

INTER-LABORATORY REPRODUCIBILITY OF A TARGETED LIPIDOMICS PLATFORM FOR ANALYSIS OF HUMAN SERUM AND PLASMA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Nyasha Munjoma¹, Giorgis Isaac², Ian D Wilson¹, Lee Gethings¹ and Robert Plumb²
¹Waters, Wilmslow, UK. ²Waters, Milford, USA

INTRODUCTION

- A general and undisputed dogma of clinical chemistry is that the levels of metabolites circulating in blood plasma are reflective of various aspects of organism homeostasis[1]
- The combination of simplified lipidomic analytical protocols, rapid developments in mass spectrometry technology, and the wide range of potential clinical and biomedical applications suggests a bright future for plasma lipidomics
- Despite the overall success to date, many researchers recognise current community practices make it difficult to harmonize published data and/or make them amenable to multi-omics approaches
- Development is also hindered by lack of communication between research and clinical communities as there is no system in place to assess and cross-correlate plasma lipidomic profiles obtained by different laboratories in various clinical settings
- Furthermore, data is often reported in arbitrary units (ion counts of peak intensity or area) even though quantification of molecule numbers (moles) is necessary for the calculation of the fraction of lipid classes and vital for the detailed interpretation and comparison of large datasets in multi-laboratory studies [2]
- Many in the lipidomics community recognised the need for standardised performance verification parameters and quality control measures for the determination of data quality since batch to batch variations are inherent characteristics of high-throughput analytics

METHODS

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

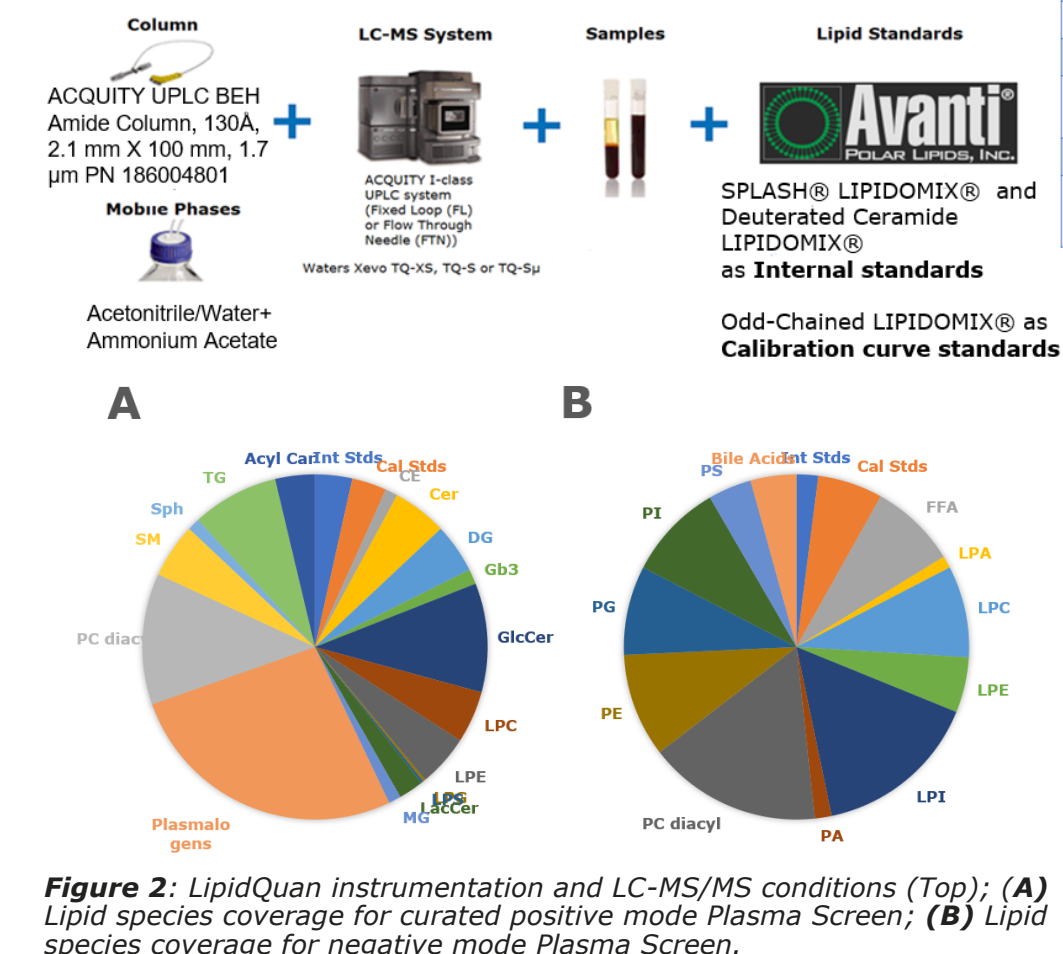


Figure 2: LipidQuan instrumentation and LC-MS/MS conditions (Top); (A) Lipid species coverage for curated positive mode Plasma Screen; (B) Lipid species coverage for negative mode Plasma Screen.

