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Application Note

Improving Feature Detection and Putative Identification For Tissue Imaging Applications Using the SELECT SERIES™ Multi -Reflecting-ToF (MRT) Mass Spectrometer

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For *in vitro* diagnostic use. Not available in all countries.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

HRMS DESI Imaging with the SELECT SERIES MRT Mass Spectrometer delivers enhanced mass accuracy and mass resolution compared to a conventional research grade oa-QToF mass spectrometer. The >200,000 FWHM mass resolution provided by the MRT mass spectrometer enables superior analyte detection and the <500 ppb mass accuracy improves putative biomarker identification.

Benefits

Analysis of wild type murine adrenal gland tissue sections using a DESI-MRT Mass Spectrometer. Demonstrating the improvement in tissue analyte detection and identification using DESI on the MRT mass spectrometer, compared to a conventional oa-QToF Mass Spectrometer.

Introduction

Mass Spectrometry based tissue imaging is a rapidly growing area of research seeing application in the life-science, pharmaceutical, medical, and food sciences. Crucial to the success of this discipline is the ability to detect changes related to disease, toxicity, contamination, quality etc and to rapidly identify the resulting putative “biomarkers” of these changes. To this end improvements in the speed, mass resolution, and mass accuracy of mass spectrometry can greatly enhance efficiency and confidence for structural identification

The SELECT SERIES MRT Mass Spectrometer is a Q-ToF mass spectrometer which possesses two gridless electrostatic mirrors, these allow the ion beam to under-go multiple reflections within the mass analyzer and extends the ion flight path to >48 meters equivalent. This gives mass accuracy (<500 ppb) and mass resolution (>200,000 FWHM), unprecedented in non-trapping instruments, with the advantage of also being unaffected by wide mass ranges or rapid scan speeds. This advancement has enabled high mass resolution capabilities for routine or large tissue imaging applications.

To demonstrate the increased mass resolution and mass accuracy now available on the SELECT SERIES MRT Mass Spectrometer, cryo-sectioned murine adrenal glands were analysedanalyzed on both a SELECT SERIES MRT Mass Spectrometer and a traditional research grade oa-QToF mass spectrometer. Adrenal glands are essential endocrine organs, lipid rich they provide cholesterol substrate for glucocorticoid, mineralocorticoid, and androgens synthesis. The aim

of this study was to gain a greater understanding of how aberrations in adrenal function may be associated with changes in lipid composition of the tissue by visualizing the structure and composition of adrenal glands.

Results and Discussion

A healthy wild-type mouse adrenal gland was cryo-sectioned onto a glass slide at a thickness of 15 µm.

The sections were analyzed by DESI-MS on a SELECT SERIES MRT Mass Spectrometer as well as a conventional research grade oa-QToF mass spectrometer operating with a resolution of 20,000 FWHM. The data was acquired with an image resolution of 25 µm pixel size in both positive and negative ionization mode.

The resulting raw data was interrogated using High Definition™ Imaging (HDI™) software was used to create visual overlays of the ionized compounds mapping the distribution of each ion within the adrenal gland. It was clear from the derived data that the medulla, cortex, and an outer region of the cortex could be clearly visualized within the tissue. Strong unique biological markers were observed for each region, in both the positive and negative ion data. The example Red/Green/Blue (RGB) overlay images show differentiating compounds for each region (Figure 1), this demonstrates that unique compound localization information can be achieved and visualized from the MRT mass spectrometer.

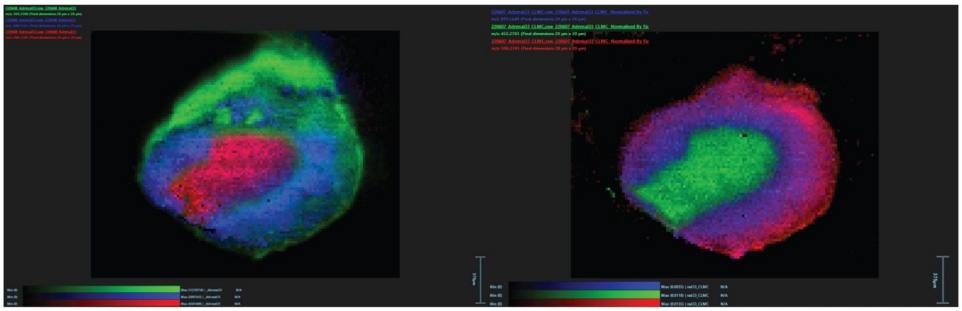

Figure 1. Example images for positive ionisationionization mode (Left) and negative ionisationionization mode (Right) (TIC normalized) of murine adrenal tissue sections analysedanalyzed by DESI MSI on the MRT.

