ADVANTAGES OF HIGH RESOLUTION ION MOBILITY ON THE CYCLIC IMS FOR DESI MASS SPECTROMETRY IMAGING

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INTRODUCTION

Desorption DESI™ is a powerful and sensitive MS ionisation technique for the profiling and imaging of metabolites and lipids direct from unmodified complex biological samples, such as mammalian tissue sections. However, the direct analysis of small molecules can be challenging due to the structural diversity and isobaric nature of these types of compounds. Ion mobility separation (IMS) has proven to enhance system peak capacity, improve specificity and separate structural isomers. Here we describe the implementation of DESI mass spectrometry imaging (MSI) where ion mobility separation was improved using a multi-pass cyclic IM travelling-wave device where the number of passes around the device can be scaled to improve resolution.

METHODS

Sample preparation

Experiments have been carried out on porcine liver and mouse brain tissue sections, produced using a cryotome and deposited on a standard microscope slide and preserved at – 80 °C until analysis by mass spectrometry.

DESI XS Conditions

Solvent delivery:	2 µL/min 98:2 methanol:water
Voltage:	0.6 kV
Nebulizing gas:	Nitrogen at 10 psi (0.7 bar)
Pixel size:	100 µm
Sprayer	New High-Performance Sprayer

MS Conditions

MS Instrument: **SELECT SERIES Cyclic IMS**
Ionization mode: DESI positive and negative ion mode
Source temperature: 100°C
Data were mined using MassLynx™ V4.2, modified DriftScope™ V2.9 and High Definition Imaging (HDI™) 1.6 software for image visualization.

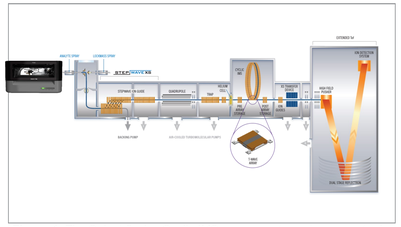

Figure 1. *The Select Series Cyclic IMS mass spectrometer geometry is illustrated here.*

RESULTS

A) Increased ion mobility separation with multi-pass DESI imaging analyzing mouse brain tissue sections.

Initial DESI imaging experiment was carried out on mouse brain tissue section with the cIM performing a single pass of ions from *m/z* 50-1,200. In a second experiment, the cIM conditions were optimised to allow the maximum of passes for the separation of phospholipids m/z 750-850 which was 7 passes.

Interrogation of the resulting data highlighted numerous examples where the power of the increased ion mobility separation enabled partial or baseline separation of isobaric species and showed different and distinct localisation in the mouse brain (figure 4).
Transfer MS/MS experiments were also conducted for the identification of the different lipids where the IMS was needed to allow the separation of the isobaric lipids (figure 5).

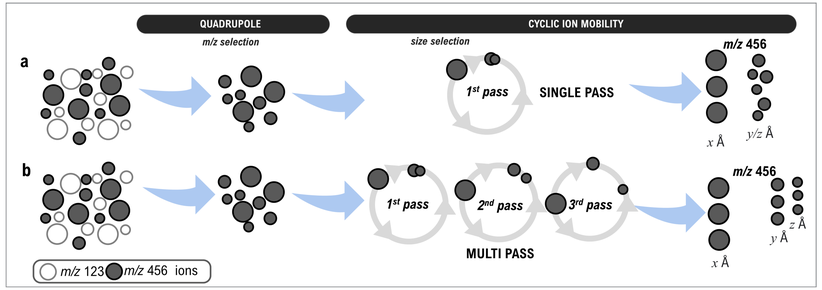

Figure 2. *A diagram showing separation of two sets of hypothetical ions at m/z 123 and 456, where each m/z consists of three populations of ions of different collision cross section (CCS). Firstly, (a) m/z 456 is selected by the quadrupole and using a single pass of the cyclic IMS device, one mobility-resolved conformer is observed with two additional overlapping species. After quadrupole selection and multiple passes of the cyclic IMS device (b) the three populations from m/z 456 with differing CCS were resolved.*

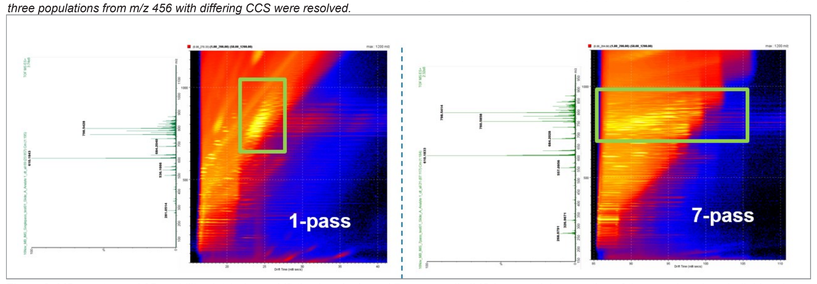

Figure 3. MS spectra and 2D mobility plot (m/z vs. drift time) a single and 7- pass cyclic IMS has partial separation of the complex phospholipid region.

