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A Study of Ion Statistics and Optimized Data Treatment for HRIM-MS and LC-HRIM-MS Data

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Introduction

Combining liquid chromatography (LC) with mass spectrometry (MS) is common practice to enable the analysis of isomeric species that MS alone cannot differentiate. Ion mobility (IM) is a gas phase separation that over the last few years has been applied with increasing frequency in front of MS and often to complement the LC dimension. An emerging type of high-resolution IM (HRIM) is Structures for Lossless Ion Manipulation (SLIM) where higher resolving power from longer, serpentine ion paths is achieved through electrode patterns on the circuit boards (Figure 1). In this study, we aim to optimize the benefits of SLIM that allow its peak capacity to rival that of LC in time scales not possible by liquid phase separations.

Figure 1. SLIM-based HRIM coupled to a 6545 LC/Q-TOF

Data Preprocessing and Resolution

Coupling HRIM to TOF mass spectrometry often results in the ion mobility separation being sampled at a higher than optimal rates. Summing adjacent TOF transients (drift bin summing - D2 trough D7) and added smoothing (drift bin moving average - d3 through d7) improves sensitivity but can lead to decreased resolving power as shown in Table 1.

Table 1. Percent decrease in resolving power for preprocessing treatment. Total Width is the product of drift bin summing and drift bin smoothing. Three SLIM wave frequency and amplitude settings are evaluated for Tune Mix ion 922 (Arrival times)

	1 bin = 0.12 msec	922 (350.8) 20_40	922 (446.3) 15_30	922 (654.1) 20_30
Preprocessing	Total Width	%decrease	%decrease	%decrease
Raw	0	0.00%	0.00%	0.00%
D2	2	0.03%	0.61%	6.36%
D3	3	0.21%	1.07%	6.45%
D5	5	0.72%	1.29%	7.99%
D2d3	6	0.52%	0.95%	8.08%
D7	7	2.26%	1.86%	8.08%
D3d3	9	1.75%	1.18%	8.54%
D3d5	15	8.02%	4.24%	9.72%
D5d3	15	8.02%	4.92%	10.54%
D7d3	21	19.84%	10.60%	11.99%
D5d5	25	27.13%	13.66%	14.63%
D7d5	35	44.19%	27.16%	22.45%

Experimental

Experimental Parameters

Experiments were performed on a beta system combining a SLIM-based HRIM device (MOBILion Systems, Inc., Chadds Ford, PA) and a 6545 LC/Q-TOF (Agilent Technologies, Santa Clara, CA). A commercial LC (1290 Infinity II Series, Agilent Technologies, Santa Clara, CA) was used for sample introduction for both flow injection and a HILIC (RX-Sil, 3.0 x 100 mm, 1.8 micron, 360 µL/min flow rate) separation prior to SLIM-MS analysis. Agilent Tune Mix as well as lipid standards (Avanti Polar Lipids, Alabaster, AL) were run to evaluate performance. Figure 2a shows arrival time EICs for the three optimized SLIM settings[1] and Figure 2b shows the EICs for the LC separation.

Figure 2a.

