High Resolution Imaging of Adrenal Glands by DESI-MS

Lisa Reid[1], Martin Palmer[1], Sheba Jarvis[2], Charlotte Bevan[2], Mark Towers[1], Emmanuelle Claude[1], Lee Gethings[1], Robert Plumb[3], Steven Lai[3]
1: Waters Corp, Wilmslow, Cheshire, UK; 2: Imperial College London, Hammersmith Hospital, London UK; 3: Waters Corp, Miford, MA, United States

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Introduction

The adrenal glands are essential endocrine organs, lipid rich they provide cholesterol substrate for glucocorticoid, mineralocorticoid and androgens synthesis. These hormones play a vital role in normal physiology, cardiovascular, metabolic health and fertility.

Adrenal malfunction can be caused by many different factors these include: genetic (e.g. congenital adrenal hyperplasia), environmental damage (e.g. Cushings syndrome), autoimmune disease (e.g. Addisons disease), hormone disruption (e.g. pituitary tumors) or by adrenal tumors[1].

The physiology of the adrenal gland (Figure 1) shows two distinct regions with a potential third region (unconfirmed):

- the inner medulla seen as a darker shading on the optical image and a Red coloration on the mass spectrometer overlaid image.
- the cortex seen as the thick grey tissue surrounding the medulla in the optical image and with a Blue coloration on the mass spectrometer overlaid image.
- possibly the adrenal capsule or the outermost layer of the cortex is seen as a light grey membrane surrounding the cortex on the optical image and a Green coloration on the mass spectrometer overlaid image.

By visualizing the structure and composition of adrenal glands we hope to gain a greater understanding of how aberrations in adrenal function may be associated with changes in lipid composition of the tissue.

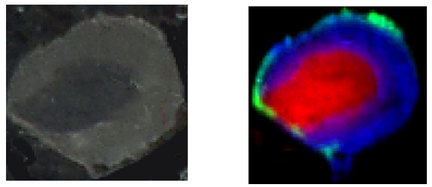


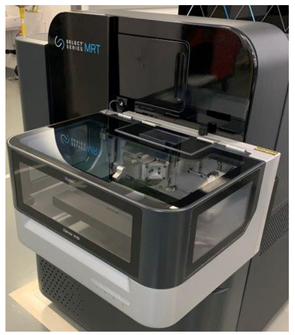
Figure 1: (Left) Showing an optical view of a sectioned adrenal gland, (Right) showing a mass spectrometer overlaid (RGB) compound localisation map.

Here we report results of a small feasibility study: using DESI imaging to investigate compound localization within the tissue regions of a normal mouse adrenal gland, in advance of studies into adrenal disease. The tissue sections have been analyzed on a conventional QToF mass spectrometer as well as a multi-reflecting ToF instrument to assess the application on each instrument type.

Experimental

Healthy wild type murine adrenal glands were cryo-sectioned onto a glass slide at a thickness of 15 µm.
The sections were analyzed by DESI-MS on a multi-Reflecting-QToF (SELECT SERIES™ MRT mass spectrometer) as well as a conventional QToF (SYNAPT™ G2-XS mass spectrometer).

Acquisitions were performed in full scan MS. The conventional QToF acquisition was performed at 25µm pixel size and 2 scans per second. The MRT acquisition was performed at 50µm pixel size and 2 scans per second.



The DESI-XS source fitted with a High Performance (HP) sprayer was set up with: 95:5% Methanol:Water solvent, 5 bar gas flow, 0.7kV capillary voltage, and an ambient transfer line.

Data were processed using High Definition Imaging (HDI™) imaging software to generate regions of interest (ROI) and peak lists. These were exported into the statistical software package Metaboanalyst to highlight marker compounds for each region of the adrenal gland. Strong biological marker compounds were then visualized using HDI.

