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Evaluating Dynamic Range of High Resolution Demultiplexing of Drift Tube Ion Mobility – Mass Spectrometry Analysis

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Introduction

The coupling of ion mobility to time-of-flight mass spectrometry has made it possible to simultaneously acquire valuable information on molecular shape and mass which can be applied to both targeted and untargeted analytical workflows. Initial traveling wave (TWIMS) and low-pressure drift tube (DTIMS) technologies produce typical resolving powers of 40 and 50, respectively.

Trapped ion mobility-mass spectrometry (TIMS) can achieve resolving powers over 100, but at the cost of substantial reduction in the mobility range and acquisition rate. Multi-pass cyclic TWIMS also demonstrates high resolving power, but again with a commensurate loss in both the mobility range and speed of analysis. Recently an extended path traveling wave system (Structure for Lossless Ion Manipulation, SLIM) has achieved resolving powers as high as 300 while maintaining a wide ion mobility measurement range and acquisition rates of approximately 1 frame per second.

Extended Hadamard multiplexed DTIMS, developed at PNNL, offers important improvements in dynamic range and signal to noise became available with the Agilent 6560 in 2016. A high-resolution demultiplexing strategy for these data became commercially available in 2020 as HRdm 1.0.

HRdm-DTIMS is achieved through the simultaneous application of advanced demultiplexing and deconvolution techniques and has demonstrated resolving powers of over 200. Unique to HRdm is an increase in resolving power without loss of drift or mass ranges, nor any decrease in acquisition rate. This makes it ideal for untargeted workflows and maximum compatibility with UHPLC and CE.

Several advances in the HRdm method are reported here. These include increasing the effective TOF transient sampling rate via interpolation, new regularization techniques, and especially compensation for instrument function and distortions. The result is significant improvement in overall performance. Here we report on resolution of isomers with varying separation and relative abundance.

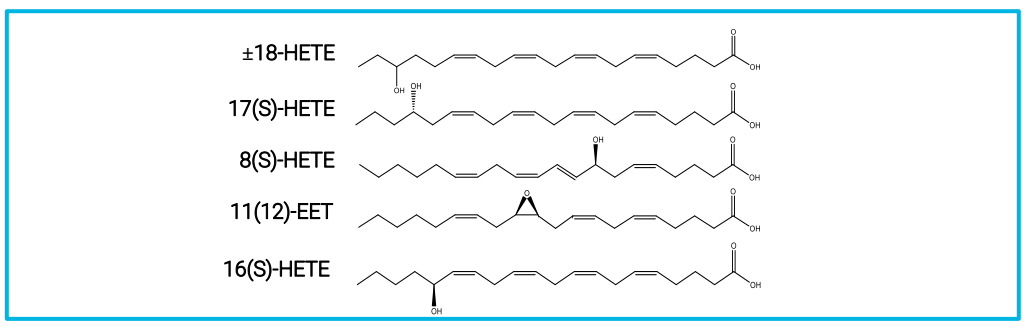

Figure 1. Isomer Structures

Experimental

Methods

Samples: Isomeric hydroxyeicosatetraenoic (HETE) and epoxyeicosatrienoic (EET) acid standards were paired based on their measured CCS differences of 1.7% (11(12)EET and 17(S)HETE), 2.7% (8(S)-HETE and ±18-HETE), 3.2% (±18-HETE and 16(S)-HETE).

Equal concentrations of each standard were prepared in LC-MS grade methanol, with the concentration of one isomer held constant while the other was serially diluted ranging from 1:1 to 1:25 (1.7% pair) 1:1 to 1:100 (2.7% pair) and 1:200 (3.2% pair) using the series 1: 1, 2, 4, 8, 16, 25, 50,100, 200.

LC: All experiments were performed on an Agilent 6560B IM-QTOF. Samples were introduced via flow injection through a stainless-steel union using an Agilent 1290 Infinity II UHPLC with a flow rate of 0.200 µL/min and an injection volume of 2 µL. Run time was 1.0 min. A mobile phase of 70% methanol in water was utilized for all standard analyses.

MS: The 6560B IM-QTOF was operated in positive ion 1700 m/z range dual gain mode with a maximum drift time of 60 ms with 20 IM-MS frames summed and saved each 1.2 sec. The drift tube entrance potential was 1200 V (12.5 V/cm) and 4-bit multiplexing using 3 msec trap fill and 0.2 msec gate open times.