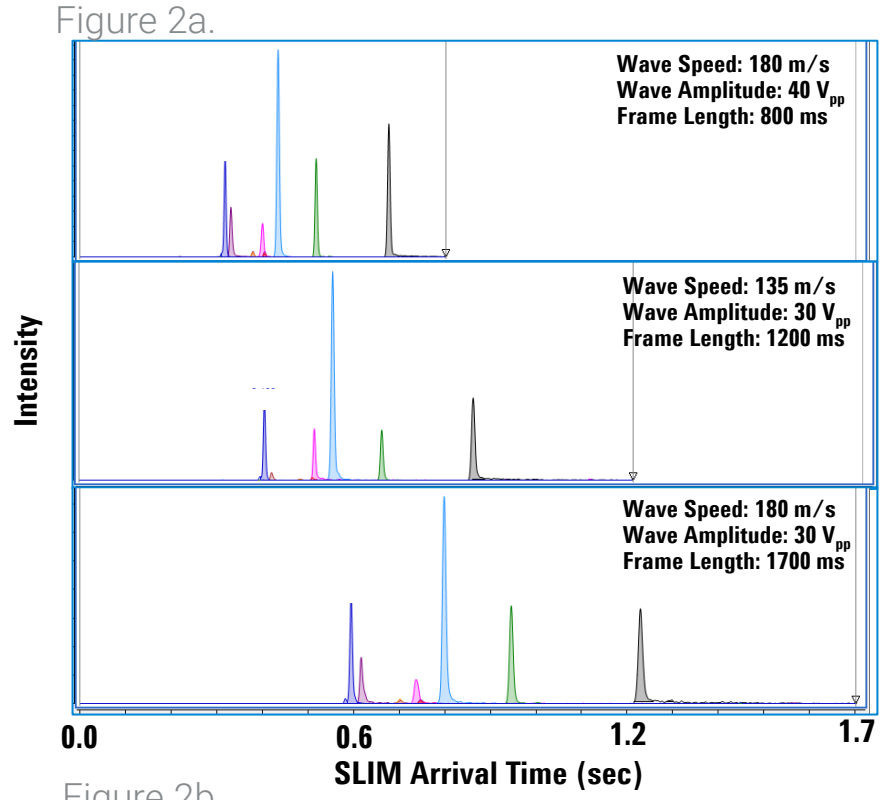

Figure 2b.

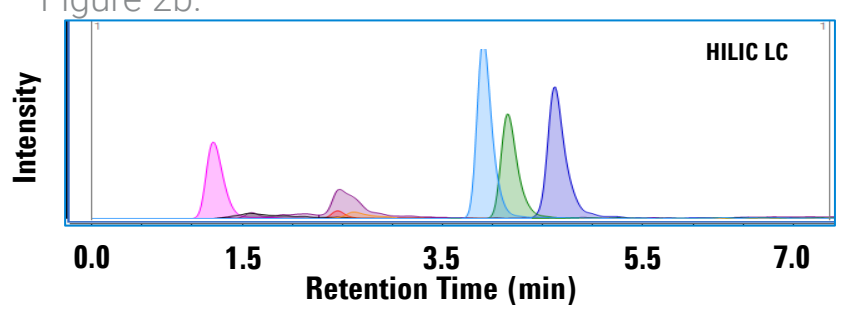

Figure 2. a) EIMs for flow injection and b) EIC for LC data

Data Analysis Parameters

The PNNL Preprocessor[2] was used to evaluate data pretreatment including IM frame (2D spectrum of IM and MS data) summing (1-55 frames), drift bin summing (1-7), drift and mass smoothing (3,5), thresholding (min20), and spike removal (1). These techniques improve peak shape and reduce data file size which improve the performance of targeted and untargeted data analysis. Skyline was used to evaluate targeted data extraction and Agilent IM-MS Browser was used to evaluate untargeted feature finding.

Peak Shape for Number of IM Frames Summed

The peak shape was evaluated for summing different numbers of IM frames. The peaks are shown in Figure 3 for an increasing number of frames (1, 3, 7, 14) for both raw (red) and preprocessed with D5d3 (blue) data. Preprocessing improves the peak shape when low numbers of IM frames are summed and provides insight into sampling and measurement timescales when applying SLIM to LC or other introduction systems.

