Improving Metabolite Identification During Imaging by Desorption Electrospray Ionization (DESI) Mass Spectrometry

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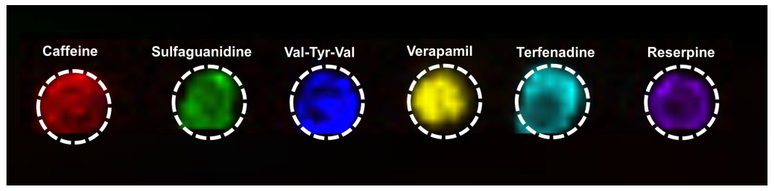
INTRODUCTION

- Desorption electrospray ionization (DESI) imaging mass spectrometry (IMS) excels at ionizing and imaging metabolites off tissue without any sample preparation such as matrix application.
- Lack of chromatographic separation or sample cleanup in IMS often confounds the identification of molecules mainly due to isobaric interferences.
- Here, we show DESI-IMS can provide confident identification by employing one of three strategies; (a) utilizing accurate mass with collision cross-section (CCS) as an orthogonal identifier for global metabolite profiling imaging, (b) using ion mobility MSMS to select a precursor ion to monitor a known product ion transitions for targeted imaging, (c) data independent acquisition (DIA) MS imaging with ion mobility separation, i.e., HDMS^E, to obtain fragmentation information.

METHODS

- **Tissue.** Rat brain was harvested and flash-frozen in liquid nitrogen (LN_2) before cryosectioning. Sections were thaw mounted on a glass slides, vacuum dried, and analyzed without any other sample preparation.
- **DESI Mass Spectrometer.** DESI imaging platform on a quadrupole time-of-flight ion mobility separation mass spec (e.g., SYNAPT G2-XS) was used for the imaging, mass drift was corrected by leucine-enkephalin lock mass and 98% Methanol and 2% Water with 0.1 % formic acid was used as DESI solvent.
- **Data Processing .** Processed by High Definition Imaging (HDI) 1.5.
- **CCS.** Drift time was calibrated for CCS values in electrospray mode using a compound mixture with known CCS values (Major Mix IMS/Tof Calibration Kit. Waters # 186008113), drift times values in the tissue in HDI was converted to CCS values.

ACCURATE MASS & CCS



	Caffeine	Sulfaguanidine	Val-Tyr-Val	Verapamil	Terfenadine	Reserpine	LeuEnK
Accurate mass	195.0883	215.0598	380.2179	455.2919	472.3223	609.2819	556.2773
Theoretical mass	195.0882	215.0603	380.2185	455.2910	472.3216	609.2812	556.2771
Mass error (ppm)	**0.52**	**-2.32**	**-1.58**	**1.98**	**1.48**	**1.15**	**0.36**
DESI exp CCS (A^2)	136	144.6	191	208.3	227.1	249.4	227.8
Ref CCS value (A^2)	138.2	146.8	191.7	208.8	228.7	252.3	229.8
% CCS difference	**-1.51**	**-1.52**	**-0.28**	**-0.14**	**-0.61**	**-1.08**	**-0.8**

Figure 1. *A series of small molecules with the known CCS value were imaged by DESI. The CCS values were calculated for the standards from their drift times showed less than 1-2 % error within 2 ppm mass accuracy for more confident identification.*

CONCLUSIONS

- Confidence in molecular identification of ions during MS imaging can be improved by utilizing collision cross-section (CCS) with accurate mass.
- Clarity of fragmentation using either MSMS or data-independent acquisition (DIA) MS imaging, such as DESI MS imaging, can be improved by employing ion mobility separation.