Putative identifications were performed by exporting the ROI spectra into MassLynx™ software where they were centroid to achieve a monoisotopic peak mass. The accurate mass was taken into the elemental composition tool to provide potential compound compositions and ppb mass error calculations. The elemental compositions were then searched through suitable databases (LIPID MAPS® Lipidomics Gateway website).
Additional confirmatory database searches were performed within the lipid imaging software Lipostar™. MSI (Molecular Discovery).

This feasibility study demonstrates DESI-MS as a suitable application for imaging adrenal glands.
A peak list was generated for each tissue region using a region of interest (ROI) tool. These ROIs were imported into a statistical analysis software for discovery of target marker compounds (Figures 3 & 4). Unsupervised PCA plots showing separation by region for the murine adrenal gland are shown: non-scaled (A), Pareto scaled (B), and the loadings plot for this analysis (C). Data generated from both mass spectrometers can be processed in this way to highlight features of interest.

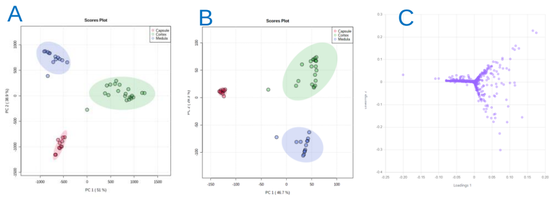


Figure 3: Statistical analyses of the three tissue regions, for a wild type murine adrenal gland. An unsupervised PCA plot with no scaling (A) Pareto scaling (B), and the associated loadings plot from the PCA analysis (C).

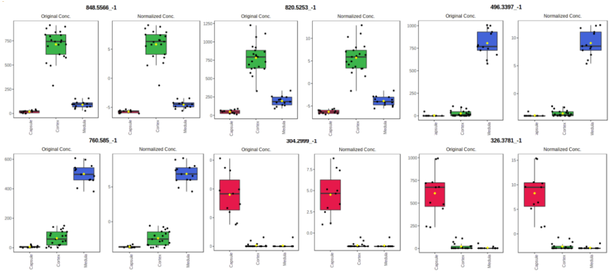


Figure 4: Statistical analyses of the three tissue regions, for a wild type murine adrenal gland. Box plots demonstrating the distribution of some prominent marker compounds.

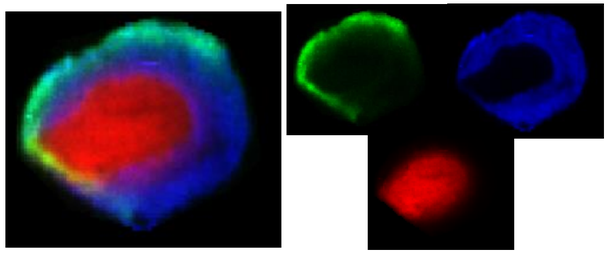


Figure 5: Showing three molecules demonstrating differential localization between the medulla (m/z 166.08615, Red), Cortex (m/z 461.28796, Green) and the capsule or outer cortex (m/z 369.35129, Blue). Combined (right) and individually (left).

Imaging software HDI was used to create visual RGB overlays of the marker compounds, clearly demonstrating the differential distribution of molecules within the adrenal gland. The medulla, cortex and outer cortex/capsule can be clearly identified within the tissue images with strong unique biological markers seen for each region (Figure 5).
Overlaid and individually shown in Figure 5 are:
Red: *m/z* 166.08615 putative ID phenylalanine (M+H+) 602ppb mass accuracy,
Green: *m/z* 461.28796 tentatively a Sterol derivative,
Blue: *m/z* 369.35159 putative ID cholesterol (M+H-H_2O) 27ppb mass accuracy.

Results

The data were compared from two mass spectrometers, a conventional QToF and the SELECT SERIES MRT mass spectrometer.

An example RGB overlay image for differentiating compounds for each region can be seen in Figure 6 this demonstrates that the same general information can be achieved by either mass spectrometer.

The RGB overlay shows: Red *m/z* 496.33957 putatively LPC 16:0/LPE 19:0 (M+H+) **383ppb** mass accuracy, Blue *m/z* 848.55609 putatively PC 38:4/PE 41:4 (M+K+) **613ppb** mass accuracy, and Green *m/z* 752.38678 currently unidentified.