LIPIDQUAN KEY FEATURES

- Robust and easy to deploy platform, reducing method development and training costs, using Quanpedia™ and dedicated SOPs.
- Rapid LC gradient (8 minute run time), fast data processing and visualization using TargetLynx™ software and third party informatics (i.e Skyline, Metaboanalyst for maximum flexibility).
- Improved identification and specificity using MRM transitions based on the fatty acyl chain fragments when applicable as well as the typical head group fragments.
- Sample preparation and data processing can readily be automated.
- Faster and more cost effective than comparable workflows.

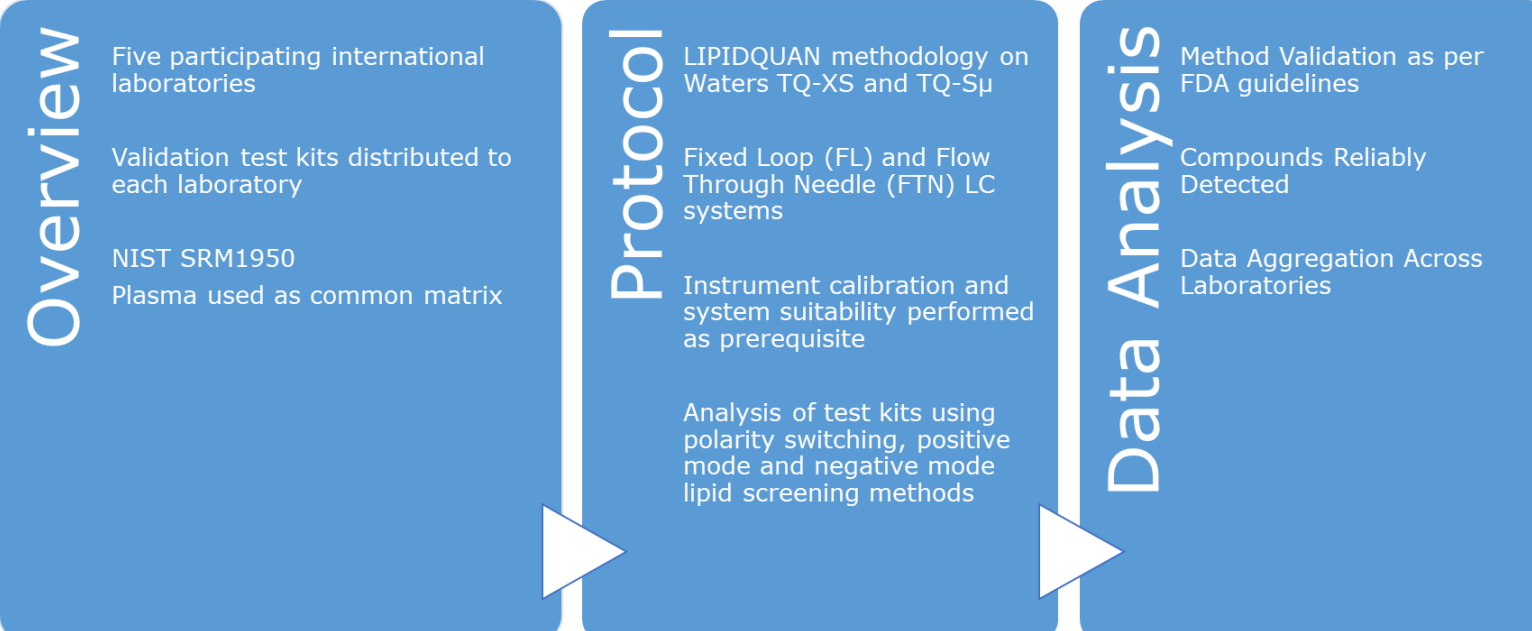


Figure 1: LIPIDQUAN platform interlaboratory cross validation study design

METHOD VALIDATION

Intra day validation assessments of the method were evaluated for various lipid classes using a polarity switching method, positive mode screen (431 MRM transitions) and negative mode screen (446 MRM transitions). A range of analytical attributes were investigated, including linearity, intra- and inter-day accuracy and precision, lower and upper limits of quantification (LLOQ, ULOQ), specificity, carry-over, matrix and other interferences.

