LipidQuan

A TARGETED LIPID PROFILING SOLUTION

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INTRODUCTION

Although advances in mass spectrometry (MS) have allowed for more in-depth lipdomic analysis, unambiguous identification and quantification has proven difficult as lipids exhibit a high number of isomeric and isobaric species. Furthermore, MS spectra often contain peaks and fragments from multiple compounds making confident identification and relative quantification of specific molecular species difficult and time consuming. As a result, the transfer of lipidomic data between laboratories is severely hindered, making multi-site study interpretation problematic.

A hydrophilic interaction chromatography (HILIC) based approach for the separation of lipids by class prior to MS analysis is a proven method of reducing identification ambiguity. An additional benefit of separating lipid species by class is that fewer stable isotope labelled (SIL) standards are required for quantification, conferring a cost saving.

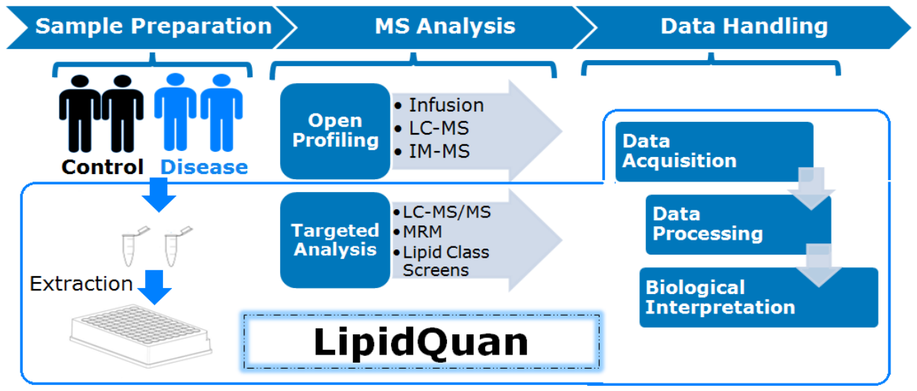


***Figure 1**: General lipidomic workflow used in most research laboratories, with the LipidQuan workflow highlighted.*

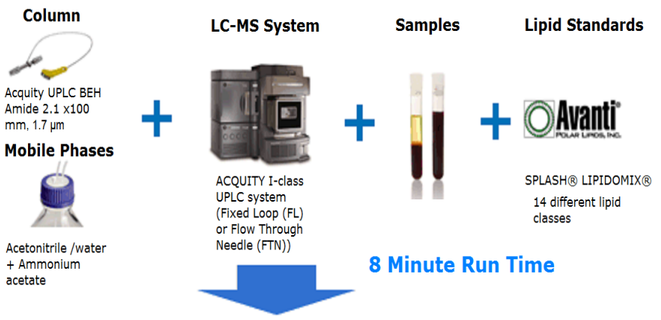
KEY FEATURES

- Streamlined and integrated lipidomics workflow (from sample preparation through to biological interpretations)
- Highly specific MRM transitions based on the fatty acyl chain fragments when applicable instead of the typical head group fragments to improve identification and specificity
- Routine targeted quantification of common lipids in plasma and serum
- Lipid class based separation reduces the number of stable isotope lipid standards (SILS) which results in significant cost saving

Sample Preparation

METHODS

Over 2000 lipid species MRMs and a selection of screening method application notes available for download @ www.waters.com/LipidQuan



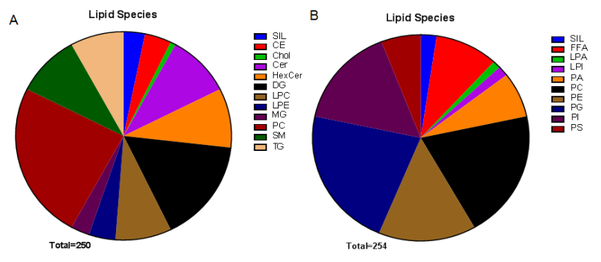


***Figure 2**: (Top) Screenshots of method Application Notes available for download at Waters Targeted Omics. (Bottom)**A**-Lipid species coverage for curated positive mode Plasma Screen . **B** -Lipid species coverage for negative mode*