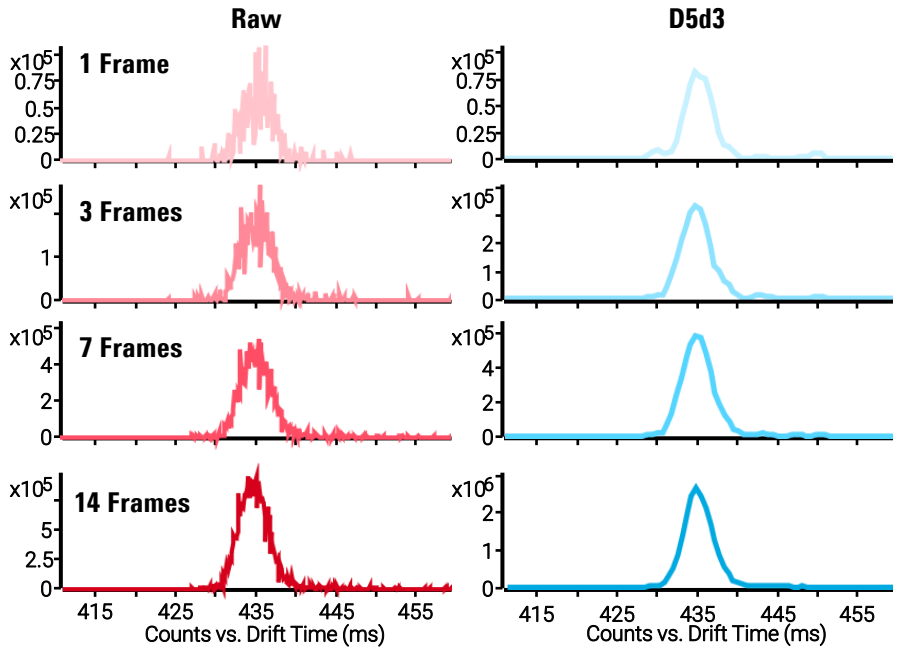

Figure 3. Peak shapes for different numbers of summed frames for raw and preprocessed data

Peak Shape for Flow Injection Data

A flow injection method was evaluated where the lipid sample was analyzed over a 2 min acquisition period. The data was acquired with a wave speed of 180 m/s and a wave amplitude of 40 V_{pp} and a frame length of 800 ms. The peak shape for both the PC 14:1_14:1 [M+H]+ and Ceramide 18:1d_18:1 [M+Na]+ are shown in Figure 4 with increasing levels of preprocessing. The Ceramide lipid is present at lower intensity and benefits more from preprocessing.

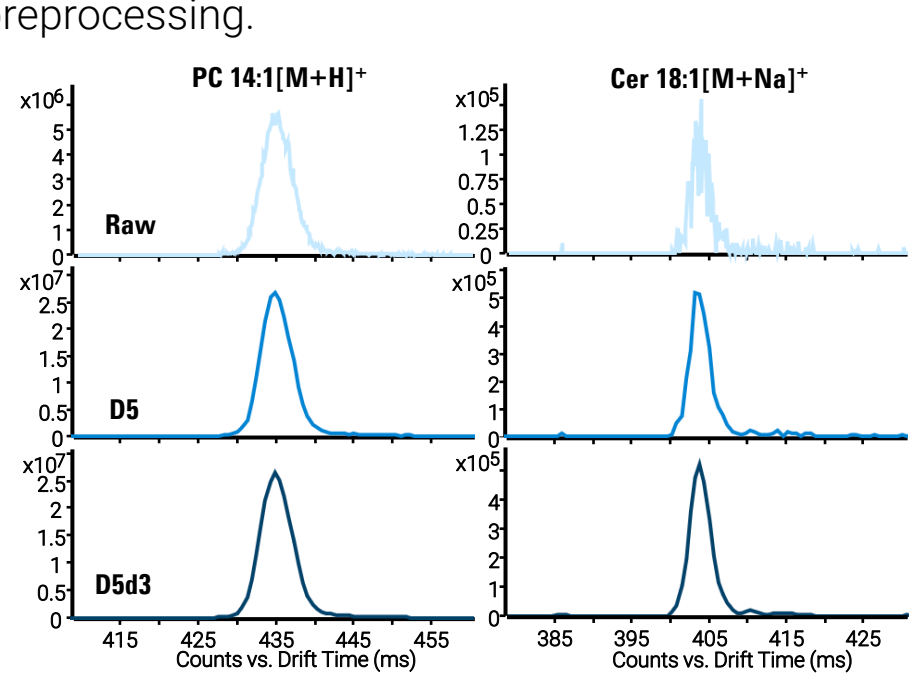

Figure 4. Peak shapes for PC 14:1 and Cer 18:1 with different processing levels

Targeted Extraction Results with Skyline

Flow injection SLIM-MS data files can be converted into LC-MS data files where the SLIM axis is mapped into the retention time space and then analyzed in SW packages that support LC-MS data such as Skyline. Different numbers of frames were summed together (1, 3, 7, 14) prior to retention time conversion to investigate the number of frames needed to generate enough data to extract the ion of interest. Data is shown in Figure 5 for both raw (top row) and preprocessed data (bottom row) to illustrate the benefit of preprocessing especially when fewer frames are available and extraction failed for the raw data at 1, 3, and 7 frames. Also note the increase in singal intensity when more frames are summed together.