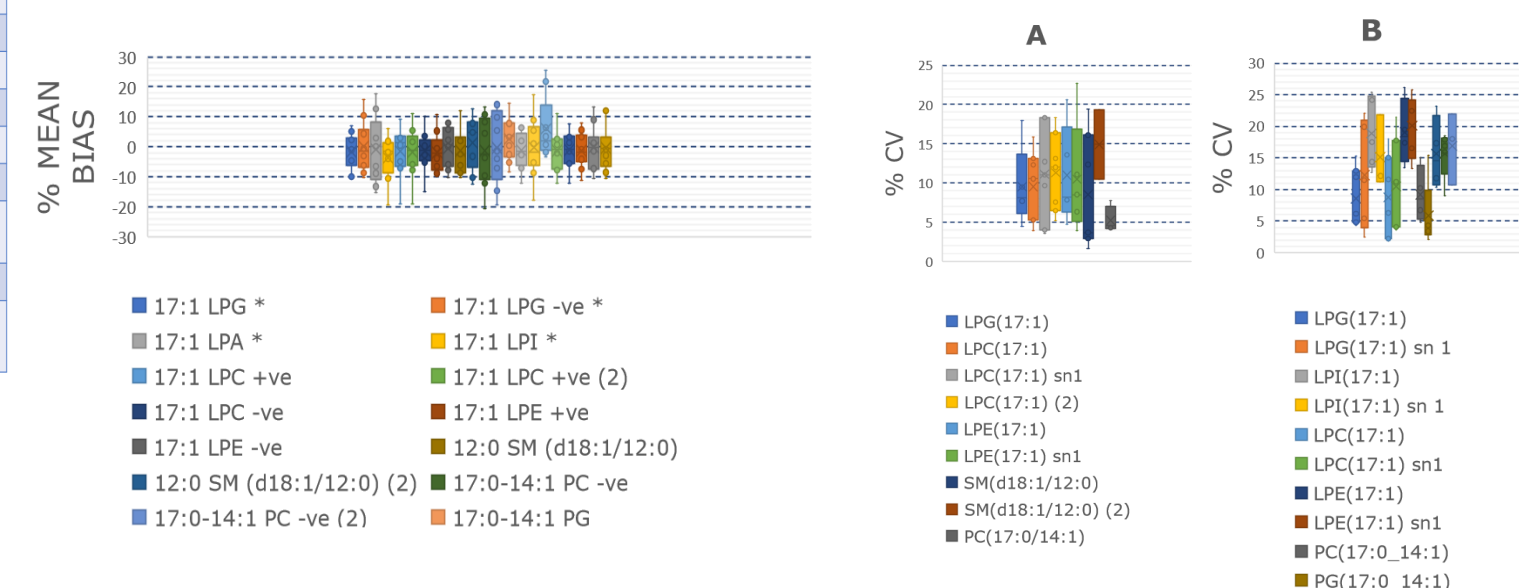


Figure 4 : Accuracy: Intra-day % mean biases of the calibration standards for the polarity switching method

Figure 5 : Precision: Intra day QC positive mode screen method CVs (n=6 for each point on the bar) (A) and the negative mode screening method CVs (B).