Agilent Low Concentration Tune Mix was diluted 1:10 and infused for 1 minute under the same conditions.

Data Processing: Each data file was first processed with the PNNL PreProcessor[2] (beta 3.1 – 2021.04.21) using 1 to 3 drift bin interpolation – increasing the number of effective TOF transients sampling the drift separation – and subsequently demultiplexed.

Resulting data files were feature detected using IM-MS Browser (10.0) and features were exported as CSV files. HRdm 2.1.4 (beta) was used with default settings (high mode) to generate high resolution demultiplexed data files from the original multiplexed and demultiplexed data files, and the CSV feature lists.

High resolution data files were then CCS calibrated using a Single Field calibration created from the tune mix infusion run.

Both standard (PNNNL) demultiplexed and high resolution (HRdm) files were processed using IM-MS Browser's feature detection to determine a feature abundance for each isomer. These abundances where then evaluated in Excel.

Modeling and Assessing Resolution Requirements

The three isomeric pairs with drift/CCS separations of 1.7, 2.7 and 3.2 percent were modeled using a standard Gaussian peak envelope with both standard (55) and high resolution (200) conditions over a range of relative abundance from 1:1 – 1:100. Figure 2 shows one of the modeling results and Table 1 summarizes the observed valleys between peaks. At an HRdm resolving power of 200, 100% valley was observed for all isomer separations as shown in Figure 3.

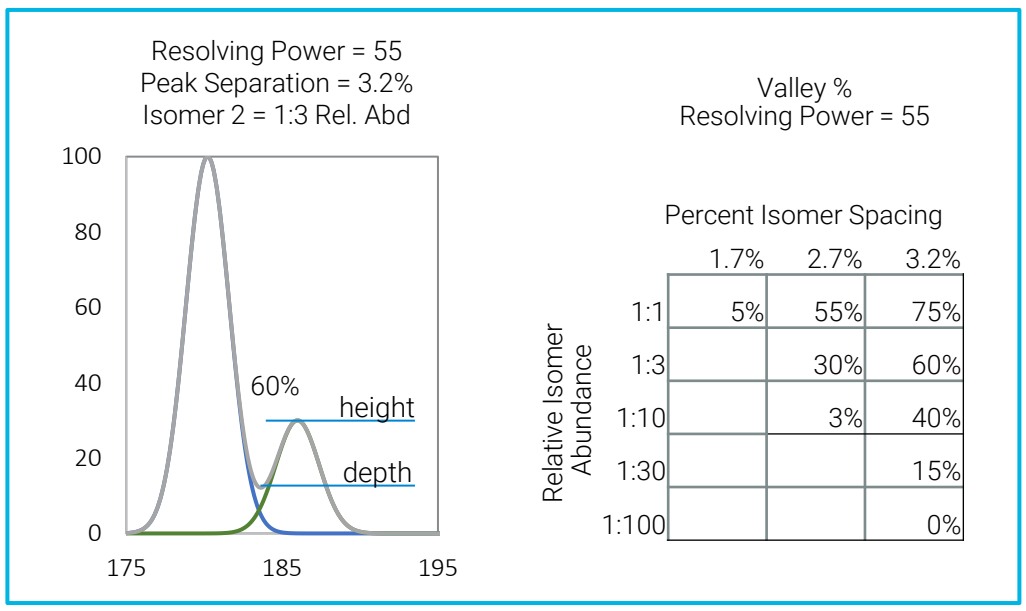

Figure 2. Valley depth between isomers. Valley % = 100 x depth / height of the lesser abundant isomer.

