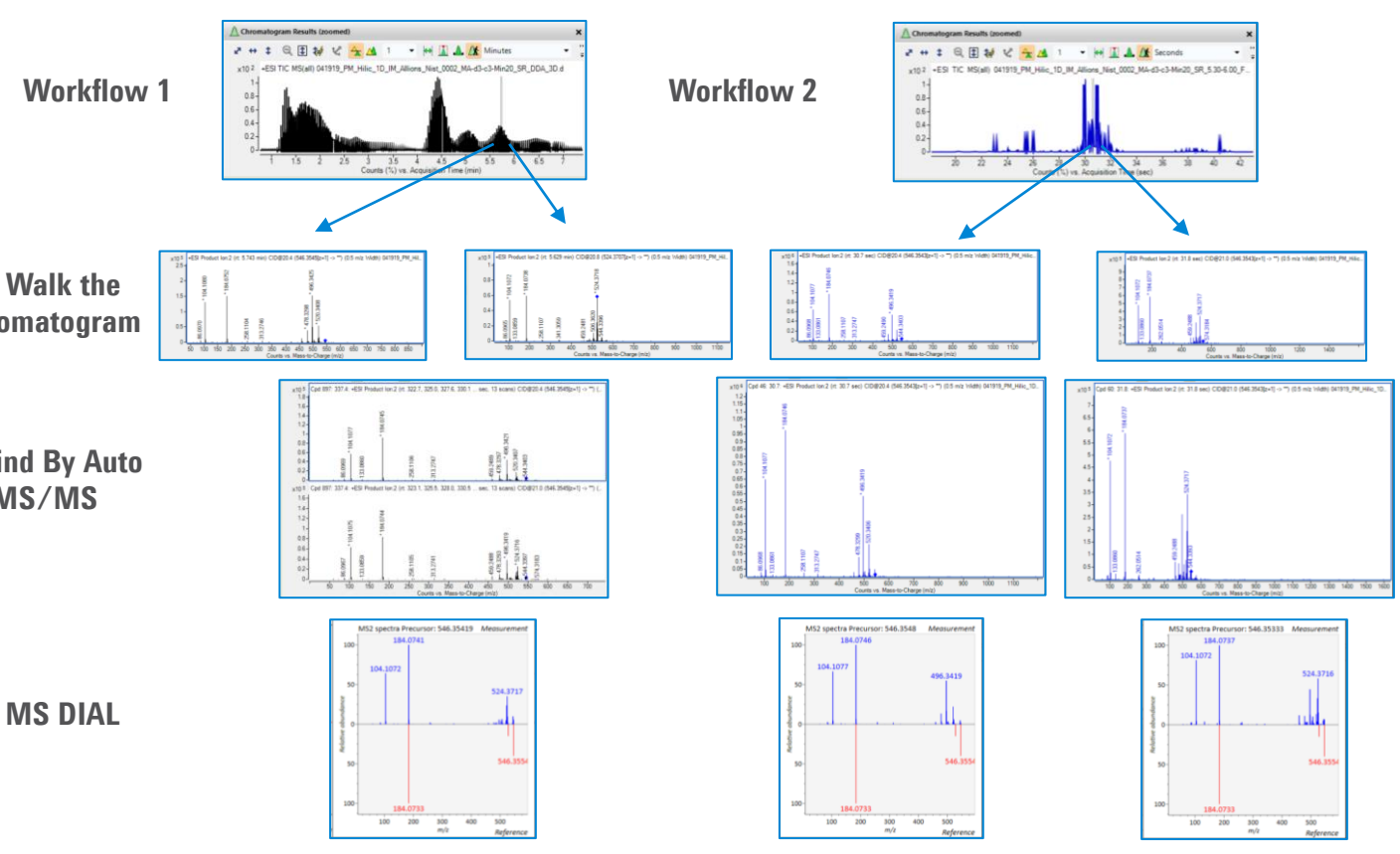

Figure 5. Results for two isomers of LPC 20:3 from the lipid extract in both (A,B) MassHunter Qualitative Analysis and (C) MS DIAL.