QUANTIFICATION OF NIST SRM 1950 USING LIPIDQUAN

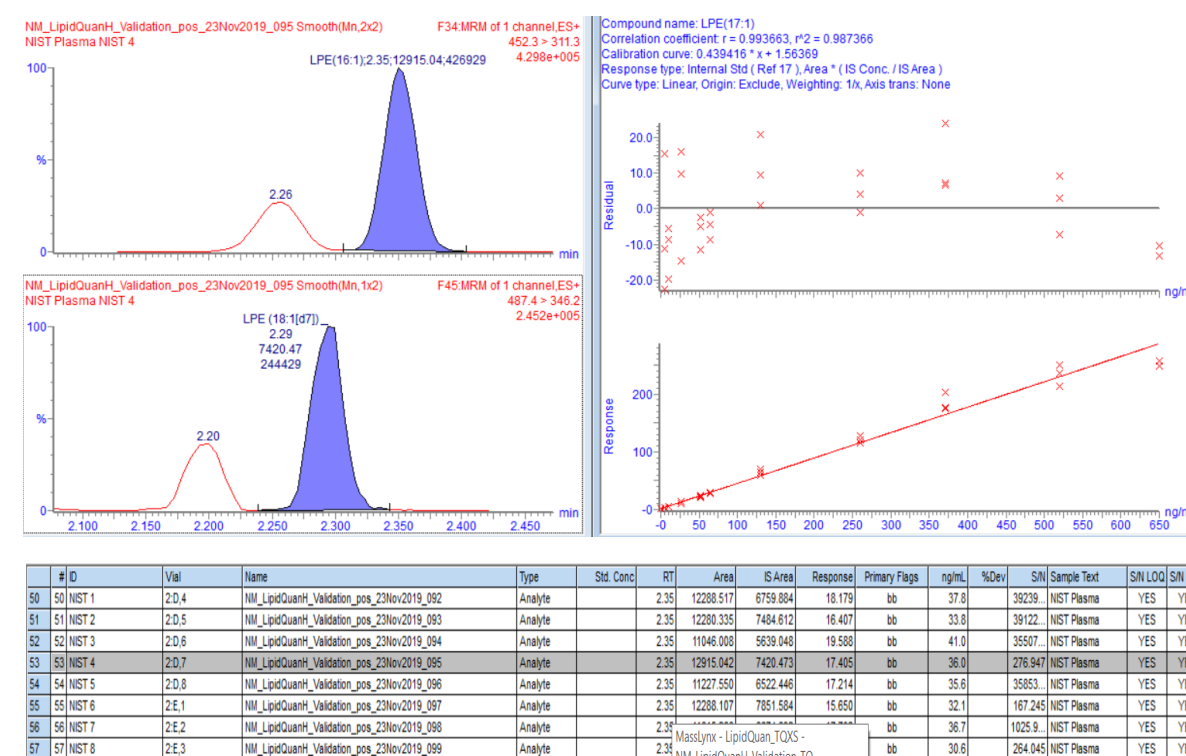


Figure 6: Quantification with TargetLynx™ uses the calibration curve and internal standards to quantify endogenous lipid species of the same class in test samples e.g NIST plasma. In this example, LPE(16:1) in NIST SRM 1950 was quantified based on the response of the LPE 17:1 calibration standards.

DATA AGGREGATION

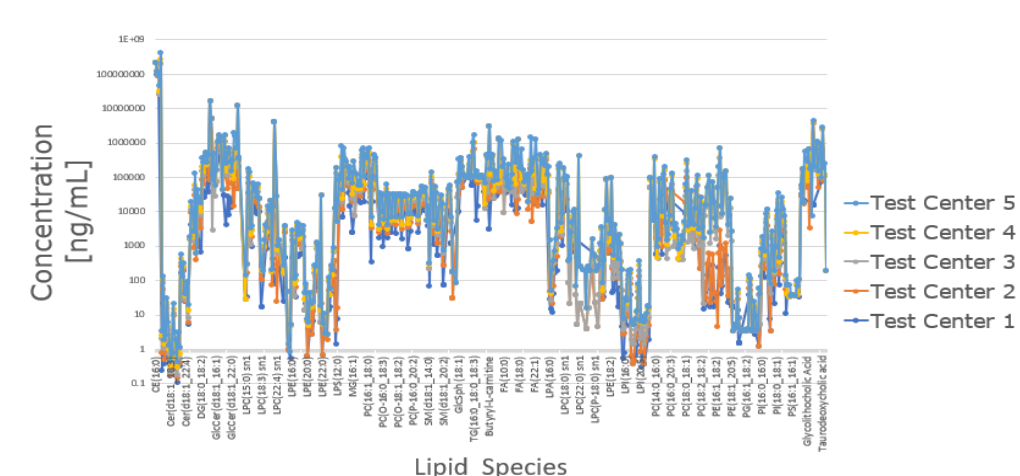


Figure 7 : Overlay of average concentrations for lipid species detected in NIST SRM 1950 (n=8) at the participating laboratories, showing good correlation of the results.

REFERENCES

- Vvedenskaya, O., Wang, Y., Ackerman, J. M., Knittelfelder, O., & Shevchenko, A. (2019). Analytical challenges in human plasma lipidomics: A winding path towards the truth. *TrAC Trends in Analytical Chemistry*, 120, 115277. <https://doi.org/10.1016/j.trac.2018.10.013>
- Burla, B., Arita, M., Arita, M., Bendt, A. K., Cazenave-Gassiot, A., Dennis, E. A., Ekroos, K., Han, X., Ikeda, K., Liebisch, G., Lin, M. K., Loh, T. P., Meikle, P. J., Orešič, M., Quehenberger, O., Shevchenko, A., Torta, F., Wakelam, M. J. O., Wheelock, C. E., & Wenk, M. R. (2018). MS-based lipidomics of human blood plasma - a community-initiated position paper to develop accepted guidelines. *Journal of Lipid Research*, 59, Jlr.S087163. <https://doi.org/10.1194/jlr.S087163>