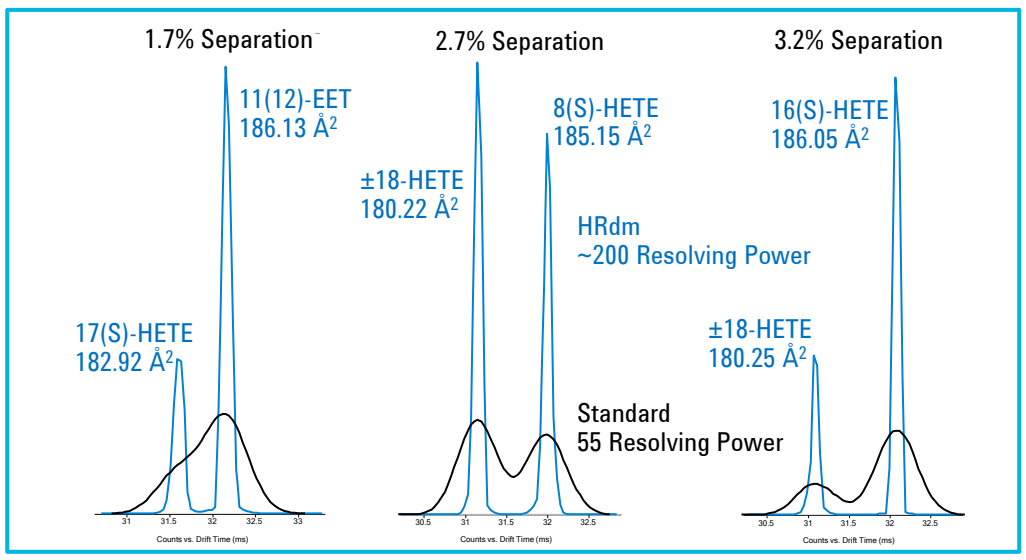

Figure 3. Peak shape and resolution for standard and HRdm demultiplexing

The modeling highlights how increased resolving power is important for even moderately well separated isomers in support of increasing dynamic range.

Results from Analytical Measurements

Experimental data was collected by analyzing each isomeric pair (1.7%, 2.7% and 3.2%) at relative concentrations ranging from 1:1 to 200:1 in triplicate. Both standard resolution and high resolution demultiplexed data sets were generated from the raw data files.

Responses for each isomer were determined using 4-dimensional feature finding, a standard feature of IM-MS Browser. The relative response of each isomeric pair was plotted against the relative concentration. With only limited exception we observed consistent relationships for all three isomer spacings.

