INVESTIGATION INTO THE USE OF TISSUE WASHING PROCEDURES AND THE SUBSEQUENT OUTCOMES FOR DESI-MS IMAGING ANALYSES

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INTRODUCTION

MALDI mass spectrometry imaging (MSI) is the most widely adopted technique for the molecular imaging of biological tissues, with DESI-MSI now a fast-growing alternative, particularly for the analysis of lipids and other small molecules. Prior to MALDI-MSI, tissue sections are often subjected to different organic and/or aqueous washing procedures. Some of the washes available have been investigated, for their ability to enhance signal in MALDI-MSI[1,2] as a consequence of removing impurities that may compete or suppress ionisation, or the improve matrix crystallization.

The aim of this study is to perform a comparable investigation into the effect of a variety of tissue washing procedures when implemented prior to DESI-MSI analysis.

METHODS

Sample Preparation

- Normal mouse brain was cryosectioned at 12 µm thickness and mounted onto Polysine coated glass slides.
- On removal from storage at −80°C, all slides were vacuum desiccated for 20 minutes.
- One of the slides of the consecutive tissue sections was left unwashed and analysed directly after desiccation for use as a control.
- Three solutions were chosen for comparison, including; Ammonium formate (50mM), ammonium acetate (50mM) and 0.1% formic acid .
- All wash solutions were stored and cooled at 4°C prior to use.
- Each of the three washes were performed using two methods: fully immersing the section in the wash solution, or pipetting a 1 mL volume (5 x 200 µL) of the wash solution over the tissue section.
- Subsequent to all washing the slides were desiccated for a further 20 minutes.
- All samples were prepared on the day of analysis.

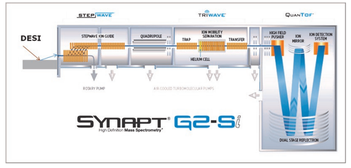

Figure 1. Schematic of the DESI source on the SYNAPT G2-Si HDMS-MS system.

Instrumentation and Setup

All analyses were performed on a Synapt hybrid Q-TOF-IMS-MS instrument, with integrated tri-wave ion guide optics used to separate ions by ion mobility in the gas phase.
The instrument was operated in resolution mode, and positive ionisation mode.

The 2D stage DESI (Prosolia) stage was mounted directly using an electrospray inlet, and an inlet capillary for collection of ions. The DESI spray solvent used consisted of 98% Methanol, 2% water and 0.1 % formic acid.The solvent was introduced at a flow rate of 1.5 µL/min with nitrogen gas pressure set to 5 bar with an applied voltage of 4.5 kV.

All image acquisitions were set up in High Definition Imaging (HDI) informatics, version 1.4. Slides were scanned using a standard flat bed scanner. Images displayed were acquired to give a final pixel size of 100 µm and a scan time of 0.5 seconds.

Data management

DESI-IMS-MS datasets were examined, with MassLynx, Driftscope, then processed and visualized by HDI, version 1.4. Multivariate analyses were performed in EZInfo (Umetrics), via Progenesis QI. All statistical analyses were unsupervised. Figure 2 demonstrates the way in which regions of interest (ROI) were exported from HDI for multivariate statistical analysis.

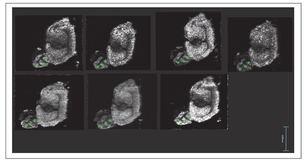

Figure 2. An image exported from HDI, depicting regions of interest (ROI) that were created for the export of data to the multivariate analysis tool (EzInfo). Each square area represents a single ROI and covers a 25 pixel area.

RESULTS

Subsequent to acquisition and processing, all imaging datasets were viewed in HDI. Figure 3 displays the drift plots of the unwashed control and each of the immersion washed sections. The circled regions emphasise the appearance of ions in the section washed with 0.1% formic acid. The corresponding images of these ions were viewed and localisation determined to be ubiquitous but specific to the tissue. To identify these ions further MS/MS experiments are required.

