QUANTIFYING THE LIPIDOME FOR RESPIRATORY DISEASE: A RAPID AND COMPREHENSIVE HILIC-BASED TARGETED APPROACH

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HIGHLIGHTS

- **Comprehensive and robust high-throughput HILIC-based LC-MS/MS method with over 2000 MRMs.**
- **Highly specific MRM transitions based on the fatty acyl chain and headgroup fragment ions.**
- **Lipid class based separation reduces the number of stable isotope lipid standards (SILS) which results in significant cost saving.**

INTRODUCTION

Respiratory linked conditions associated with chronic obstructive pulmonary disease (COPD), asthma, and infection are increasing with significant associated socio-economic costs.

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.

Here we describe a comprehensive and high-throughput HILIC-based LC-MS/MS method for the separation and quantitation of both polar and non-polar lipid classes (Figure 1); (www.waters.com/targetedomics).

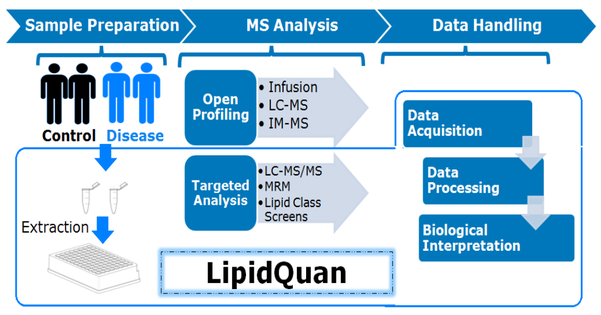

Figure 1. *General lipidomics workflow used in most research laboratories, with the LipidQuan workflow highlighted.*

METHODS

SAMPLE PREPARATION

A simple sample preparation procedure was adopted using protein precipitation with a pre-cooled isopropanol (IPA) at 4 ºC (1:5, plasma:IPA).

INSTRUMENT CONDITIONS

LC Conditions:

LC system:	ACQUITY UPLC I-Class with FTN or Fixed Loop		
Column:	ACQUITY UPLC BEH Amide (2.1x100mm, 1.7 µm)		
Column temp:	45°C;	Injection volume: 2 µL	
MP A:	95/5 ACN/Water (10 mM ammonium acetate)		
MP B:	50/50 ACN/Water (10 mM ammonium acetate)		
Gradient:	0.1% to 20.0% B for 2 minutes, then 20% to 80% B for 3 minutes followed by 3 minutes re-equilibration		

MS Conditions:

MS systems:	Xevo TQ-XS or Xevo TQ-S micro		
Ionization mode:	ESI (+/-);	Capillary voltage:	2.8kV (+)/1.9kV (-)
Acquisition mode:	MRM		
Source temp.:	120 °C;	Desolvation temp.:	500 °C
Cone gas flow:	150 L/hr;	Desolvation flow:	1000 L/hr

INFORMATICS

A LipidQuan Quanpedia method file that contains the LC conditions, MS method (with over 2000 MRM transition), and associated TargetLynx processing method (including retention times) was generated.

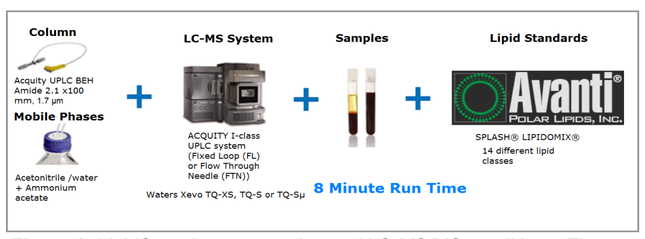

Figure 2. *LipidQuan instrumentation and LC-MS/MS conditions. The LipidQuan Quanpedia method file contains LC conditions, MS method with over 2000 MRM transitions and processing methods.*