Putative identifications were assigned by generating theoretical elemental composition predictions based upon the accurate mass resolution provided by the MRT mass spectrometer (Figure 7). The proposed formula was then searched through online databases for potential compound identification.

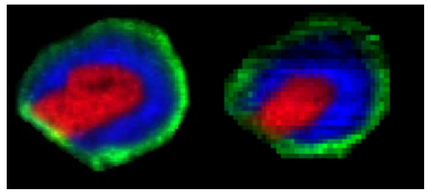


Figure 6: Showing conventional QToF RGB Image overlay (left) and MRT mass spectrometer RGB overlay image (right), with the same three compounds shown as significant using PCA analysis.

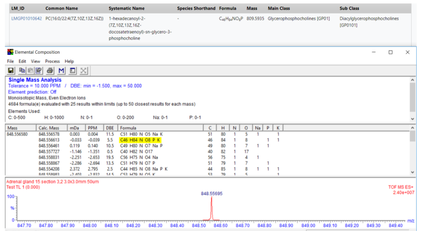


Figure 7: Showing an example elemental composition search within MassLynx software and the corresponding LIPID MAPS database match for the proposed composition from MRT DESI MSI dataset..

The conventional QToF provided ~20,000 FWHM mass resolution and the SELECT SERIES MRT mass spectrometer provided >200,000 FWHM mass resolution. The MRT mass spectrometer therefore enables greater spectral separation of compounds with very similar molecular masses, unresolved by the conventional QToF mass spectrometer.

An example spectra from both mass spectrometers is shown (Figure 8). The spectra on the conventional QToF produces a single *m/z* of 806.55, however, when this signal was investigated using the SELECT SERIES MRT mass spectrometer, the increased mass resolution separated this *m/z* into three distinct masses: 806.51038, 806.55902 and 806.56897.

For each *m/z* 806.6 signal the putative identifications are as follows:

m/z 806.55 = Potential IDs, including but not limited to: PC 38:6 (M+H+), PE 41:6 (M+H+), PS 37:0 (M+H+) , PC 36:3 (M+Na+)

m/z 806.51007 = PE 35:4 (M+K+) **508 ppb** mass accuracy
m/z 806.55829 = PE 36:4 (C13, M+Na+) **260 ppb** mass accuracy
m/z 806.56909 = PE 38:6 (M+H+) **422 ppb** mass accuracy

When these masses are visualized (Figure 9 A) on the conventional QToF, the single spectral peak demonstrated a strong signal across both the medulla and the cortex of the adrenal gland, it appears no differentiating localization was seen for the lipid.

When this same signal was investigated within the MRT data it was observed that of the three lipid species seen all show differential tissue localizations (Figure 9).

Two signals: *m/z* 806.51038 (B) and *m/z* 806.5590 (C) appear to localize predominantly within the cortex region of the adrenal gland. B demonstrates lower levels within the medulla and C does not appear to be present within the medulla.

The signal at *m/z* 806.56897 (D) appears to localize predominantly within the medulla of the adrenal gland with a lower level seen within the cortex. (Figure 9).

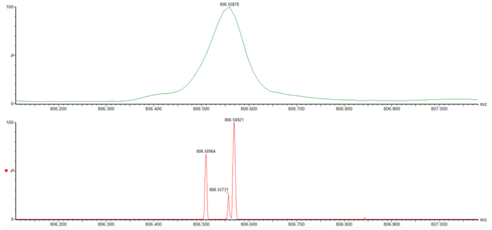


Figure 8: Showing MRT mass spectrometer spectra (Green), and conventional QToF spectra (Red).

