High Throughput HDMS^E Blood Product Lipidomic Screening Using a DESI Inlet

Lisa Reid[1], Mark Towers[1], Lee Gethings[1], Royston Goodacre[2] Ian Wilson[2] & Paul Richardson[2]
[1] Waters Corporation, Wilmslow, Cheshire, UK
[2] Department of Biochemistry and Systems Biology, Institute of Systems, Molecular, and Integrative Biology, University of Liverpool, UK

INTRODUCTION

In biomedical research, to profile blood products for large cohort lipidomic studies may consist of long LC/MS analyses requiring several weeks of instrument time. Consequently, increasing the potential for instrument failure, unwanted batch effects and sample degradation.

DESI imaging is traditionally used to investigate compound localization within tissue where, by visualizing the structure and molecular composition of tissues, a greater understanding of organ structure and changes due to disease may be obtained.

Here we have examined the feasibility of using DESI as an alternative high throughput direct analysis method for performing lipid profiling. This technique has been applied to screen sera obtained from a UK human population as part of the Human Serum Metabolome study (HUSERMET).

METHODS

Serum samples (*n*=500) from the HUSERMET study (Stockport, UK) were protein precipitated 1:4 v/v with IPA, and 2 µL of the supernatants pipetted onto Teflon spots on a glass microscope slide.

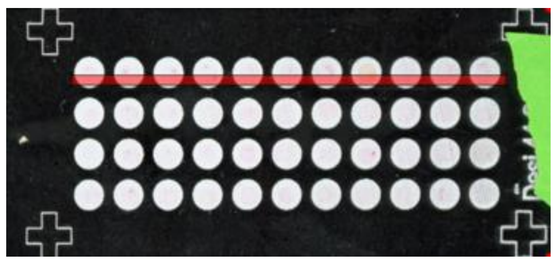

Figure 1: Example slide image with a single pass acquisition highlighted in red.

The slides were analyzed by DESI-HDMS^E on a SYNAPT^{TM} G2-*XS* oa-QToF mass spectrometer, a single line pass was performed through the center of each Teflon spot (**Figure 1**).

DESI XS setup:
0.6kV capilliary voltage,
5 bar gas flow,
98% MeOH 2% Water,
2 µL per minute flow rate,
heated transfer line at 300ºC

Detector: SYNAPT G2-*XS* mass spectrometer (Waters).
Positive ionisation mode: Step size 50µm.
HDMS^E 8 scans per second.
Negative ionisation mode: step size 100µm.
HDMS^E 2 scans per second.

Data can be processed with the following software platrforms: MassLynx^{TM}, DriftScope^{TM} High-Definition Imaging (HDI^{TM}), Progenesis^{TM} QI and UNIFI^{TM}

RESULTS

Each slide of 44 samples required 8 minutes acquisition time giving a total acquisition for all 500 samples of <3 hours per polarity. **Figure 2** shows a typical total ion chromatogram (TIC) from a line of 11 spots. Acquisition speed could be increased if desired, however, on this occasion the scan rate was intentionally limited to ensure excellent signal strength and maximise the information generated per sample.

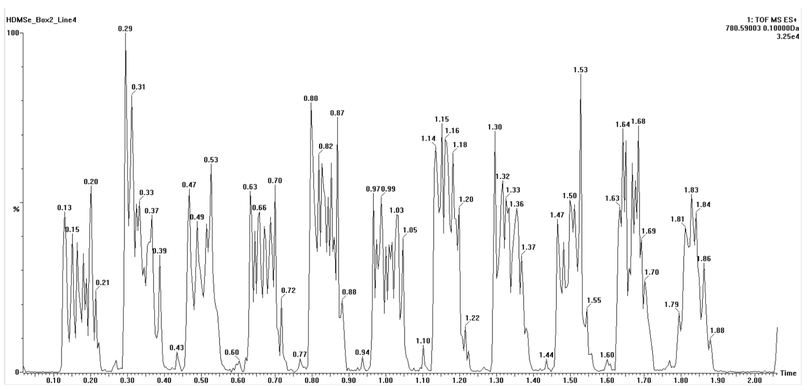

Figure 2: Example XIC from a line of 11 sample spots m/z 780.6 putatively identified as PC 37:3 (mass accuracy -2 ppm).