RESULTS

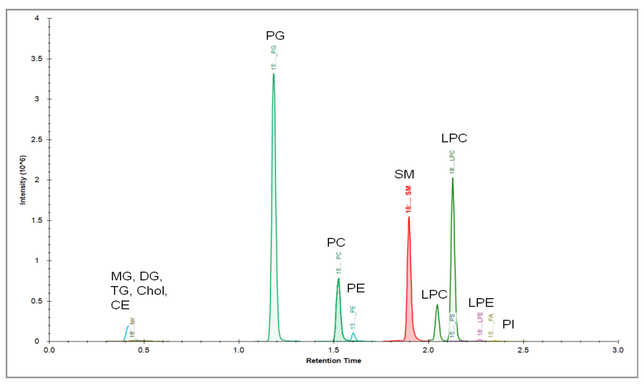

Figure 3. *Positive ion mode chromatogram representing HILIC separation of the SPLASH LIPIDOMIX™ lipid standard mixture.*

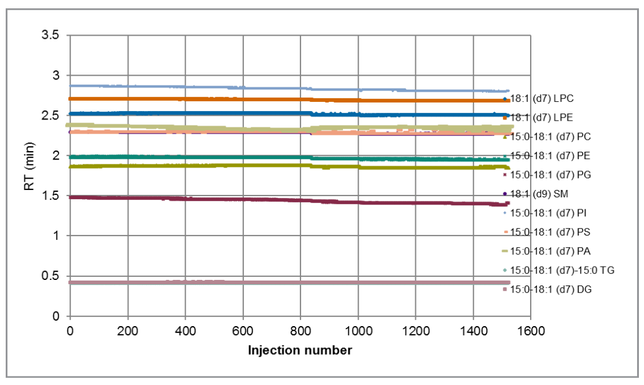

Figure 4. Average retention time (n=1500) *of SPLASH LIPIDOMIX™ lipid standard mixture spiked into NIST 1950 plasma with RSD's <2%.*

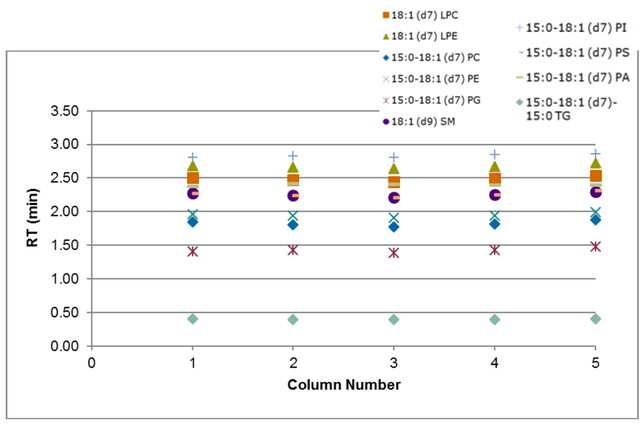

Figure 5. *Average retention time (n=1500) of SPLASH LIPIDOMIX™ lipid standard mixture in IPA using five columns from different batches with RSD's <2%.*

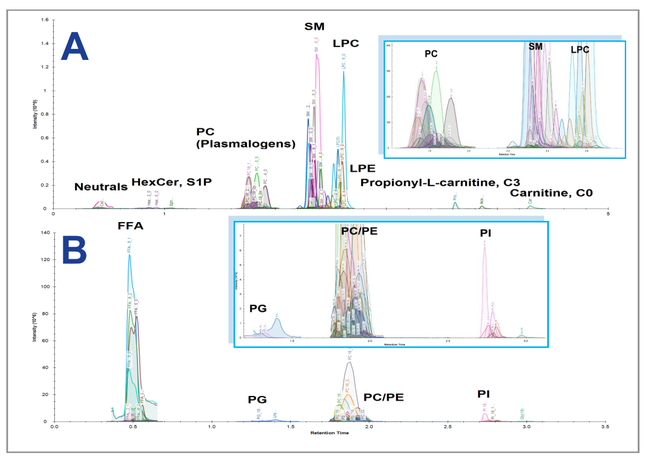

Figure 6. *Example chromatogram of plasma samples analysed using LipidQuan platform.* ***(A)*** *Positive mode screen (with zoomed insert) and* ***(B)*** *Negative mode screen (with zoomed insert) of various lipid classes.*

