Screening Metabolomics of Extracellular Vesicles using the Xevo™ MRT Mass Spectrometer

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INTRODUCTION

A metabolomics study is designed to discover metabolic changes of any organism. The metabolome is very complex, containing 1000's of compounds and identification of small molecule metabolites remains a significant challenge, with many analytes having similar molecular weights. Here we demonstrate the advantages of improved mass resolution and sub ppm mass accuracy offered by the Xevo MRT Mass Spectrometer (MS) for metabolomic studies. The benefits of this approach are demonstrated using a small feasibility metabolomics study aiming to construct a comparative metabolite profile for extracellular vesicles (EVs) and matrix bound vesicles (MBVs) obtained from MC3T3 pre osteoblasts under osteogenic culture conditions. In an aim to understand the two subtypes potentially varying roles in skeletal mineralization.

Data were acquired using the Xevo MRT MS (Figure 1) coupled to an ACQUITY™ Premier System, using a BEH™ Amide (1x100 mm) Column and a rapid four minute (99-50% B) HILIC gradient.

Figure 1: The Xevo MRT Mass Spectrometer

Data acquisition was controlled by waters_connect™ Software Platform, data visualization through the MSToolkit application within the waters_connect Hub and statistical data processing performed using MARS software (MassAnalytica™, Barcelona, Spain).

RESULTS AND DISCUSSION:

Confident identification of potential biomarkers can be challenging in metabolomic studies with many small molecules sharing very similar molecular masses. By increasing mass resolution it is possible to spectrally separate more of these compounds. The Xevo MRT MS delivers powerful mass resolution capabilities, demonstrating a small molecule (<500 Da) mass resolution of ~70,000 full width half maximum (FWHM) (Figure 2).

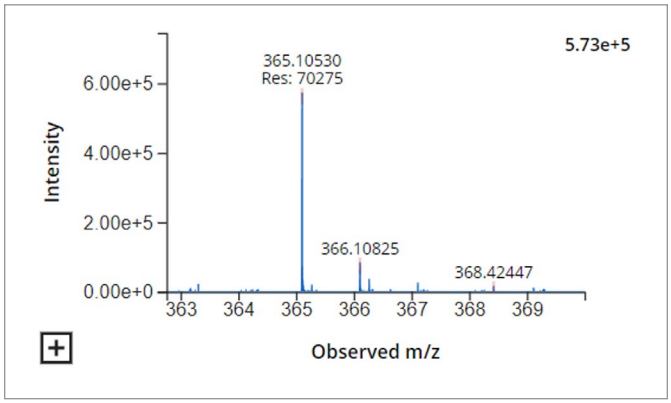

Figure 2: Putatively identified 3-b-Galactopyransyl glucose with a mass resolution of ~70,000 FWHM

The EVs and MBVs (and a system QC of commercially available NIST urine standard) separated well by unsupervised PCA analysis (Figure 3).

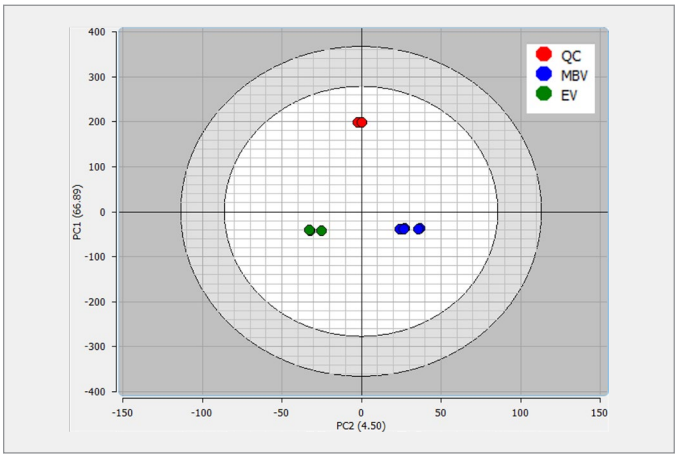

Figure 3: PCA plot showing clear separation between the EVs and MBVs

Features of interest were selected using a Volcano plot (Figure 4).

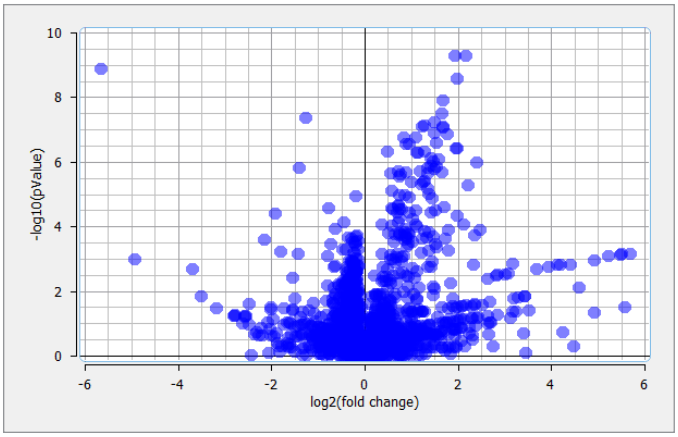

Figure 4: Volcano plot, allowing for visualization and selection of analytes contributing to group differentiation

Within metabolomic studies features of interest will predominantly be of low molecular weights, meaning mass accuracy tolerances can be exceeded with only slight mDa mass shifts. This can affect confidence when putatively identifying features of significance, separating sample phenotype.

The data were processed within MARS software investigating profile differences between the EVs and MBVs. MARS was able to putatively identify compounds of interest with sub-ppm mass accuracy (Figure 5).

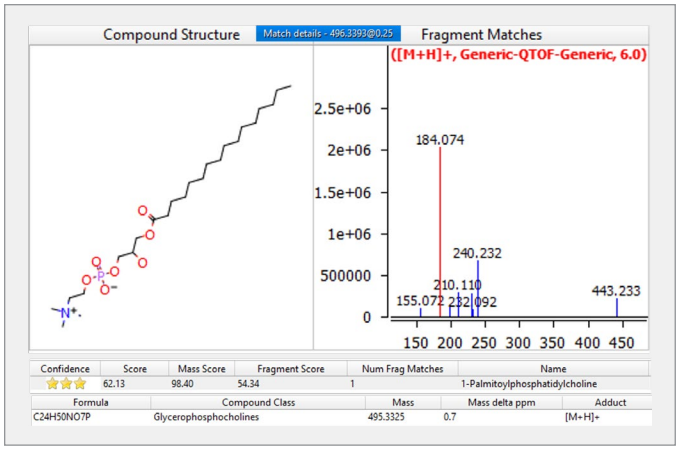

Figure 5: 1-Palmitoylphosphatidylcholine (LPC 16:0) putatively identified within MARS at 0.7 ppm mass accuracy

The main classes of features identified as significant for sample differentiation were predominantly upregulated in the EVs, and consisted of Glycerophospholipids, Fatty Acyls, Glycerolipids, and Carboxylic Acids.

CONCLUSIONS:

Here we demonstrated the combination of HILIC chromatography on an ACQUITY™ Premier System, coupled to the Xevo MRT MS for the rapid, accurate metabolomic analysis of biological samples. The Xevo MRT MS is a routine benchtop system providing mass resolution of up-to 100,000 FWHM and sub ppm mass accuracies.

The MARS software enables accurate multivariate statistical analysis and identification of features of interest through database searches.

The workflow described allows for the rapid acquisition and processing of metabolomic data. Offering a powerful solution suited to discovery OMICS application questions.

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