The mass spectra in Figure 4 highlight the changes in the ions detected as a result of the various washing procedures. It should also be noted that the overall signal intensity appears increased to the greatest extent in the spectrum exported from the section washed in 0.1 % formic acid. This is contrary to results presented in previous MALDI related studies, but can be explained as there is already acid applied to sections analysed using MALDI as part of the matrix.

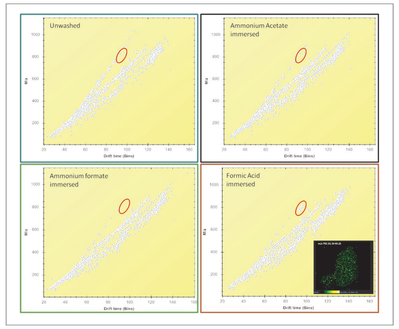

Figure 3. Drift plots taken from HDI for each of the immersion washes and the unwashed sample (as labeled on plots). Each plot contains an area circled in red, this highlights a number of ions specific to the 0.1 % formic acid wash. An insert of an ion image (m/z 792.55, Dt 95.21) corresponding to one of the ions within the highlighted region of the 0.1 % formic acid wash drift plot is also shown. The ion image displayed shows local-isation specific to the tissue region only.

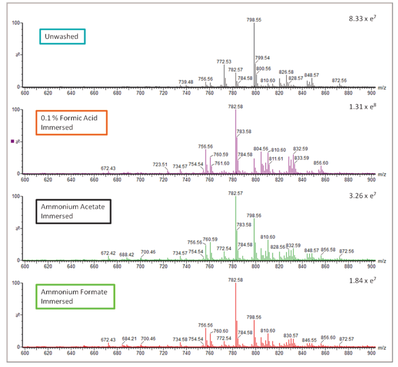

Figure 4. Mass spectra across the m/z range of 600-900 for the unwashed, control sample and each of the immersion washes (as labeled). Imaging data was TIC normalised in HDI software, and the spectra above generated from a 2400 pixel ROI export from each sample. In each case the ROI export encompassed all features of the brain sections for consistency. Intensity values given to the top right of each individual spectrum relate to the most intense signal across the mass range shown.

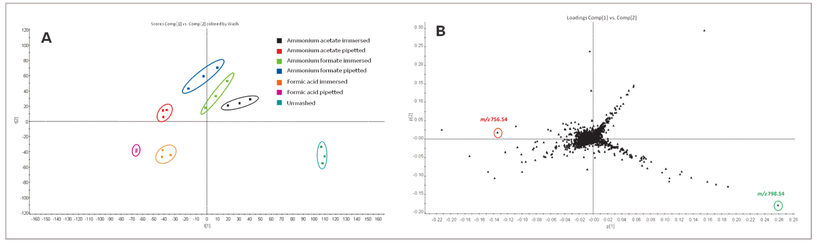

Figure 5. The unsupervised PCA score plot (A) and Loadings plot (B) resulting from the ROI that were exported as shown in Figure 2 are displayed here. Two ions of interest are highlighted and labeled.

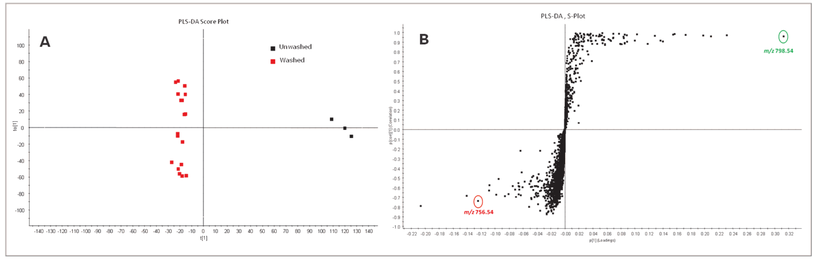

Figure 6. The supervised PLS-DA score plot (A) and S-plot (B) resulting from the ROI that were exported as shown in Figure 2 are displayed here. Two ions of interest are highlighted and labeled, just as for Figure 5.