Data generated shows excellent signal strength across all samples generating a per sample summed spectra signal of mid e5 for positive mode data, and mid e4 for negative mode data (**Figure 3**).

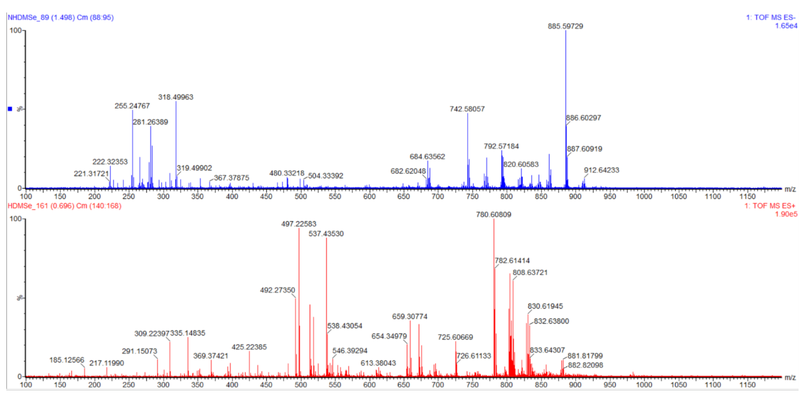

Figure 3: Example spectra combining the scans over a single sample spot in negative ionisation mode (top) and positive ionisation mode (bottom).

There is the potential for a drying pattern or “coffee ring” effect to be seen when any fluid is dried on a surface, this could possibly impact sample comparison. It was demonstrated that, as these samples have been extracted using IPA – a highly volatile solvent, there is no obvious evidence of a drying pattern that may cause signal variances.

A number of sample spots were imaged in their entirety to investigate the presence of drying effects (**Figure 4**).

These acquisitions also showed no significant evidence of a pattern, or gradient created as the sample dried. Should a less volatile solvent be required, any signal gradients generated should be nullified through the summation of the spectra for each spot.

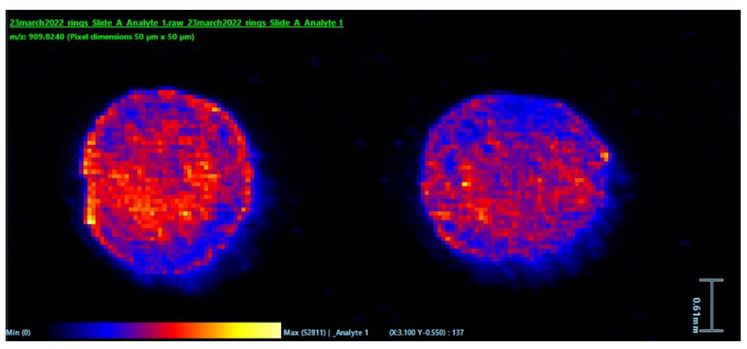

Figure 4: Example sample spots showing an abundant lipid (putatively TG 57:3). Left image is an air-dried sample and right image is a lyophilized sample. The lipid signal intensity appears stronger on the air dried sample spots.

Differences can be seen between samples when their spectra is visually compared, also any samples with contamination that may impact an LC analysis can be identified (**Figure 5**).