RESULTS & DISCUSSIONS

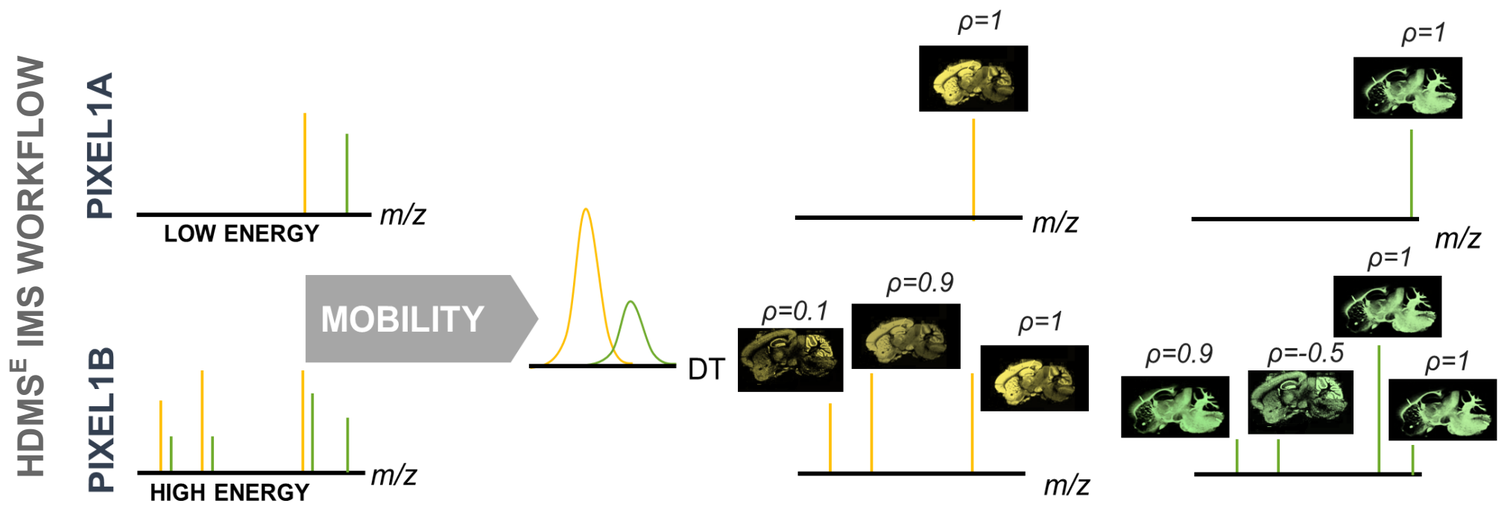


Figure 2. *HDMS^E is a data-independent acquisition method, where two sister functions, one at low energy giving molecular profile and another high energy with fragmentation information, are acquired for each pixel. Two functions are aligned by matching the precursor ion in the low energy function with the remnant precursor ion in the high energy function, drift time window, and spatial correlation (ρ) of fragments with their precursors.*

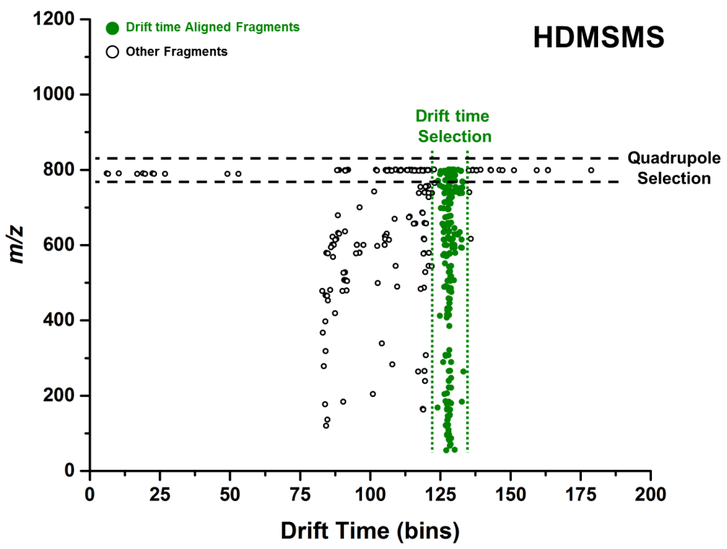


Figure 3. *The mobility plot of ion mobility MSMS or HDMSMS is shown here. In HDMSMS, ions are first filtered by quadrupole based on its m/z value and then separated in ion mobility cell to remove precursor and fragmented.*