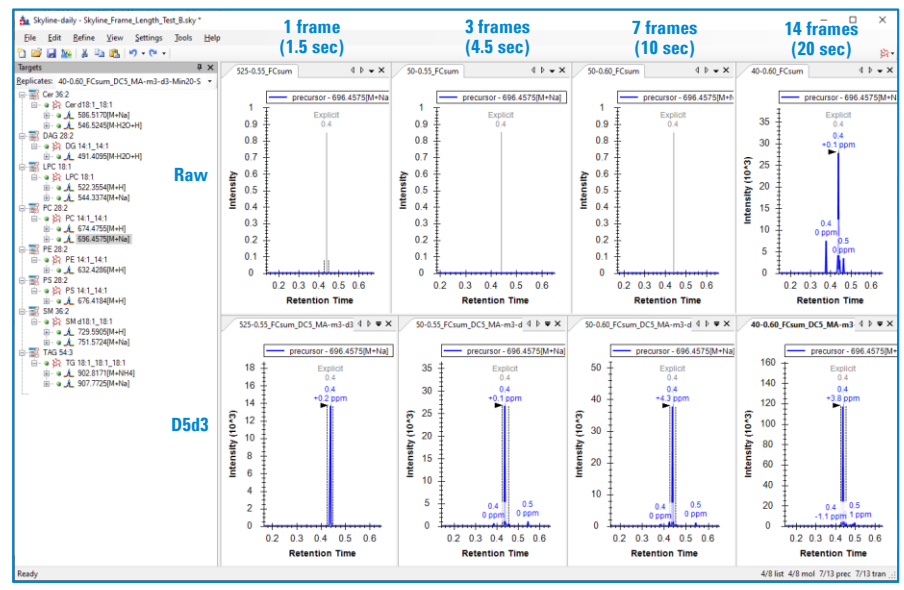

Figure 5. Skyline results for flow injection data across varying number of frames both raw and preprocessed

Skyline also supports 4D LC-IM-MS data. Results are shown in Figure 6 for raw, D5, and D5d3 preprocessed data and a reduction in peak noise. Targeted data extraction is possible for both raw and preprocessed data. The extracted window shown on the IM heat map is determined from the analytes' CCS which is reverse converted to SLIM arrival time based on the newly implemented polynomial calibration.

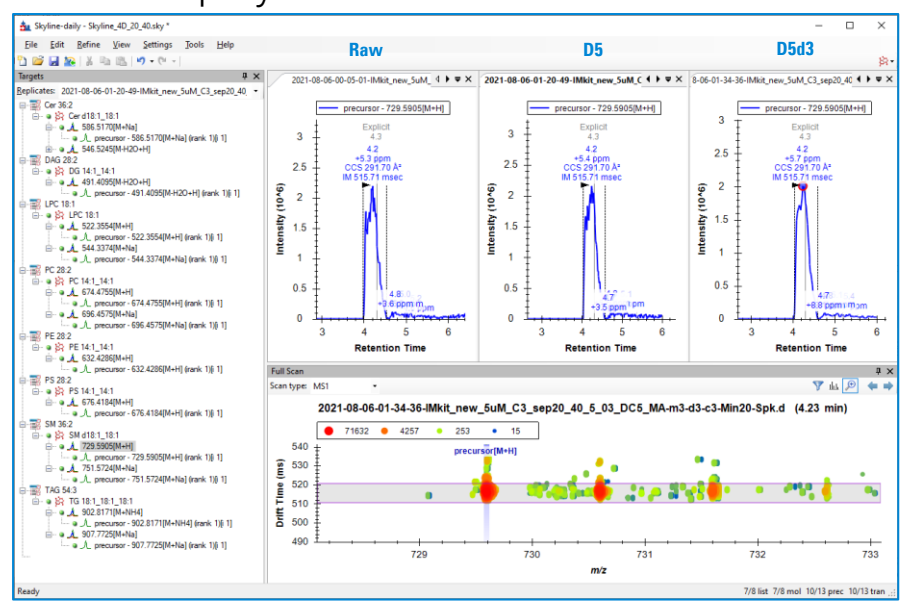

Figure 6. Skyline results for LC-IM-MS data

Results and Discussion

Untargeted Data Analysis for IM-MS Data

Data sets for untargeted feature analysis evaluation were acquired in triplicate for each instrument setting tested. The data files were subsequently processed with varying degrees of data preprocessing which are indicated in the following stacked bar charts with different shades of colors. First, untargeted feature finding results were evaluated for summing various numbers of IM frames. Figure 7 shows that as the number of frames decreases the lower intensity lipids are not found for the raw (red) data file. If preprocessing (blue) is applied, most of the lipids can be found down to summing only 7 IM frames.