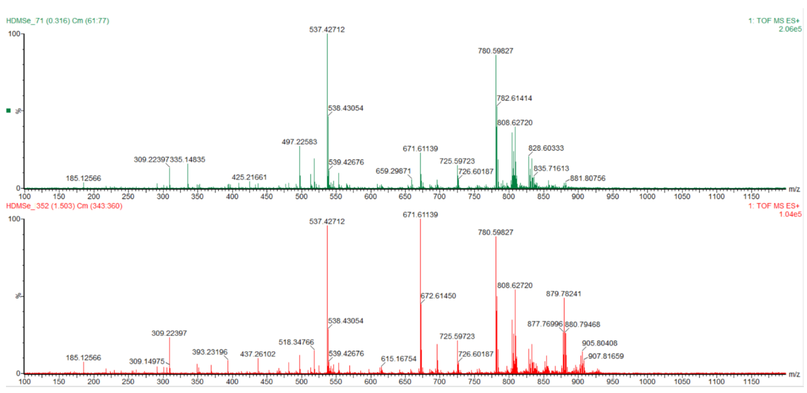

Figure 5: Example spectra of two positive mode sample acquisitions with visually different lipid profiles.

Summed spectra from a sample spot can be opened within DriftScope software to visualize the ion mobility separation and extract individual signals (**Figure 6**).

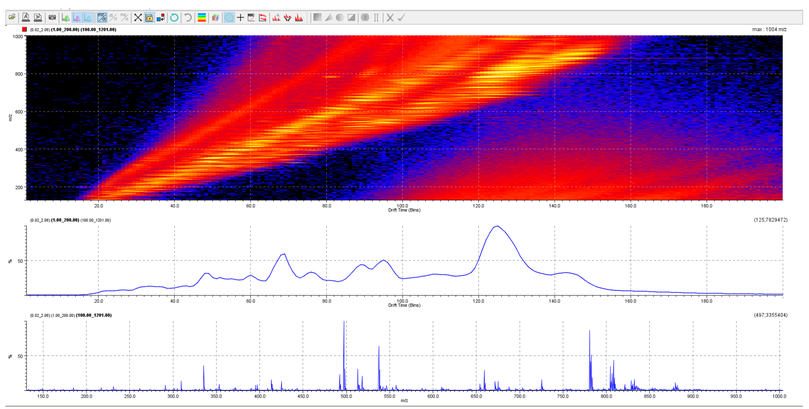

Figure 6: Example drift plot of a single sample positive mode acquisition created using DriftScope software.

Sample acquisitions can be imported into UNIFI software for library searching and visualization if desired (**Figure 7**). UNIFI software is able to use database searching and match compounds based upon mass accounting for single or an average of multiple adduct species. It can also include drift time information for increased confidence in database matches. **Figure 7** shows a typical UNIFI software view.

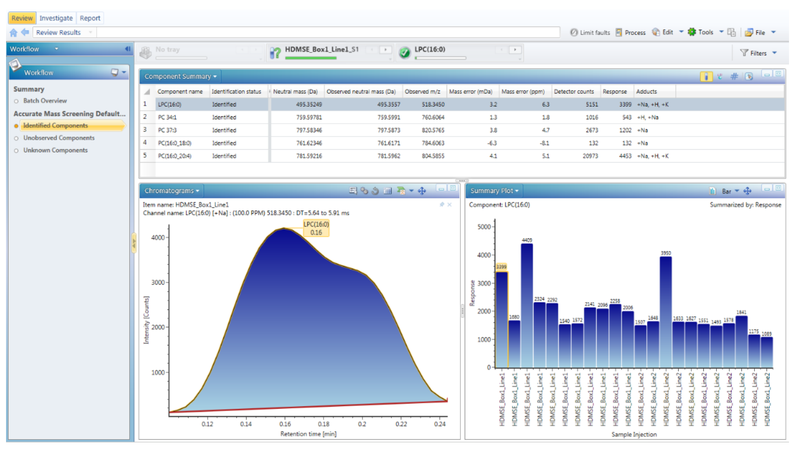

Figure 7: Example UNIFI software view showing the lipid LPC (16:0) demonstrating m/z match for the observed signal compared to the theoretical values. The table provides information on mass error, signal strength, response (area) and the observed adducts. The chromatogram window displays the XIC and the integrated ‘peak’. The Summary plot shows the selected lipid response across all samples.

