HIGH THROUGHPUT LC/MS PLATFORM FOR LARGE SCALE SCREENING OF BIOACTIVE LIPIDS IN HUMAN PLASMA/SERUM

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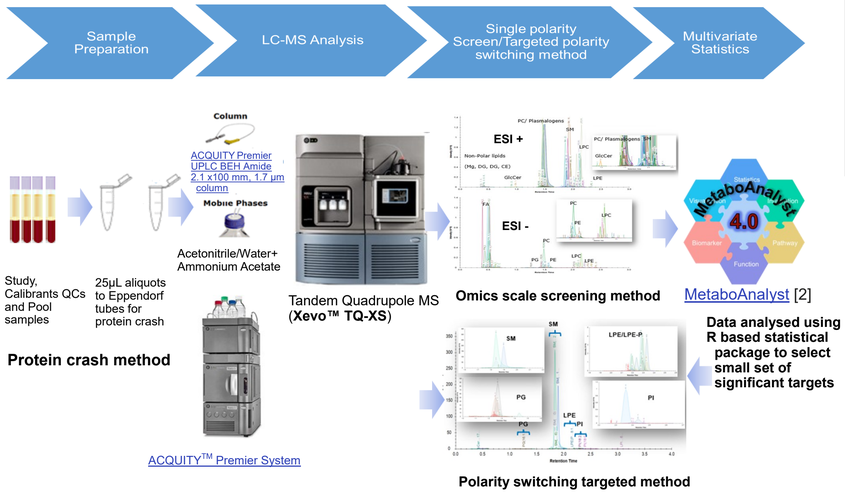
INTRODUCTION

Lipids play a key role in many biological processes and their accurate measurement is critical to unravelling the biology of diseases and human health. Currently, lipidomic analysis has two general approaches; i) targeted analysis, often used to study signal processing and hypothesis-driven pathway analysis or ii) global analysis (untargeted) approaches used to obtain a qualitative view of changes in the lipidome. The strategies/methodologies employed are dependent on the aims and scope of the underlying research question and have their advantages and disadvantages. As described by Cajka and Fiehn [1], these approaches can be characterised based on the number of detected lipids and the reliability of quantification. To aid this, a high throughput method for the semi quantitative screening of over 2000 lipids has been developed. Using a library of over 4000 MRM transitions, we describe an accessible, robust, high throughput semi-quantitative omics screen for the phospholipid components of human plasma/serum lipidomes. This methodology integrates many of the advantages of global lipid analysis with those of targeted approaches. We also demonstrate how, having used the method as an initial screening, it can easily be adapted for more targeted analysis and accurate quantification for a prostrate cancer study.

METHODS

The Liquid Chromatography, Mass Spectrometry and TargetLynx™ Processing Files can be downloaded via the Waters Targeted Omics website. The Targeted Omics Library contains over 2000 lipids which can be used to generate methods using the Quanpedia file provided. In order to implement these methods, a BEH™ Amide Column and ACQUITY™ UPLC™ System are required for chromatographic separation. This solution can be used with any of the available Waters tandem quadrupole instruments. Commercially available standards can be used as internal standards and calibrants as described in the downloadable LipidQuan™ User Guide.



Targeted Omics Library used to generate methods
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Key Points

- Lipids eluted according to class via HILIC chromatography, reducing isomeric/isobaric interferences and the number of calibrant and internal standards required for quantification (i.e., cost reductions).
- For the assay described here a two-stage approach was employed
  - i. Single polarity screens for approximately 500 pre-selected bioactive lipids
  - ii. Polarity switching method derived from stage one, from which the bioactive lipids can be more accurately quantified

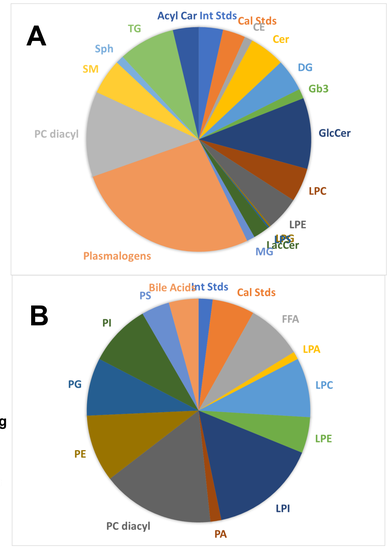


Figure 1. Initial omics scale screens of approx.450 lipids in positive (431 MRM transition) and negative electrospray ionization mode (446 MRM transition) were assessed for reproducibility, sensitivity, and dynamic range using analysis times of 8 minutes

