Exploration of Single Cell Lipidomics with a Novel Multi-reflecting Q-Tof Platform

Scarlet A Ferrinho[1, 2]; Preeti Mourya[1]; Shazneil Briones[1]; Nyasha Munjoma[2]; Richard Lock[2]; David Heywood[2]; Robert S Plumb[2]; Lee A Gethings[2]; Olivier Cexus[1]; Paul A Townsend[1]
[1]University of Surrey, Guildford, United Kingdom; [2]Waters Corporation, Wilmslow, United Kingdom

Introduction

The development of cutting-edge mass spectrometers with unbeknownst sensitivity, resolution and speed has led to single-cell OMICs gaining significant momentum in recent years.

Here we present a single-cell lipidomic workflow, established with lipid standards and cultured human cell lines using an ACQUITY™ Premier liquid chromatography (LC) system coupled to a Xevo™ MRT Mass Spectrometer. We investigate a variety of column conditions and MS parameters for increased chromatographic resolution, speed, sensitivity and robustness.

Optimisation

Parameter investigation

- Column chemistry: **C18**, C30, C8
- Column length: 50, **100**, 150 mm
- Column diameter: **2.1** and 1 mm
- Gradient length: 3.7, 6, **12**, 19, 28, 29 mins
- Flow rates: 0.1-**0.4** ml/min
- Scan speeds of 1, 5, **10** , 20 Hz

A dilution series of EquiSPLASH® (Avanti Polar Lipids) at a concentration of 0.5-1000 ng/mL was used to benchmark LC and MS performance with varying parameters. Conditions highlighted in **bold** were chosen for subsequent experiments.

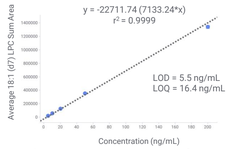


Figure 1: The average sum area of 18:1 d7 LPC at a concentration of 1000 – 5 ng/ mL. 1 µL injection, 12-minute gradient, 1 Hz, C18 CSH™ (1.7 µm 2.1x100 mm).

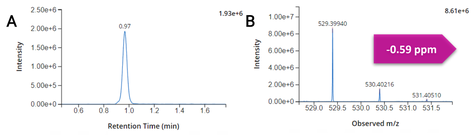
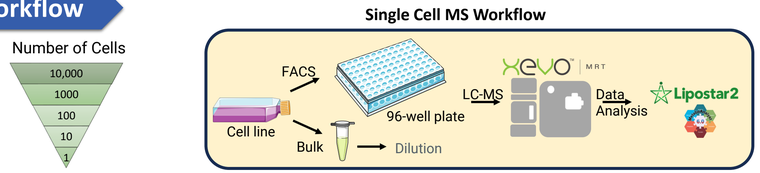


Figure 2: (A) Extracted ion chromatogram (EIC) of 18:1 d7 LPC and (B) corresponding mass spectrum. 50 pg, 12-minute gradient, 10 Hz, MS^E, C18 CSH (1.7 µm 2.1x100 mm).

Workflow



THP-1, C1R and Jurkat cell lines were chosen for investigation. Bulk cells were extracted in IPA containing EquiSPLASH (100 ng/mL) and diluted to a concentration of 10,000, 1000, 100,10 and 1 cell/ µL.

In addition, cells were dispensed into 96-well plates by fluorescence activated cell sorting (FACS) in numbers of 1, 10 and 100 cells per well and were extracted in IPA containing EquiSPLASH (10 ng/mL).

The lipidome was explored using the Xevo MRT MS, and data analysis was performed with software packages including Progenesis™ QI (Waters Corporation), MetaboAnalyst[1], and Lipostar (MassAnalytica™).

Results

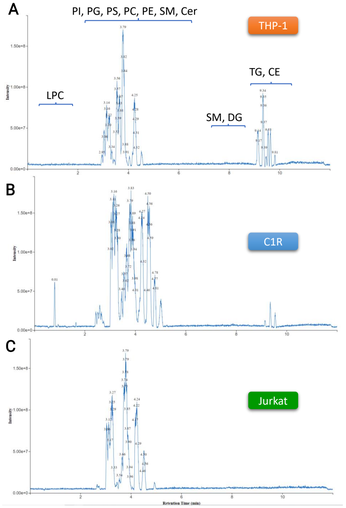


Figure 3: BPI chromatograms of (A) THP-1, (B) C1R and (C) Jurkat (10,000 bulk cell extract) cells.

1 The BPI of THP-1, C1R and Jurkat shows a clear difference in the nature and abundance of various lipid classes (Figure 3). The different profiles of these cells is also corroborated by PLS-DA plots (not displayed) which reveal a discrete placement of these cell types in relation to one another in addition to the blank, media, internal standards and QC samples.