Reprocessing was performed to align the masses across all datasets, and ROI were exported from the cerebellum of each section (as shown in Figure 2). Unsupervised PCA (Figure 5) and supervised PLS-DA (Figure 6) were performed and both analyses confirm the clear differences in detection between the unwashed control and the other, washed models. Similarly to the drift plots in figure 3, the PCA results appear to indicate that the ROI exported from sections washed with 0.1% formic acid as displaying the most significance in the differences of ions detected. Two of the most outlying ions (*m/z* 756.54 and 798.54) shown in both loadings plots above were selected and the corresponding images are shown in Figure 7 below. The ion image of *m/z* 798.54 (previously identified as an $[M+K]^+$ ion) is much more intense for the unwashed tissue section, this is likely due to the removal of potassium during the washing steps. Additionally, the images for *m/z* 756.54 show improvement subsequent washing, particularly in those washed with formic acid, which is expected for $[M+H]^+$ species.

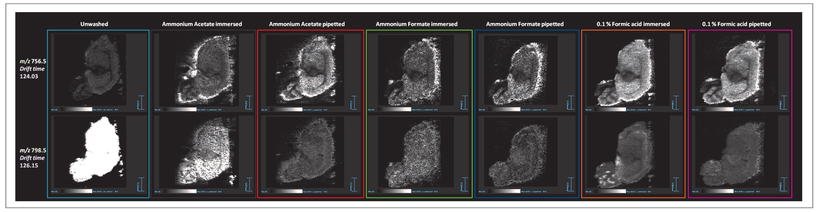

Figure 7. The corresponding ion images for m/z 798.54 (an example of $[M+K]^+$) and 756.54 (an example of $[M+H]^+$) are shown for each of the washed samples and for the unwashed control. All images are displayed on the same intensity gradient.

In Figure 7 the images appear to suggest that there may have been some delocalization as a result of washing. This will be investigated through the examination of data acquired to a final pixel size of 40 µm. The images in Figure 8 show that although there may be some delocalization associated with the washes performed here, it was still possible to detect and depict ions with discreet localisation. Again, the ion images below also display significant improvement subsequent to washing.

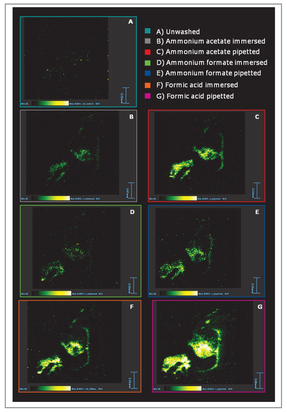

Figure 8: Ion images generated from m/z 643.51 in each data-set (as labeled in the Figure). All images are displayed on the same gradient scales.

CONCLUSION

- **Just as for MALDI-MSI tissue washing procedures improve the detection of $[M+H]^+$ species.**
- **The most beneficial washing solution amongst those tested here was found to be 0.1 % formic acid.**
- **Experiments have been conducted at higher spatial resolution (40 µm pixel size) to investigate any delocalisation effects.**
- **Future work will include the continuation of these experiments on further mouse brain sections with analysis in negative mode ionisation in addition to the inclusion of other tissue types.**
- **MS/MS will be performed to establish the presence of and to identify any adduct ions.**

References

1. Angel, P.M., Spraggins, J.m., Baldwin, H.S., Caprioli, R. (2012) Enhanced sensitivity for high spatial resolution lipid analysis by negative ion mode matrix assisted laser desorption ionization imaging mass spectrometry, *Analytical Chemistry*, **84**, 1557-1564.

2. Wang, H. J., Liu, C.B., Wu, H. (2011) A simple desalting method for direct MALDI mass spectrometry profiling of tissue lipids, Journal of Lipid Research, 52, 840-849.

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