The high mass resolution provided by the MRT mass spectrometer, >200,000 (FWHM) revealed the fine isotope structure of the putative biomarkers and delivered <500 ppb mass accuracy allowing for more confident deduction of elemental composition. This increased mass accuracy allowed the number of potential elemental compositions to be significantly reduced. Thus, increasing confidence in the derived identifications. Putative structural identifications of three lipids were assigned by generating theoretical elemental composition predictions based upon the accurate mass resolution provided by the MRT mass spectrometer (Figure 2). The proposed formula was then searched through online databases for potential compound identifications.

In the RGB overlay (Figure 1, A) positive mode ionization shows the following signals: Red *m/z* 496.33972 putatively identified as LPC 16:0/LPE 19:0 ($M+H^+$) with a 20 ppb mass accuracy, Blue *m/z* 848.55695 putatively identified as PC 38:4/PE 41:4 ($M+K^+$) with a 39 ppb mass accuracy (Figure 2), and Green *m/z* 369.35156 putatively identified as Cholesterol ($M-H_2O+H^+$) with a 54 ppb mass accuracy. The RGB overlay (Figure 1, *B*) negative mode ionization shows: Red *m/z* 500.27826 putatively identified as LPE 20:4 ($M-H^-$) with a 120 ppb mass accuracy, Blue *m/z* 899.56519 putatively identified as PI 39:4 ($M-H^-$) 356 ppb with a mass accuracy, and Green *m/z* 452.2783 putatively identified as LPC 13:0 ($M-H^-$) with a 133 ppb mass

accuracy.

The impact of the increased MS resolution provided by the MRT is illustrated with the lipid *m/z* 806.55. The conventional oa-QToF mass, operating with a ±50 mDa tolerance window, produced 13 possible compound identifications with the best match being PC 38:6 ($M+H^+$). The MRT mass spectrometer was able to distinguish this signal as three distinct masses with the putative identifications (centroid mass values): *m/z* 806.51007 as PE 35:4 ($M+K^+$) with a 508 ppb mass accuracy, *m/z* 806.55829 as PE 36:4 ($C13_2$ $M+Na^+$) with a 260 ppb mass accuracy and *m/z* 806.56909 as PE 38:6 ($M+H^+$) with a 422 ppb mass accuracy.

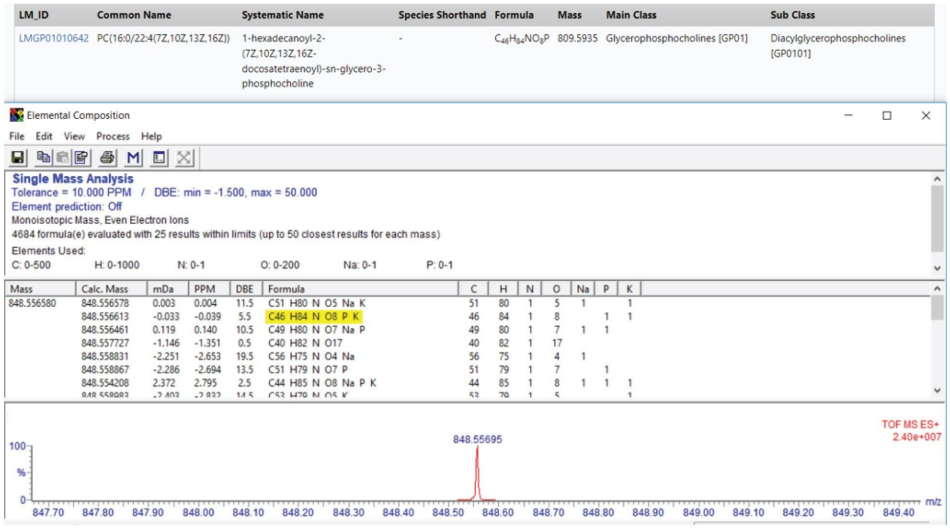

Figure 2. An example elemental composition search within MassLynx software and the corresponding LIPID MAPS[3] database match for the proposed composition from the MRT DESI MSI dataset.

An example spectra obtained from both mass spectrometers is shown (Figure 3). The spectra produced by the conventional oa-QToF produces a single ion signal with an *m/z* of 806.55. However, when this signal was investigated using the MRT mass spectrometer the increased mass resolution provided by this mass spectrometer enabled the separation of the observed *m/z* signal into three distinct

(near) baseline separated mass/charged features (non-centroid masses): *m/z* 806.50964, *m/z* 806.55829 and *m/z* 806.56726.