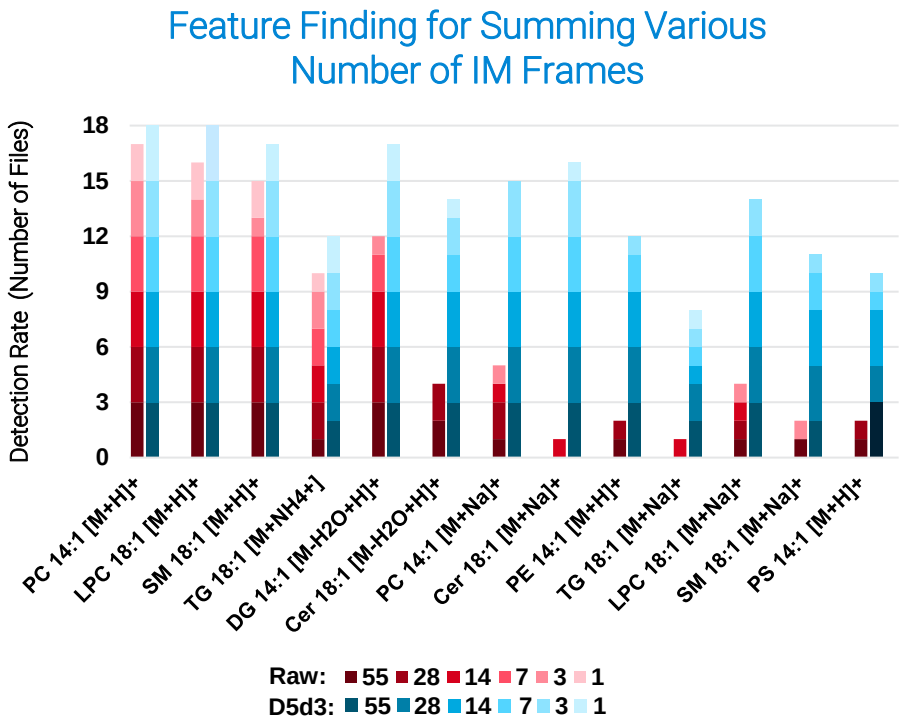

Figure 7. Feature finding results for IM-MS data across various frames of data summed for both raw (red) and preprocessed (blue) data

Figure 8 shows results for flow injection data for a wave speed of 180 m/s and amplitude of 40 V_{pp} with an IM frame length of 800 ms. The lipids are listed in order of decreasing intensity. For more intense lipids (PC $[M+H]^+$ and LPC $[M+H]^+$), feature finding was successful across all 15 data files. For less intense lipids, feature finding was more successful with increased drift summing and smoothing as shown by the darker blue shades below.