ROBUST LC CONDITIONS

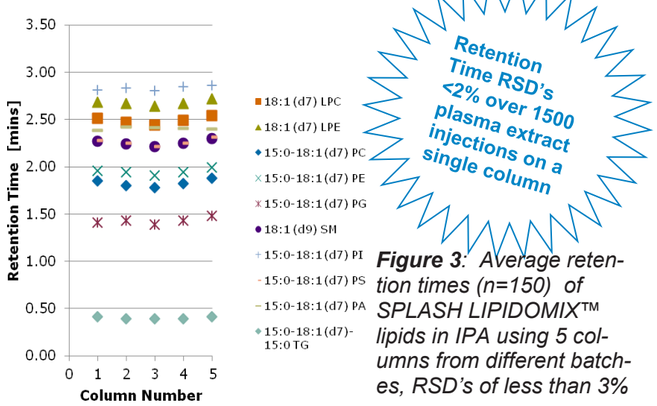


***Figure 3**: Average retention times (n=150) of SPLASH LIPIDOMIX™ lipids in IPA using 5 columns from different batches, RSD's of less than 3%*

MS Analysis

CHROMATOGRAPHY

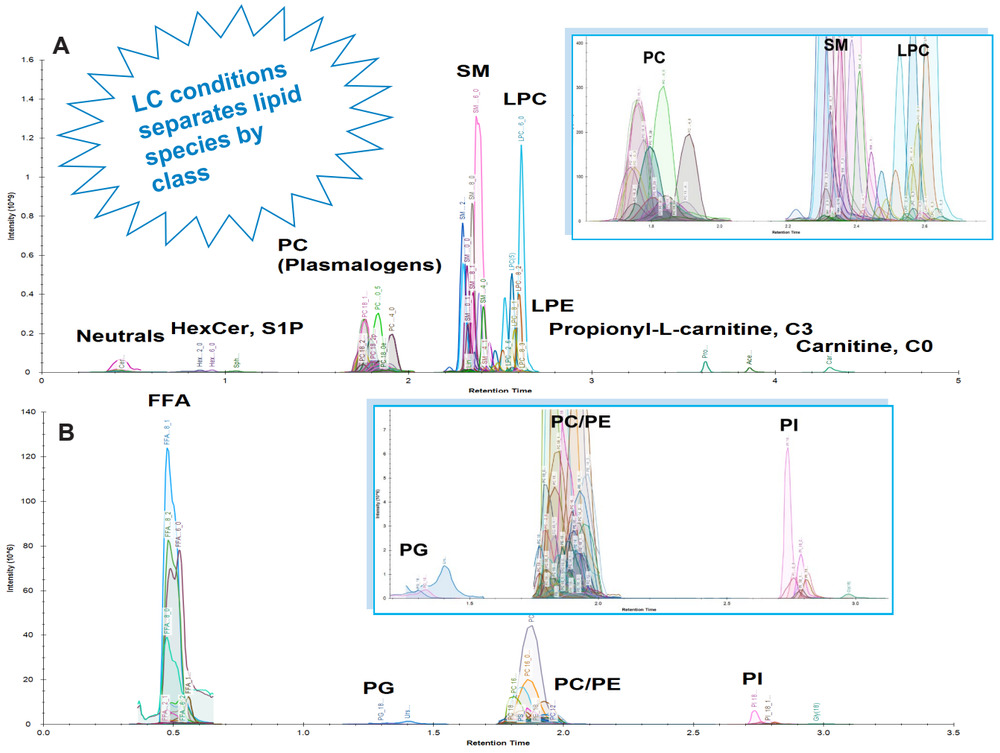


***Figure 4**: Example chromatogram of plasma samples analysed using LipidQuan platform. **A** is a positive mode screen (with zoomed insert) and **B** is a negative mode screen (with zoomed insert) of various classes described in Figure 2*