RESULTS

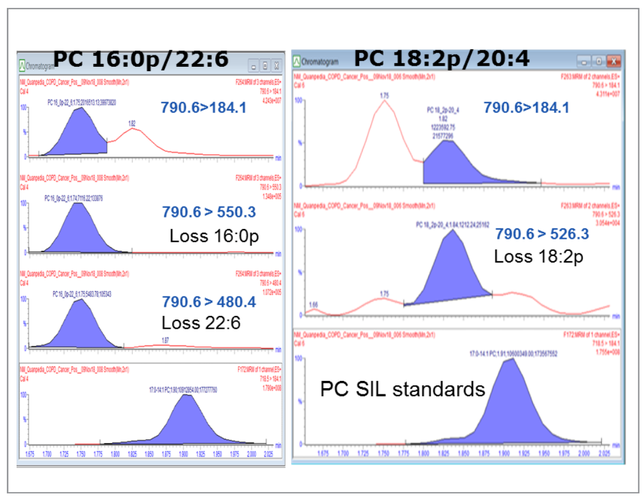

Figure 7. *LipidQuan improves isobaric lipid species identification by using both fatty acyl and headgroup MRM transitions for confirmation. Example, PC (16:0p/22:6) and PC (18:2p/20:4) have precursor m/z 790.6 and can not be distinguished using only the head group transition (m/z 184.1).*

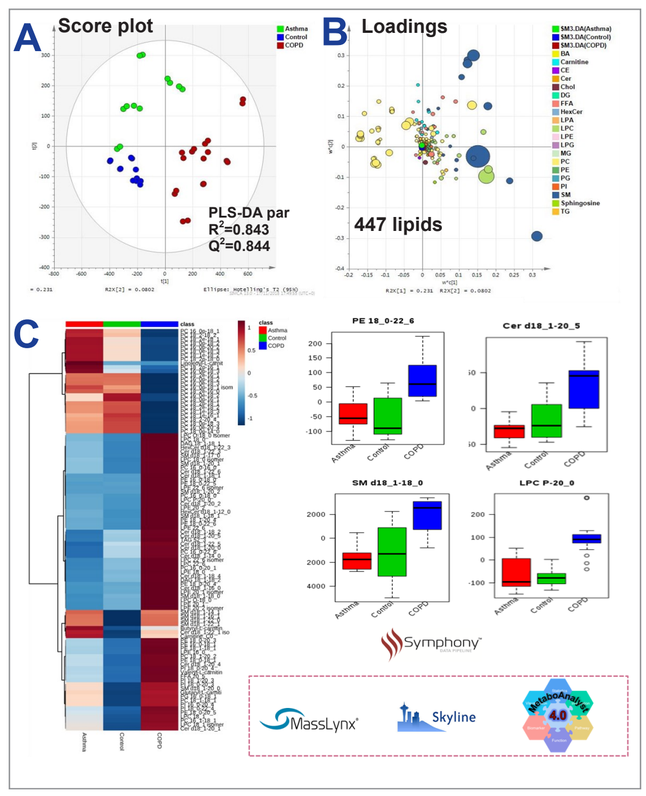

Figure 8. *LipidQuan data from a COPD/Asthma study was statistically analysed using* ***(A)*** *SIMCA-P+ and* ***(B)*** *Metaboanalyst statistical packages via Symphony data pipeline to enable biological interpretations. (C) Hierarcjical clustering of the top 100 lipid species (ANOVA/t-test with FDR < 1%) highliting the average differential expression acroos the three groups. The box plots show example of altered lipid species on the different cohort samples.*

CONCLUSION

- **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.**
- **Fast data processing using TargetLynx or open source software such as Skyline.**
- **Data visualization using SIMCA-P+ (Umetrics) or MetaboAnalyst.**

Acknowledgements

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References

1. Munjoma, N., Isaac, G., Plumb, R., Gethings, L., (2019) Quantifying the Lipidome for a Respiratory Disease Study Using LipidQuan: A Rapid and Comrehensive Targeted Approach., Application Note (720006542EN).
2. Isaac, G., Munjoma, N., Gethings, L., Plumb, R., (2018) LipidQuan for Comprehensive and High-Throughput HILIC-based LC-MS/MS Targeted Lipid Quantitation., Application Note (720006402EN).

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