2

2 Furthermore, a clear separation could be seen between 1, 10 and 100 cells for both the bulk cell dilution and cells prepared by FACS (Figure 4).

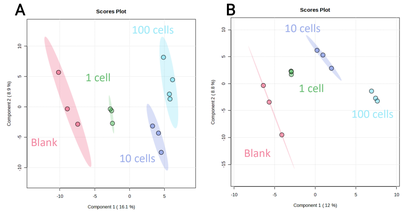


Figure 4 (A) PLS-DA plot of C1R cells prepared by FACS, and (B) PLS-DA plot of C1R cells prepared by a bulk cell dilution. Lipids were identified by progenesis QI, and statistical analysis and image creation was achieved with MetaboAnalyst.

3

3 Lipostar annotated various lipid classes with high confidence as exemplified by TG 52:4 in Figure 5 which had a confidence score of 89.76.

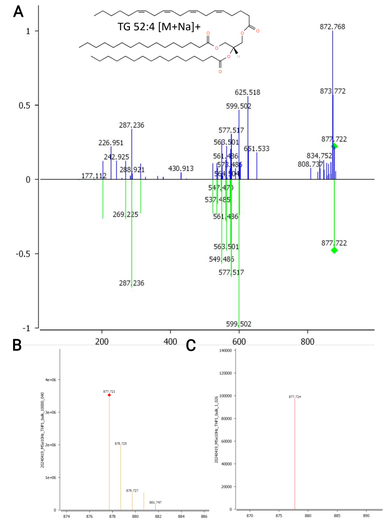


Figure 5: (A) Matched fragmentation (blue, raw MS2, green theoretical) of TG 52:4 with a confidence score of 89.76. THP-1 bulk cell dilution annotated using Lipostar with the MS/MS validator tool. (B) MS1 data from THP-1 (10,000 cells) and from (C) THP-1 (1 cell).

4

4 Annotated lipids could be explored from the raw data using LC-MS Toolkit as exemplified by PC 36:2 (Figure 6) which could be seen across the dilution series.

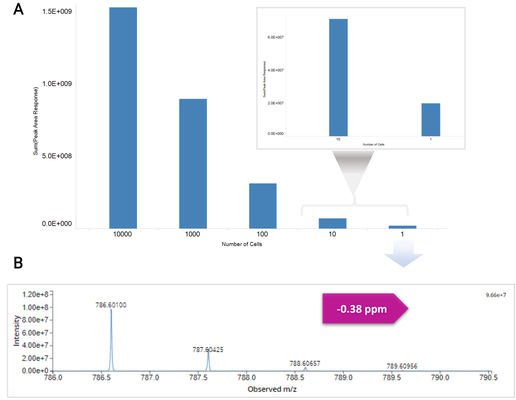


Figure 6: (A) The sum intensity of PC 36:2 ([M+H]+, calculated m/z 786.6007) of a diluted bulk cell extract from 10,000 cells to a single cell from THP-1. (B) Representative MS1 spectrum of PC 36:2 is also shown with a sub 1 ppm mass accuracy of 380 ppb.

5

5 Lipid features from THP-1 (10,00 cells) highlights the in-sample dynamic range of lipids extracted from the cell, and the capacity to detect these over 5 orders of magnitude using the Xevo MRT MS as shown in Figure 7.

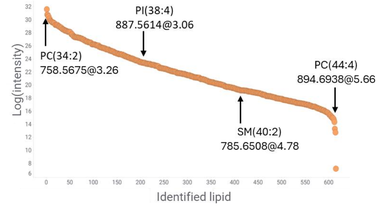


Figure 7: Dynamic range of lipids present within THP-1 cells (10,000 cells) as detected using the Xevo MRT MS. The quantitation curve consists of lipid features with tentative identifications, which were identified using an in-house database.

Conclusion

- Single cell lipidomics is achievable on the Xevo MRT MS with excellent resolution, sensitivity, dynamic range and mass accuracy.
- FACS provides a streamline workflow to prepare cells for single cell MS.
- A streamlined LC-MS workflow is presented that allows for single cell analysis on various cell types.

This work has been funded by Innovate UK (Knowledge Transfer Partnerships) and Waters Corporation.

1- Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A., MacDonald, P., Wishart, D., Li, S., and Xia, J. (2024) MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation Nucleic Acids Research (doi: 10.1093/nar/gkae253)

UNIVERSITY OF SURREY | Waters™ | Innovate UK | Knowledge Transfer Partnerships