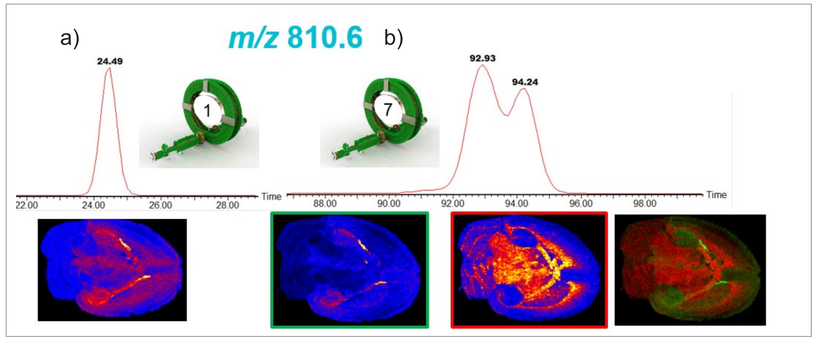

Figure 4. (a) In single pass DESI imaging, for m/z 810.6, a single mobility peak was observed with a drift time of 24.49 ms showing the ubiquitous localization throughout the brain tissue section. (b) Two conformers were observed for same m/z value after seven passes, at drift time 92.93 and 94.24 ms showing distinct distribution of two molecules within the mouse brain.

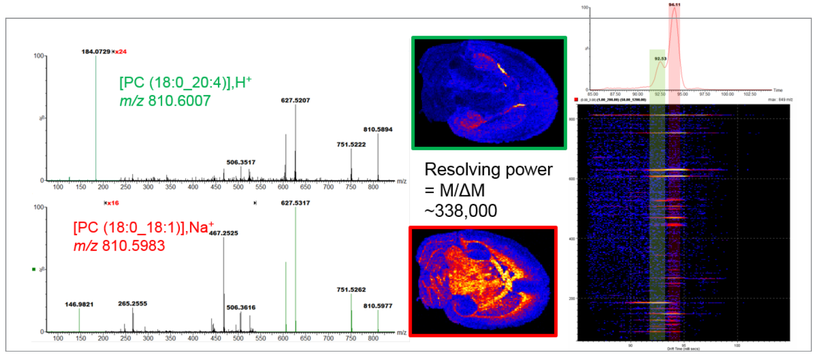

Figure 5: MS/MS of m/z 810.6 after seven passes in cyclic IMS can be aligned to two detected peaks. Based on fragmentation, the first peak can be assigned to a protonated PC (18:0_20:4) with the accurate mass of 810.6007 with chemical formula of $C_{46}H_{84}NO_8P$. The second one is identified as a sodiated PC (18:0_18:1) with the accurate mass of 810.5983 and chemical formula of $C_{44}H_{86}NO_8P$. The mass difference between the two isobaric species are 2.4 mDa apart and the theoretical mass resolving power needed to discriminate them is 338,000 Da.

B) Separation of bile acid conjugate isomers in ESI (standards) and DESI imaging (porcine liver tissue sections)

Bile acid conjugates (BA) are important participants in the absorption of lipids. Analysis is difficult because of their isomeric structure. For example TDCA and TCDCA at *m/z* 498.2889 gave similar MS/MS spectra when standards were analysed by ESI (figure 6). For direct analysis experiment such as DESI imaging, high resolution IMS was necessary to differentiate the isomers. From 13-pass in the cyclic IMS, a separation in IMS is observed with an appropriate drift time that was compatible with the DESI imaging timing (figure 7).

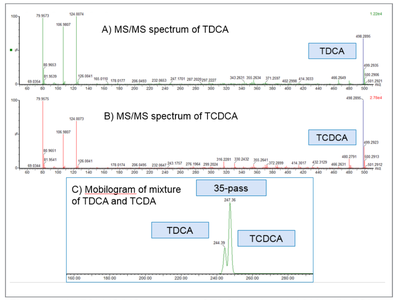

Figure 6. (A) ESI MS/MS spectrum of TDCA (m/z 498.2889). (B) ESI MS/MS spectrum of TCDA (m/z 498.2889), showing similar MS/MS spectra. (C) Extracted mobilogram of the mixture TDCA and TCDA almost baseline separated after 35 passes within the high resolution cyclic IMS.

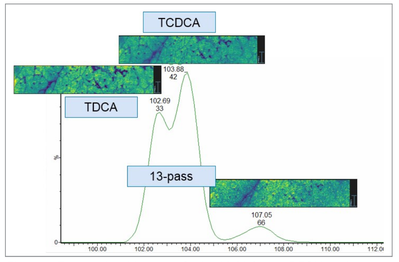

Figure 7. Extracted mobilogram of m/z 498.29 from a DESI imaging cyclic IMS experiment with 13-pass acquired from a porcine liver section, showing the ion images of isomers TDCA (drift time of 102.69 ms) and TCDA (drift time of 103.88 ms).

CONCLUSION

- **DESI imaging workflow is compatible with Cyclic ion mobility for single pass and multi-pass image acquisitions.**
- **Multi-pass ion mobility separation in Cyclic MS increased the peak capacity of the DESI imaging experiment**
- **High-resolution ion mobility separated isobaric lipid species and isomeric bile acid conjugates.**
- **Tandem MS performed after multi-pass ion mobility separation was able to provide characteristic fragmentation pattern for separated ions leading to more accurate and confident identification and localization of the molecule.**

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