Results and Discussion

High Resolution Demultiplexing Workflow Improvements

The High Resolution Demultiplexing[3] (HRdm) workflow has been simplified so that the interpolation, demultiplexing, feature finding, and high resolution demultiplexing all happen in a single step for the user. HRdm calls the PNNL PreProcessor in the background to perform interpolation and initial demultiplexing. It then implements feature finding and then applies the high-resolution deconvolution. This reduces the number of software packages that a user must interact with from three to one.

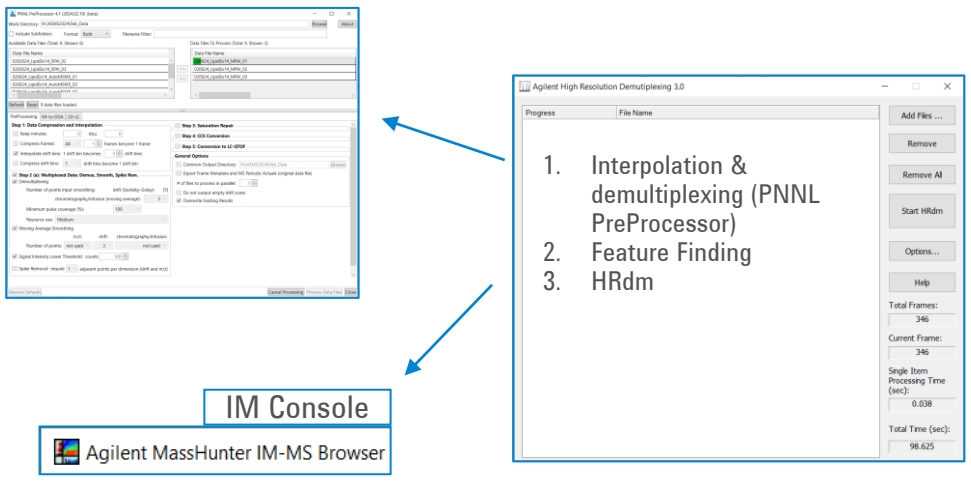

Figure 6. Automated HRdm 3.0 Workflow

Comparing Lipid Data with Single Pulse and Multiplexed Data Acquisition

The NIST SRM 1950 (different extraction) was run with both single pulse and multiplexed All Ions IM/MS. The multiplexed data was processed with HRdm 2.0 and with the Automated HRdm 3.0 workflow with an interim feature finder. Results in the number of lipid IDs from Lipid Annotator (yellow) and MS DIAL (green) are shown below in Figure 7A for Workflow 1, with TG lipids (blue) shown separately. Lipid Annotator has strict scoring which results in fewer lipid IDs especially for TGs where not all precursors are IM resolved. The smaller number of HRdm lipids vs. DeMP lipids for Workflow 1 is due to narrower bands of extracted fragment ions (based on the narrowed FWHM of precursor from HRdm processing). The RT section where TG lipids elute was summed and processed with Workflow 2 to show benefits from HRdm deconvolution in Figure 7B.