IMPROVED LIPID IDENTIFICATION

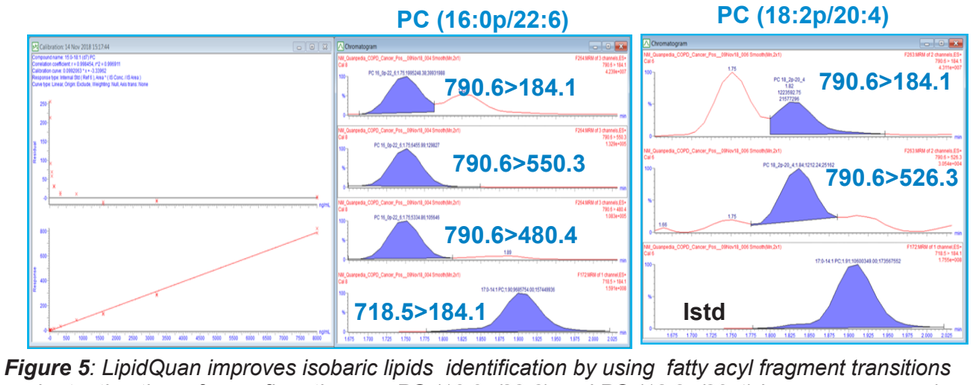


***Figure 5**: LipidQuan improves isobaric lipids identification by using fatty acyl fragment transitions and retention times for confirmation e.g. PC (16:0p/22:6) and PC (18:2p/20:4) have precursor m/z 790.6 and can not be distinguished using only the head group transition (m/z 184.1)*

Data Handling

MULTI-VARIATE STATISTICS

Data processing can be performed using TargetLynx or open source software such as Skyline. Since lipids of the same class elute in discreet bands, stable isotope lipid standards can used for more accurate qauntification of endogenous lipids. Multi-variate statistics can be performed on processed data using packages such as SIMCA-P+ (Umetrics) or MetaboAnalyst through a Symphony data pipeline.

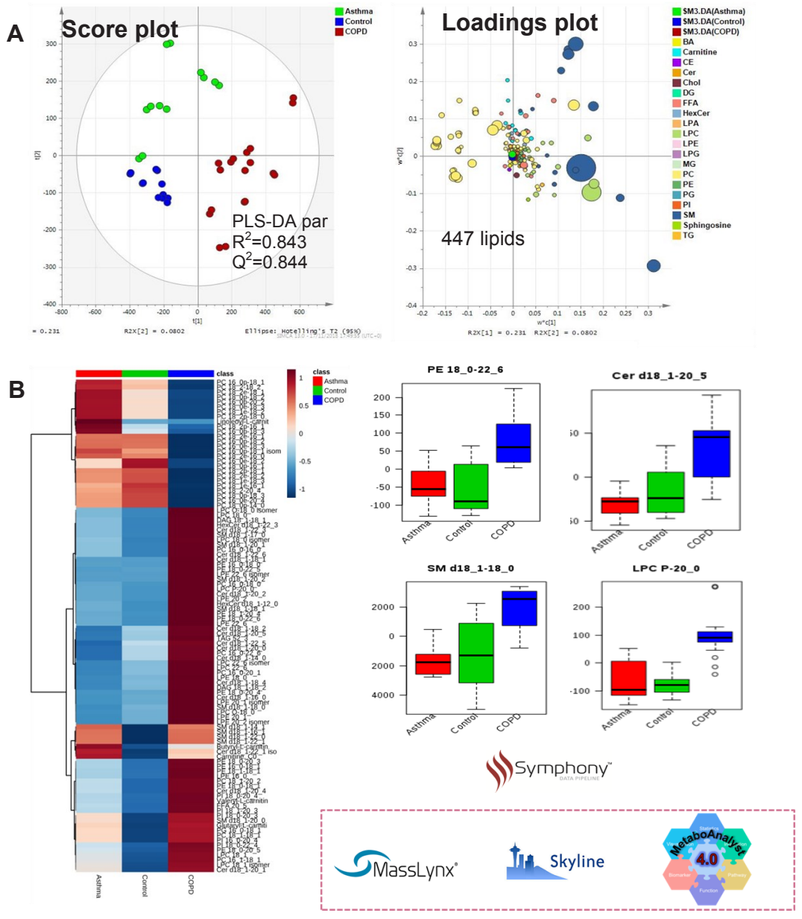


***Figure 6** : LipidQuan data from a COPD/Asthma study was statistically analysed using SIMCA-P+ **A**) and Metaboanalyst (**B**) statistical packages via Symphony data pipeline to enable biological interpretations*

Acknowledgement

The authors would like to thank Steve Lai (Waters) for help with method development as well as J Will Thompson (Assistant Research Professor of Pharmacology & Cancer Biology (Duke University)) for advice and constructive comments.