COLLISION CROSS SECTION ENABLED DESI ION MOBILITY MASS SPECTROMETRY IMAGING

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INTRODUCTION

Mass spectrometry imaging (MSI) determines the localization of compounds on samples, including tissue sections and drug delivery systems.

Desorption Electrospray Ionization (DESI) provides a complimentary direct sampling technique to traditional MALDI MSI methods. DESI MSI integrated with ion mobility separation based on structure/shape differences (HDMS Imaging) improves peak capacity, confirms target ion identification, and adds fragmentation specificity.

Collision cross section (CCS) is a fundamental property available from ion mobility spectrometry (IMS) that reflects the molecular structure; therefore, combining CCS with accurate mass increases the confidence in compound identification, particularly in matrices (like tissues during MS imaging) where no chromatography is used.

The workflow for obtaining CCS values from DESI HDMS Imaging experiments is provided. Benchmarks against CCS reference standards and endogenous tissue peaks from mouse brain sections demonstrate that experimental CCS values differ by < 2% of the literature values.

SAMPLES

Waters reference and calibration standards:

Leucine-Enkephalin lock mass (P/N 186006013)

- 400 ng/µL in water

Major Mix ion mobility/ToF CCS calibration standard (P/N 186008113)

- Multiple components in acetonitrile;water solvent

Standards for CCS benchmarks:

Caffeine, sulfaguanadine, Val-Tyr-Val, verapamil, terfenadine, reserpine (Sigma Aldrich),

- Stock solutions (1 mg/mL) in methanol or 80:20 methanol:water
- Dilutions to 5 to 50 ng/µL in methanol
- 1 µL spotted on Prosolia 24-well sample plate

Tissue sample for Mass Spectrometry Imaging:

Fresh-frozen 15 µm thick section of mouse brain on standard glass slide

METHODS

Ion mobility-mass spectrometry (HDMS) MSI

Source:	Waters modified 2D DESI stage (Prosolia, US)
Mass Spectrometer:	SYNAPT G2-S*i* ion mobility QToF (Figure 1).

DESI conditions:

- 95:5 methanol:water with 0.1% formic acid (v) at 3 µL/min
- Nebulizing gas pressure of 4.5 bar nitrogen
- 2.5 kV sprayer voltage

Polarity:	Positive
Mass range:	50 -1,200 m/z; 0.5 s per MS scan
MS Imaging Pixel size:	100 µm

Data management

MSI data were acquired using MassLynx 4.2. Experimental parameters were defined, raw files processed, and HDMS imaging data visualized using High Definition Imaging (HDI) 1.4 software. CCS values were calculated in MS Excel using exported peak fit data from HDI 1.4.

MASS SPECTROMETRY IMAGING

Ion mobility-mass spectrometry instrumentation for MS Imaging

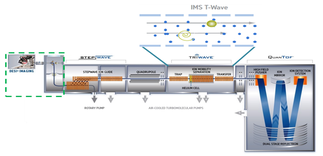


Figure 1. Schematic of the DESI SYNAPT G2-Si QToF mass spectrometer with ion mobility shape/structure separation prior to ToF MS

Mass Spectrometry Imaging (MSI) basic concept

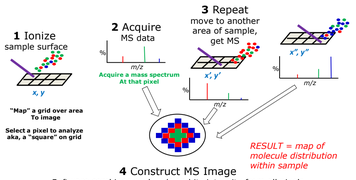


Figure 2. Illustration of how to do Mass Spectrometry Imaging (MSI).

DESORPTION ELECTROSPRAY IONIZATION MS IMAGING SOURCE

- A shower of charged ESI solvent droplets focuses into a beam by a high pressure gas flow (N_2)
- The beam washes the surface to desorb the analytes on the sample.
- Desorbed analytes were then ionized and carried into an inlet capillary that transferred the ions to the MS for analysis.

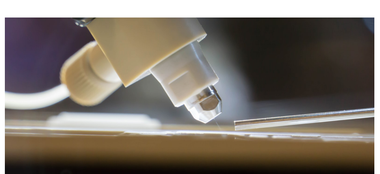


Figure 3. DESI as an ion source for MS Imaging (Photo courtesy of Samuel Dapore-Schwartz, Waters Corp.)

ION MOBILITY SPECTROMETRY

Shape/structure separation with ion mobility before MS

- Ion mobility separation is based on an ion's structural size and shape so it is a complementary separation method to MS.
- Traveling Wave IMS (TWIMS) in the SYNAPT uses a DC pulse moving down a series of elements like a wave as seen in Figure 4, guiding the ions against a counter flow of neutral gas (nitrogen here).
- The larger, bulkier molecular structures (red) will not move as easily through the gas flow as smaller, more compact ones (yellow).

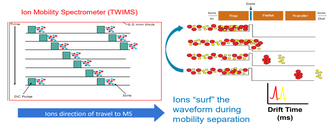


Figure 4. Ion mobility separation using traveling wave IMS (TWIMS)[1]

COLLISION CROSS SECTION (CCS)

Determining the CCS which is a fundamental property of the ion

Ion mobility drift is related to the ion's average rotational cross section, i.e., the average "target size" of the ion colliding with the drift gas.