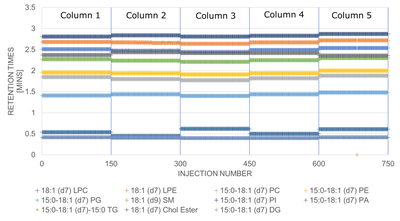


Figure 2. Evaluation of column to column retention time variability. 150 injections on each of the five different batches of stationary phases were performed . There were small differences between the mean retention times obtained for the different batches of 1.7µm ACQUITY BEH Amide Stationary Phase (though there was good reproducibility for the data for each column

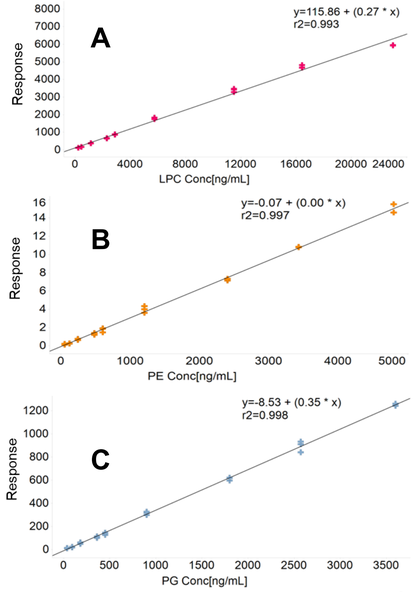


*Figure 3. Example standard calibration curves representing LPC (ES+) R^2= 0.99 (**A**), LPE (ES+) R^2= 0.99 (**B**) and PG (ES-) R^2= 0.99 (**C**)*

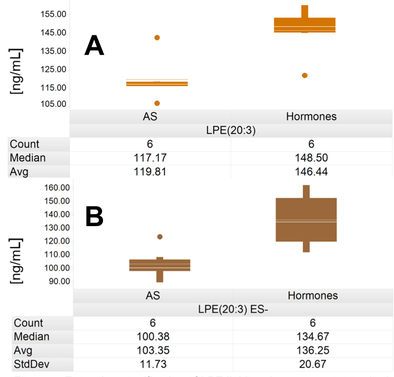
RESULTS

Robustness testing to assess retention time reproducibility and the impact of different batches of stationary phase on retention times was performed as part of the method development process (Figure 2).

Method validation was based as far as practicable on the FDA "Guidance for Industry" on Bioanalytical methods Intra- and inter-day reproducibility, accuracy, dynamic range, stability, carry over, dilution integrity and matrix interferences were assessed for the omics scale screen and the polarity switching method. Example calibration curves from the polarity switching method shown in Figure 3. Intra day mean biases and precision of the omics screening methods shown in Figure 4.

PROSTRATE CANCER APPLICATION

- Prostrate cancer (PCa) is 40% of all cancers worldwide,[3] but blood tests for prostate-specific antigens (PSA) are known to be less accurate[4,5]
- Lipids have been identified as potential PCa biomarkers [6,7] e.g. LPEs
- Application of LipidQuan Targeted Lipidomics Workflow to detect and quantify key lipids as a proof of concept



A	AS	Hormones
	LPE(20:3)	
Count	6	6
Median	117.17	148.50
Avg	119.81	146.44

B	AS	Hormones
	LPE(20:3) ES-	
Count	6	6
Median	100.38	134.67
Avg	103.35	136.25
StdDev	11.73	20.67

*Figure 5. Example quantification of LPE lipids using surrogate standards LPE(17:1). The box plots show upregulation of LPE(20:3) in hormone therapy treated patients (n=6) vs. Prostate cancer active surveillance (AS) patients (n=6) in positive (**A**) and negative modes (**B**)*

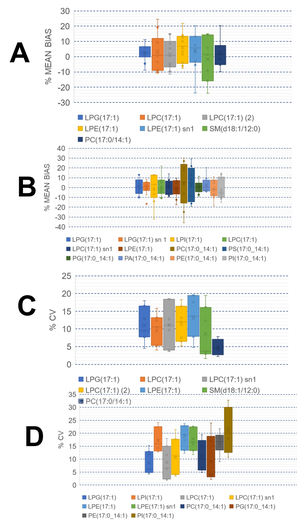


Figure 4: Intra-day % mean biases of the calibration standards for the positive ion (n=3) (A) and negative ion (n=3) (B) methods is provided. Intra-day QC CVs for positive mode CVs (n=6 for each point on the bar) (C) and negative (D). Failed ULOQ and LLOQC CVs in negative mode were excluded.

CONCLUSION

- The method showed >3-orders of magnitude linearity and sufficient sensitivity to detect lipids at endogenous levels in human plasma/serum
- The short analysis time makes the methodology ideal for the analysis of large cohorts typically observed in population "omics" studies
- LC methodology was demonstrated as being robust for 1500+ injections and was transferrable across columns prepared from different batches of the same stationary phase.
- Methodology applied to prostate caner cohort to differentiate between treatment therapies

References

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