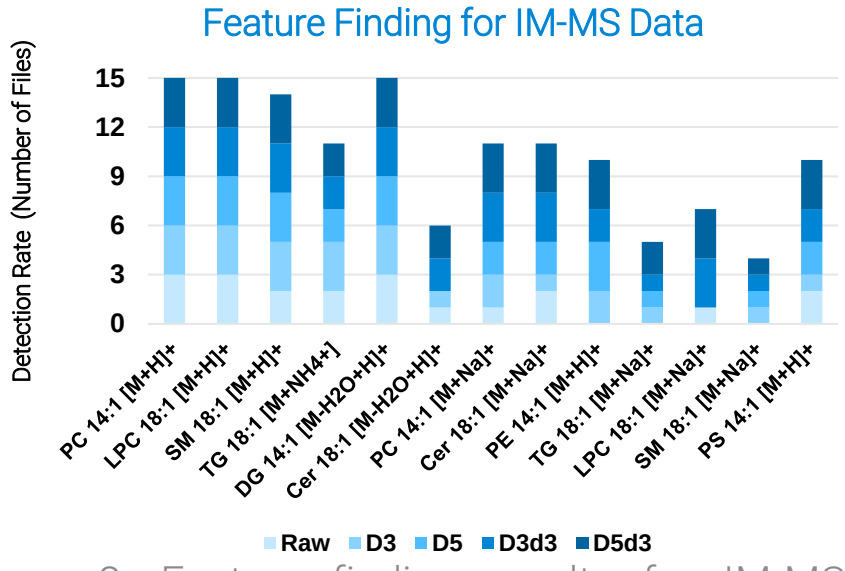

Figure 8. Feature finding results for IM-MS data across various preprocessing settings

Untargeted Data Analysis for LC-IM-MS Data

Feature finding was also applied to LC-IM-MS data across three different wave settings and frame lengths (800 (blue), 1200 (yellow), and 1700 (green) ms). Feature finding is more successful for lower concentration lipids when more preprocessing is used. A slight drop off in feature finding is observed for the longest frame length of 1700 ms for LC analysis.

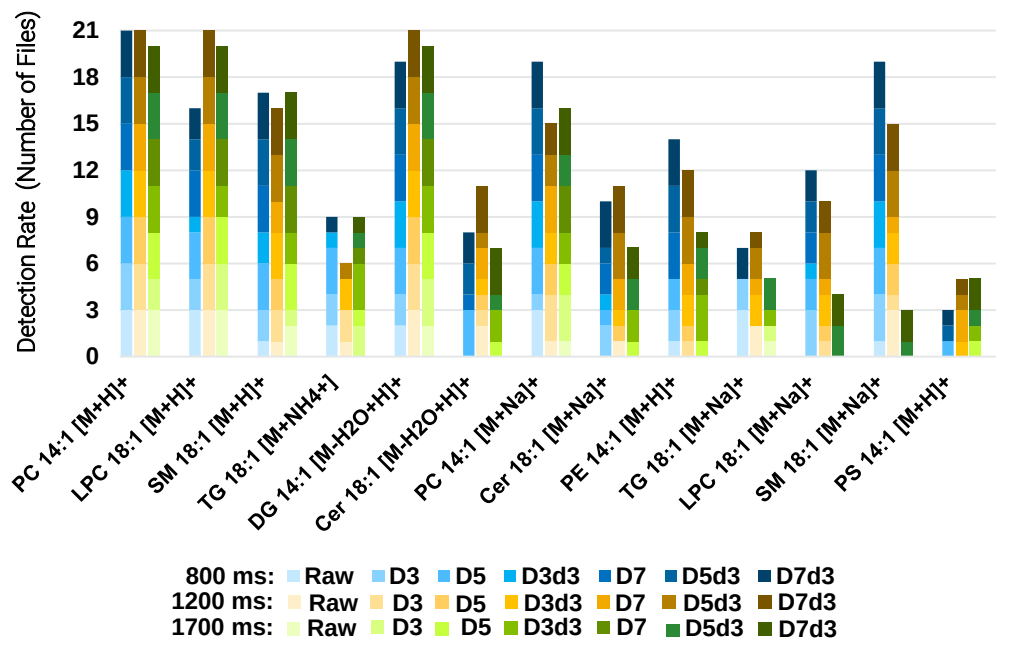

Figure 9. Feature finding results for LC-IM-MS data across various preprocessing settings

Conclusions

In this study we evaluated the benefit of data preprocessing for both targeted and untargeted data analysis for a novel SLIM-based HRIM device.

- Data preprocessing techniques such as drift bin summing and smoothing are critical for untargeted workflows
- Targeted analysis also benefits from data preprocessing techniques when ion statistics are low
- Increasing the measurement period and summing IM frames is key to improving detection limits when ion statistics are low

References

[1]J. C. May et. al. Resolving Power and Collision Cross Section Measurement Accuracy of a Prototype High-Resolution Ion Mobility Platform Incorporating Structures for Lossless Ion Manipulation. Journal of the American Society for Mass Spectrometry 2021, 32, 4, 1126-1137.

[2]A. Bilbao et. al. A Preprocessing Tool for Enhanced Ion Mobility-Mass Spectrometry-Based Omics Workflows. Journal of Proteome Research 2021.

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