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 Caike and Fiehn [1], these approaches can be characterized 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.

METHODS

The Liquid Chromatography, Mass Spectrometry and TargetOmics® Processing Files can be downloaded via the Waters® TargetOmics website. The Targeted Omics Library contains over 2000 lipids which can be used to perform semi-quantitative screens 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, using the method as an initial screening, it can easily be adapted for more targeted analysis and accurate quantification for a prostate cancer study.

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

PROSTATE CANCER APPLICATION

Prostate cancer (PCa) is 40% of all cancers worldwide [2] but blood tests for prostate-specific antigens (PSA) are known to be less accurate [3]. Lipids have been identified as potential PCa biomarkers [4,5,6,7]. Application of the TargetOmics® LipoQuantTM Targeted Lipidomics Workflow to detect and quantify key lipids as a proof of concept.

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 studies. LC methodology was demonstrated as being robust for 1500+ injections and was transferable across columns prepared from different batches of the same stationary phase. Methodology applied to prostate cancer cohort to differentiate between treatment therapies.

References


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