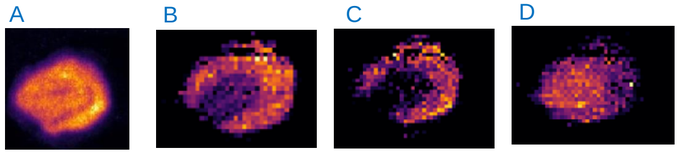
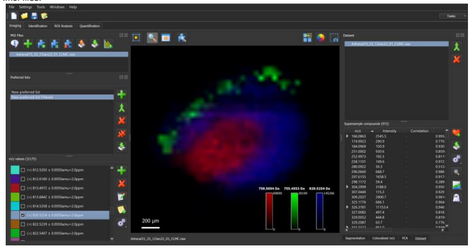


Figure 9: Showing compound map for m/z 806.55 Da on the conventional QToF mass spectrometer spectra (A), and the 806.51038 Da (B), 806.55902 Da (C) on the MRT mass spectrometer.

In addition to utilising HDI and/or MassLynx software for data mining, peak picking and visualisation data from both the SELECT SERIES MRT and SYNAPT-XS can be imported into Lipostar MSI imaging software. This package can be used for all aspects of data processing for mass spectrometry imaging applications including statistical analysis and database searching complete with structural information when provided the appropriate .mol files.



A screenshot taken from the Lipostar MSI imaging software, showing a RGB overlay of three compounds demonstrating differential localization.

Some masses of interest were also selected for manual MS/MS acquisition to generate fragmentation patterns. This information was used to increase confidence in putative identification. Only a small focussed acquisition was performed on the region of tissue containing the marker compound therefore no images were created from this acquisition.
One mass chosen for MS/MS fragmentation evaluation was *m/z* 166.06 for which the fragments generated match a hypothesised identification of phenylalanine (Figure 11).

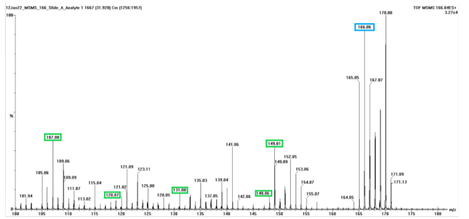


Figure 11: Showing the spectra obtained from MS/MS analysis of mass 166.06 Da. Demonstrating fragmentation matches to the theoretical fragmentation pattern inside the green boxes and the precursor ion in the blue box.

Conclusions

This feasibility study demonstrates DESI-MS as a suitable tool for imaging adrenal glands.

Data can be collected on conventional QToF mass spectrometers or - as demonstrated - on a high mass resolution SELECT SERIES multi-reflecting ToF capable instrument. The images produced by the DESI inlet provide excellent image quality in terms of image resolution and spatial separation information.

The data acquired using this technique can easily be imported into image processing software including but not limited to: HDI and Lipostar MSI, for visualization. Further investigation can be performed through the use of statistical software such as Metaboanalyst or within Lipostar MSI, and putative identification is possible using database searches manually, or through dedicated software.

The high mass resolution provided by the SELECT SERIES MRT mass spectrometer reveals fine isotope structure and delivers ppb mass accuracy allowing more confident deduction of elemental composition. With the number of potential elemental compositions reduced, confidence in identifications is vastly improved. For the above example, the conventional QToF mass with a ±50 mDa tolerance window produces 13 possible compound identifications, the SELECT SERIES MRT separated this signal as three distinct masses: putatively identified as PE (38:4)+K+, *(C13$_3$)*PC (36:4)+Na+, and PC (38:6)+H+, within an average 397 ppb mass accuracy.

If desired the same tissue sections can be re-analyzed via DESI to generate targeted fragmentation information in the form of MS/MS acquisitions, analyzing a single mass of interest with quad selection and subsequent fragmentation providing a cleaner high energy spectra than obtained through full scan acquisitions. Or tissue sections analyzed by DESI can be taken for histological staining due to the non-destructive nature of the technique.

Reference
[1] US Department of Health Sciences, NIH (National Institute of Child Health and Human Development), Adrenal Gland Disorders https://www.nichd.nih.gov/health/topics/adrenalgland/conditioninfo/types

Download scientific posters at waters.com/posters