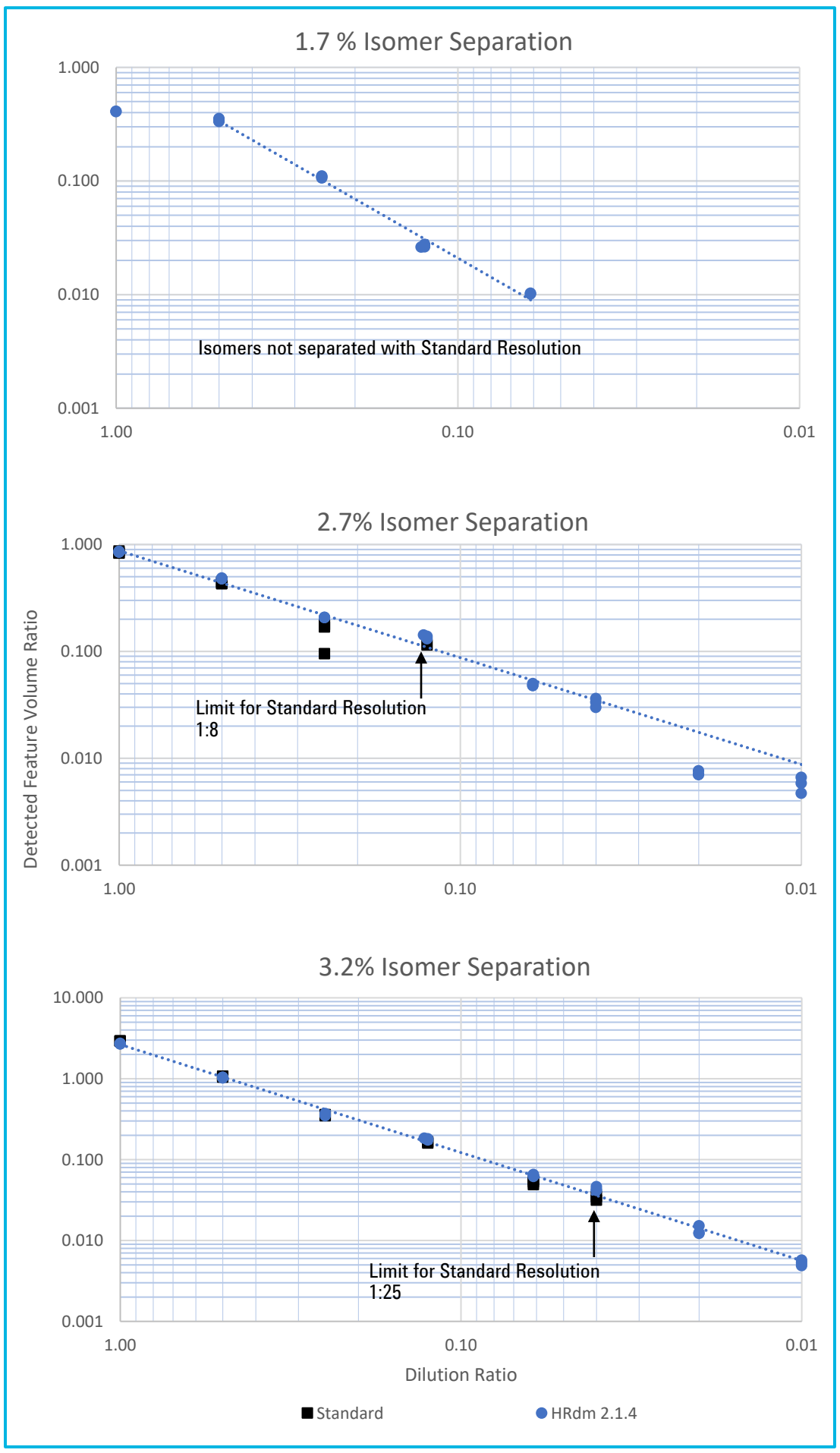

Figure 4. Response ratio for isomer with increasing relative dilution. Black points plot standard resolution, blue points plot high resolution results.

Results and Discussion

As anticipated from the resolution modeling, standard mode resolution was unable to separate and detect isomers spaced by only 1.7%. At 2.7% spacing separation and detection was possible over to a dilution ratio of 1:8. For the isomer pair that differs in CCS by 3.2% it was possible to separate and detect the isomer pairs with dilution ratio up to 1:25.

Performance was significantly improved when processing the data with high resolution demultiplexing. With an isomer spacing of 1.7%, individual isomer detection was achieved with dilution ratio up to 1:16. With 2.7% and 3.2% separation this increases to dilution ratios up to 1:100.

In addition to examining how HRdm contributes to isomer separation and detection we examined the resolving power and CCS assignment across the isomer relative dilution series. Figure 5 plots the average observed resolving power as a function of the relative dilution.

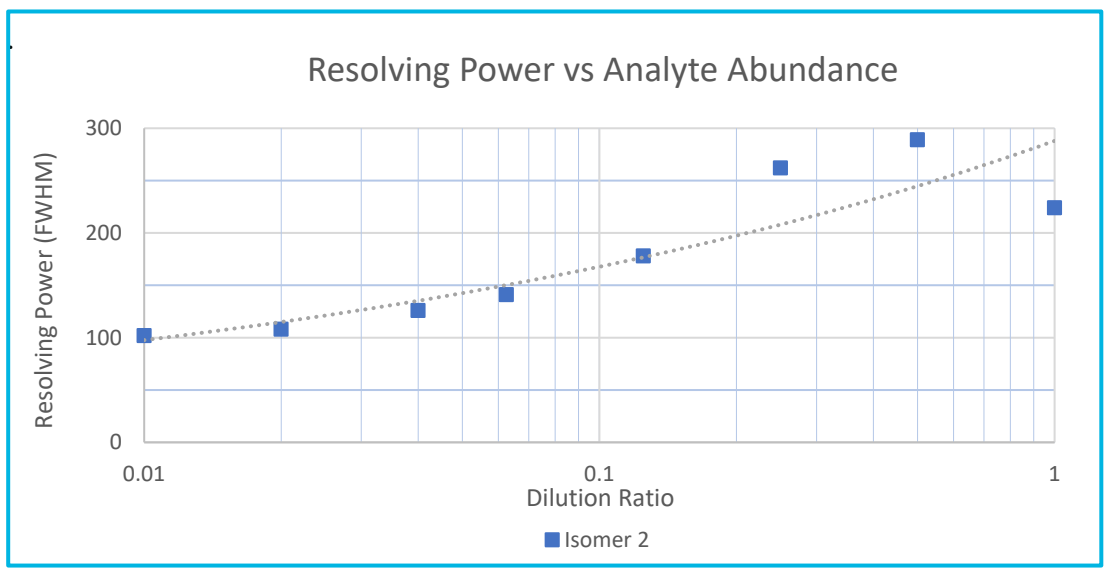

Figure 5. Average observed FWHM resolving power for HRdm data as a function of relative intensity.