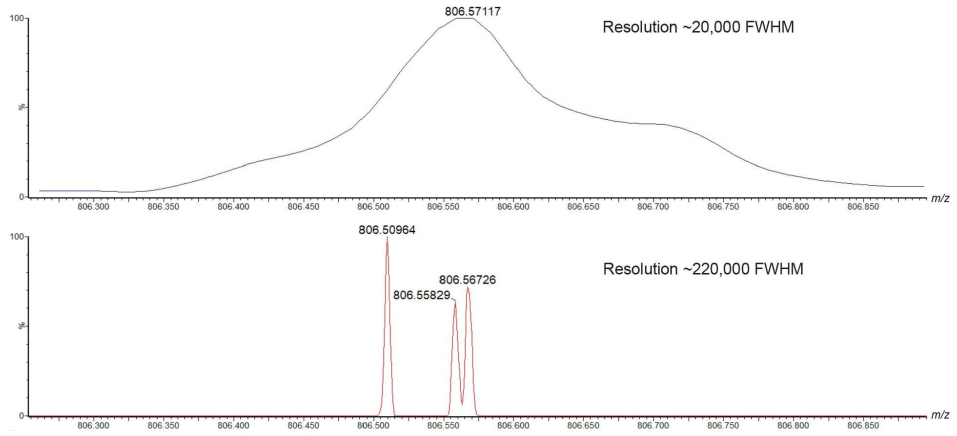

Figure 3. Representative mass spectra for a narrow m/z range produced from murine adrenal gland tissue analysis on the conventional oa-QToF mass spectrometer (Top) and the SELECT SERIES MRT Mass Spectrometer (Bottom).

When these masses were visualized using the HDI software, the *m/z* 806.55 ion seen by the conventional oa-QToF demonstrated a strong signal across both the medulla and the cortex of the adrenal gland, appearing as a non-localized or non-differentiating lipid marker (Figure 4A). When this same signal was investigated using the MRT data, three separate lipid species were detected; each showing differential tissue localizations (Figure 4 B, C, D). Two signals: *m/z* 806.51007 (B) and *m/z* 806.55829 (C) appear to localize predominantly within the cortex region of the adrenal gland. Signal B demonstrates lower levels within the medulla, whilst signal C does not appear to be present within the medulla. The signal at *m/z* 806.56909 (D) appears to localize predominantly within the medulla of the adrenal gland, whilst a lower level is observed within the cortex.

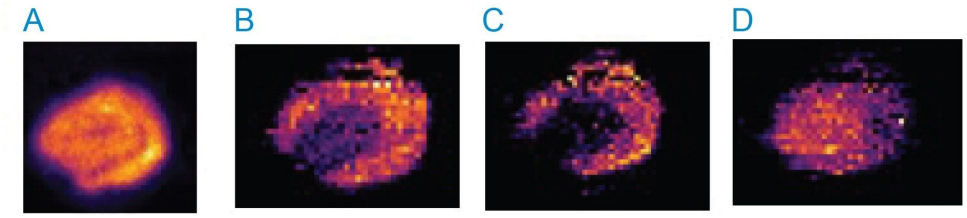

Figure 4. Compound map for m/z 806.55 on the conventional oa-QToF mass spectrometer (A), and individual compound maps for the m/z 806.51007 (B), m/z 806.55829 (C) m/z 806.56909 from the MRT mass spectrometer spectra.

The data generated by the MRT mass spectrometer is compatible for processing in a number of different software packages, including Waters software; such as MassLynx™ and HDI, as well as third party software such as LipoStar™ MSI (Molecular Discovery). The workflow used to process the adrenal tissue sections for this application included MassLynx investigation for spectra quality determination and elemental composition tools. HDI for compound maps, enabling the tissue images to be generated as well as peak list generation for each tissue region, enabling statistical analysis for discovery of target marker compounds within MetaboAnalyst[2] online statistical software.

Conclusion

This application demonstrates that DESI-MSI on the SELECT SERIES MRT Mass Spectrometer is a suitable tool for imaging mammalian tissue, as illustrated in this example with the analysis of murine adrenal glands. The high mass resolution provided by the SELECT SERIES MRT Mass Spectrometer reveals fine isotope structure and increases the number of features observed by separating more ions with closely matching molecular weights. Delivering a <500 ppb mass accuracy the number of potential elemental compositions is reduced, thus increasing the confidence in the derived putative identification via database searches (e.g. LIPID

MAPS™ Lipidomics Gateway website), or through dedicated software. The data acquired using this technique can easily be processed through MassLynx software, imported into image processing software such as HDI and further investigation can be performed via statistical software such as Metaboanalyst.

References

1. US Department of Health Sciences, NIH (National Institute of Child Health and Human Development), Adrenal Gland Disorders.
2. Ref: Pang, Z., Zhou, G., Ewald, J. *et al.* Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat Protoc* (2022). https://doi.org/10.1038/s41596-022-00710-w <https://doi.org/10.1038/s41596-022-00710-w> .
3. Update of the LIPID MAPS® comprehensive classification system for lipids. Fahy E., Subramaniam S., Murphy R., Nishijima M., Raetz C., Shimizu T., Spener F., van Meer G., Wakelam M., and Dennis E., *Journal of Lipid Research* 50, S9-S14 (2009).

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