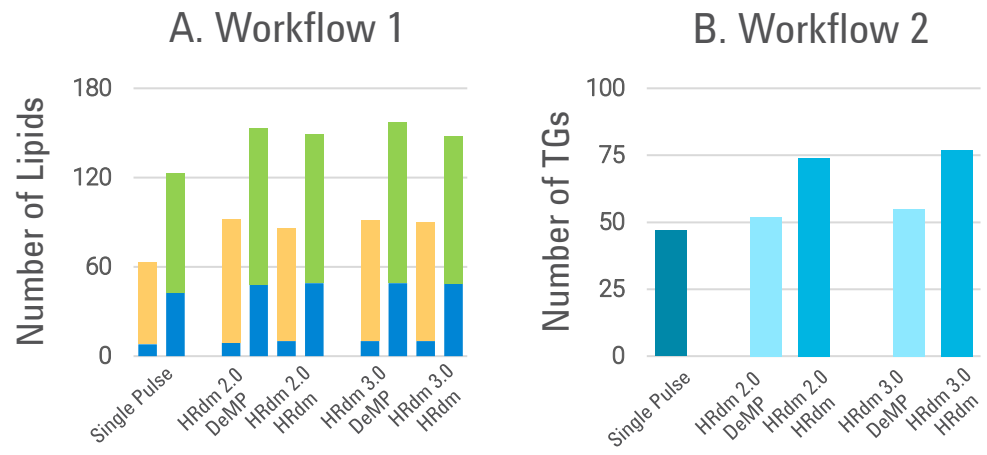

Figure 7. Results for multiplexed data files with IM-to-DDA conversion for A. Workflow 1 and B. Workflow 2

Future Workflows in PNNL PreProcessor

A current area of investigation in the PNNL PreProcessor is to take comprehensive or hi-res 2D-LC MS data and convert it into LC-IM-MS data. 2D-LC MS data can then be processed with IM feature finding. A PFAS standard was acquired with both a 2D-LC MS and LC-IM-MS method and 12 of 14 standards were found in both data files. Further investigations are planned to evaluate mass accuracy and applying IM peak detection to chromatographic peak shapes.

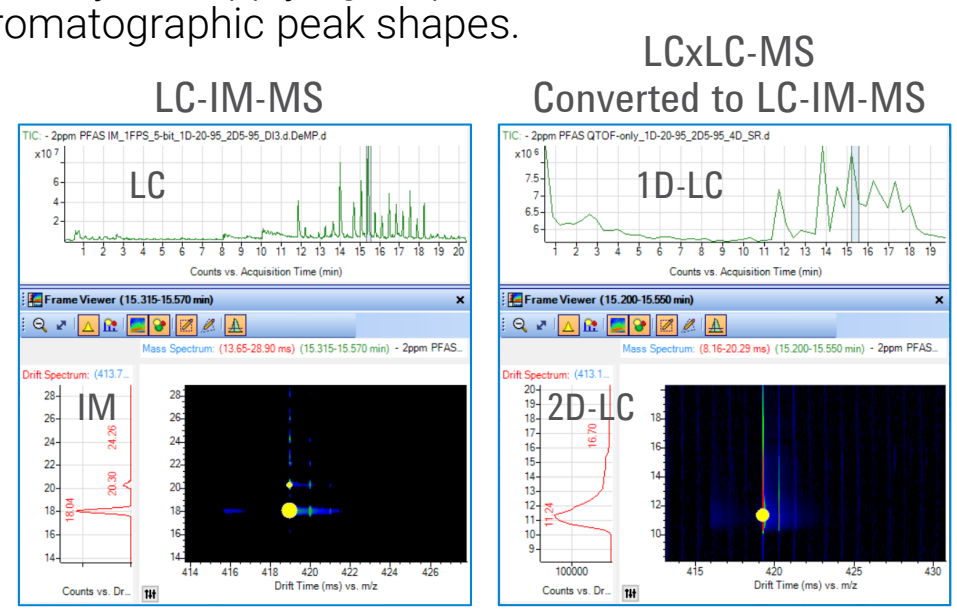

Figure 8. Extending IMFE to converted 2D-LC-MS data

Conclusions

- IM-to-DDA conversion increases number of lipid identifications
- Separate DDA events are reported for IM isomers in Workflow 1, but Workflow 2 is recommended to verify they both appear in downstream data analysis
- Automated HRdm workflow with new feature finding simplifies workflow and is supported in IM-to-DDA conversion
- Multiplexed All Ions data shows increase in number of IDs with Workflow 1, but Workflow 2 is needed to see increase in number of IDs for HRdm
- 2D-LC-MS data converted to LC-IM-MS data allows for analysis with 4D ion mobility feature finding

References

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