Data can be exported into Progenesis QI software to perform multivariate analysis and database searching. Confirming that this workflow is able to generate a list of potential “biomarkers” and look for phenotypic differences in the study population. As this study consisted of healthy individuals, there were no significant biomarkers identified for the dataset (**Figure 8**).

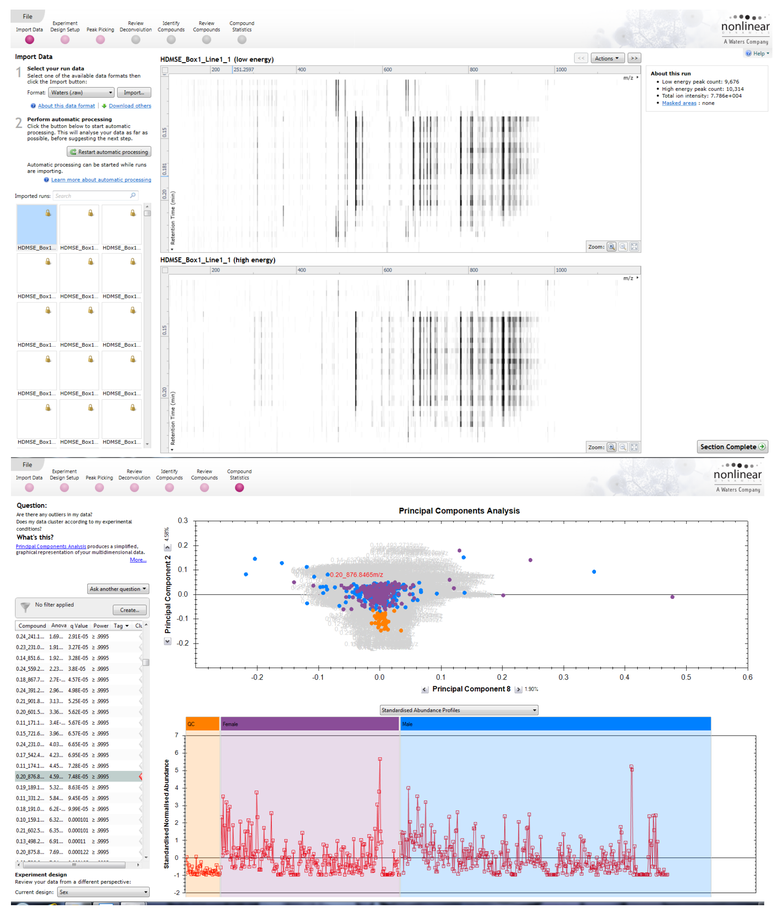

Figure 8: Example Progenesis QI software screenshots: Positive mode acquisition data import tab (top), and the statistical analysis tab (bottom) showing PCA generation and a compound abundance profile (m/z 876.8 putatively identified as TG 52:2 (mass accuracy +2.7 ppm)).

CONCLUSIONS

DESI can be used as a high throughput basic screening technique for the lipidomic analysis of small volumes of serum, with individual sample analysis <15 seconds per sample.

The full workflow described, employs minimal sample preparation maintained as identical to the method for LC/MS, enabling storage and subsequent LC analysis if desired.

Benefits include: reduced potential for time-related batch effects or sample degradation during analysis and drastically reduced file size, thus improving both storage demands and processing time.

DESI pre-screening can be used to indicate potential issues such as contamination or the presence of incorrect sample type, which could have devastating effects on subsequent LC/MS analyses.

The data generated provides an overview of sample quality, reveals fold changes in lipid profiles and could be used to facilitate a more in-depth subset analysis by LC/MS.

We highlight that good data quality can be achieved using rapid data acquisition and show how these data can be imported into peak processing and/or statistical software packages depending upon application goal.