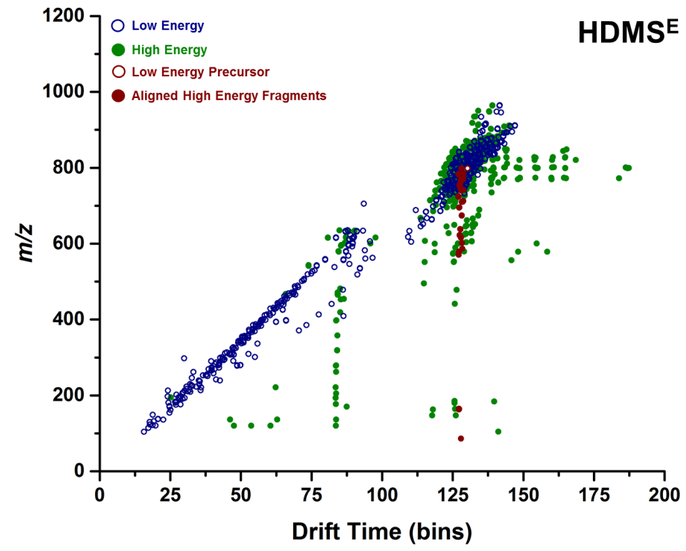
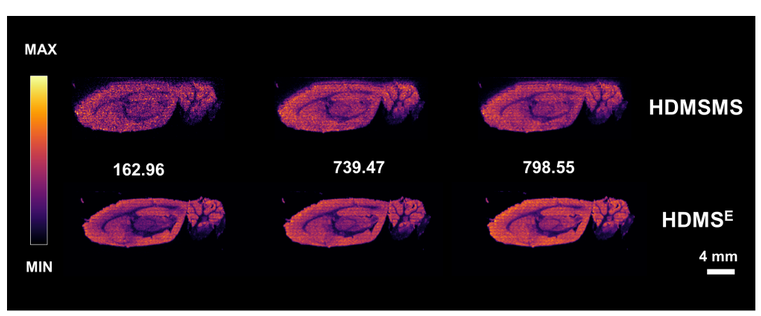


Figure 4. *The mobility plot of data-independent acquisition, HDMS^E, is shown. HDMS^E allows getting fragmentation information along with the profile for more confident identification by mobility and spatial alignment of fragment ions with its precursor.*



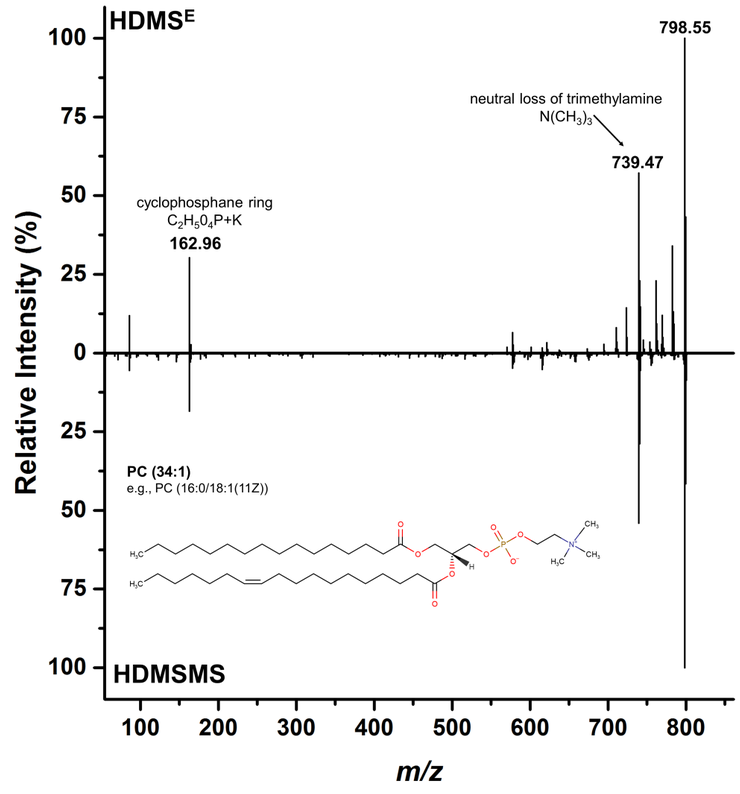


Figure 5. *A comparison of fragmentation information obtained from HDMSMS and HDMSE for a lipid ion, m/z 798.55, is shown using a mass spectra mirror plot. Both used the same drift time window for alignment of the precursor with its fragment ions. Both HDMSE and HDMSMS was successful at giving a similar fragmentation pattern for major fragment ions. The inset at the top shows the acquired images. DESI HDMSMS was run on the same sample after HDMSE DESI IMS acquisition*

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