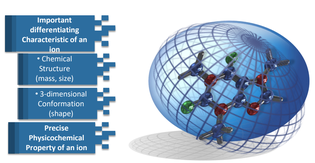


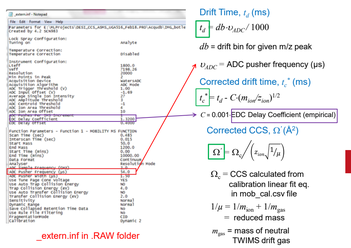
Figure 5. Rotationally averaged CCS measured by IMS

1. WORKFLOW - MEASURING CCS

Figure 6. Workflow for generating list of accurate mass m/z and drift bins from DESI MS Imaging data with ion mobility (HDMS Imaging)

2. WORKFLOW - CALCULATING CCS FROM MS IMAGING

Calculating the CCS from TWIMS Drift Time and Accurate Mass[2,3]



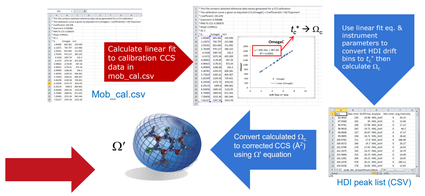


Figure 7. Use the equations to the left with the linear fit equations from mob_cal.csv to convert the drift bins in HDI peak list to corrected CCS in Excel

RESULTS

Benchmarking DESI HDMS Imaging CCS calculation for standards

Figure 8 shows DESI HDMS images for a series of CCS reference standards spotted onto a well plate. The adjusted CCS values were calculated from the imaging data using the workflow above. These CCS values agree with the known values to better than 2% with this method.

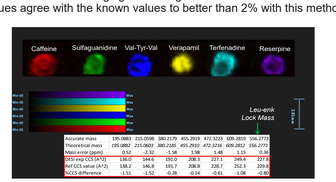


Figure 8. Adjusted CCS values in Å² determined from DESI HDMS Imaging using the workflow presented.

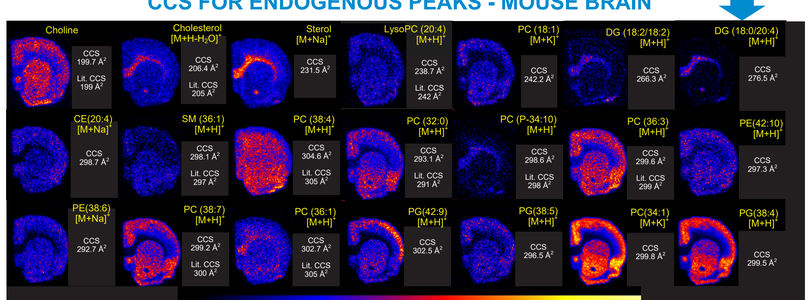
RESOLVING ISOBARIC PEAKS WITH ION MOBILITY

- In Figure 9 (right), database search gave the same identification for the left and right pairs of peaks because their accurate mass m/z alone matched the database m/z value to < 4 ppm.
- However, the pairs of ions shown had very different CCS, indicating that they are, at minimum, different structures and possibly different species.
- MS resolving power of $m/\Delta m$ as high as 870,000 would be required to differentiate these ions.
- The ions are all easily resolved on a standard QToF with ion mobility pre-separation before the MS analysis.

DESI HDMS IMAGING OF MOUSE BRAIN TISSUE

- Numerous endogenous lipids and metabolites, were detected on tissue as shown in the gallery below for a representative set of the types of compounds detected.
- CCS calculated using the current workflow agreed with the literature values[4,5] shown to better than 2%, identified using Metlin and LipidMAPS.

CCS FOR ENDOGENOUS PEAKS - MOUSE BRAIN



3. WORKFLOW - TENTATIVE PEAK ID

Finding tentative peak identifications for MS Images

Tentative peak IDs can be obtained for peaks observed in the MS Imaging data by searching exported accurate mass m/z .csv file from HDI vs. online databases within search criteria of ± 10 mDa.

RESOLVING ISOBARIC PEAKS WITH ION MOBILITY

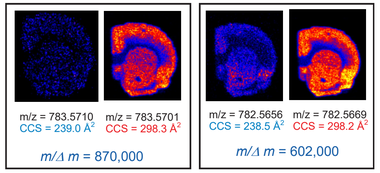


Figure 9. Ion mobility spectrometry resolution of isobaric structures through differences in CCS.

CONCLUSIONS

- **A simplified workflow was illustrated for determining corrected CCS from DESI MS imaging data with ion mobility enabled.**
- **Drift bin and accurate mass m/z values from peak fitting in HDI and the CCS calibration parameters in mob_cal.csv were used in Excel to calculate the CCS for ions imaged.**
- **CCS values calculated with this workflow agree to better than 2% for calibration standards and known lipids in literature.**
- **CCS values for the abundant lipids and other endogenous compounds were determined, some of which are not readily available in the literature.**

References

1. Waters Pub. #720004176EN, April 2012.
2. Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Anal. Chem.* **2010**, *82*, 9557.
3. Pagel, K.; Natan, E.; Hall, Z.; Fersht, A. R.; Robinson, C. V. *Angew. Chem. Int. Ed.* **2013**, *125*, 379.
4. Paglia, G., et al. *Anal. Chem.* **2015**, *87*, 1137.
5. Paglia, G., et al. *Anal. Chem.* **2014**, *86*, 3985.

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