The resolving power increase achieved by HRdm is a function of the available signal. This is seen in Figure 5, where resolving powers of the less abundant isomer drop from approximately 200 at high intensity to 100 at lower abundances. The resolving power of the higher abundance isomer remains unchanged (not shown).

Lastly, we examined the precision of CCS measurement over the relative isomer concentrations. Figure 6 shows the results over the full relative concentration range for the 3.2% separation. As the concentration of the diluted isomer decreases, there is a shift away from the more abundant isomer. For the isomers separated by 3.2% the maximum shift in CCS is 0.4%. For the isomer pair separated by 2.7% the maximum is 0.8% and for 1.7% the maximum is 0.9%.

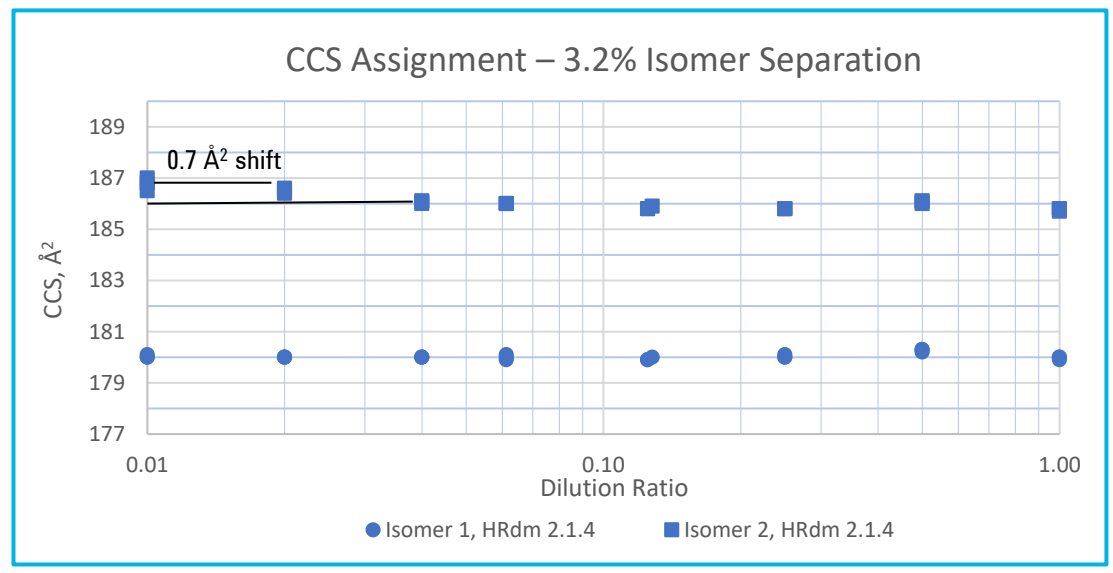

Figure 6. CCS assignment and reproducibility for wide range relative abundance.

In addition to enhanced drift peak resolving power we also observed improvements in peak fidelity. Occasionally small shoulder or artifact peaks previously observed are largely removed with updates to the instrument function, new regularization techniques, and compensation for small multiplexing distortions.

Conclusions

New algorithmic improvements to HRdm 2.0 make significant contributions to the analytical performance available to ion mobility-mass spectrometry.

- High resolving power without compromising data acquisition rates or ion mobility range in support of untargeted workflows.
- Extended dynamic range for the measurement of isomeric pairs.
- Both high and standard resolving power results can be obtained from a single run.

References

[1]Jody C. May, et. al. Resolution of Isomeric Mixtures in Ion Mobility Using a Combined Demultiplexing and Peak Deconvolution Technique. Anal. Chem. 2020, 92, 14, 9482–9492.

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

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