Optimization and Evaluation of Global LCMS Lipidomics for use in Clinical Diagnostics

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Introduction

Global lipidomics in clinical diagnostics can provide valuble insigth into lipid metabolism, lipid signaling, and lipid-associated diseases. Global lipidomics is however restricted by the analytical challenges posed by the chemical diversity of lipids. Clinically relevant lipids exhibit substantial variations in both LogP values and concentration ranges, sometimes spanning several orders of magnitude. To be effectively employed as a diagnostic tool in clinical settings, a global lipidomics method must meet several criteria: it should accurately annotate a wide array of lipids, exhibit robustness, and possess sufficient sensitivity to detect lipids of clinical relevance.

Global Lipidomics LCMS Method

In this study, we present an optimized global lipidomics method leveraging liquid chromatography mass spectrometry (LCMS), with a C30 column. For sample preparation 2-propanol (IPA) was used as extraction solvent.

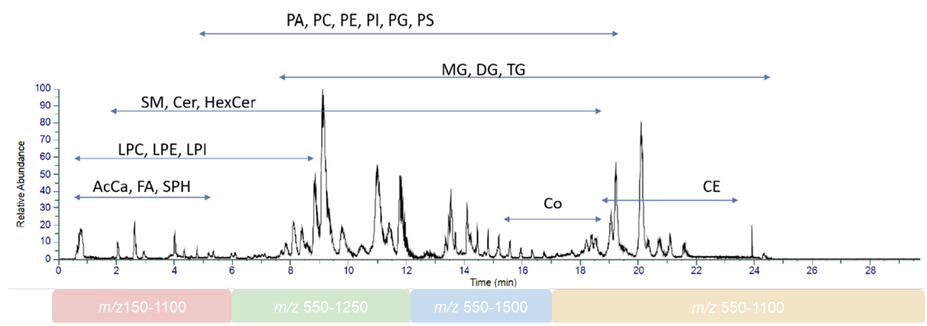

Figure 1: Elution windows for the different lipid sub classes including scheduled DDA *m/z* ranges.

Optimization and Evaluation

Optimization: The method, initially set up based on Jankevics et al 2021 (1), was optimized using an Equisplash (Avanti Lipids) and human serum. Human serum was used to establish the elution time frames, seen in **Figure 1**. A comparison of serum from a healthy volunteer, analyzed using DDA (Top 5 and 10) and scheduled DDA (Top 5 and 10) in positive and negative ionization mode showed that scheduled DDA was generally superior, see **Figure 2 (Right)**. Accordingly, scheduled DDA (Top 5) was selected as the optimal data acquisition method for the final clinical diagnostics method.

Evaluation: For evaluation, the Lipidomics Standard Initiative (LSI) guidelines (2) were followed, assessing the reproducibility of retention time and peak area in EDTA plasma, serum and DBS, for 90 lipid species across 50 patient samples. Overall the method showed a high degree of reproducibility. See **Figure 2 (Left)** for retention time repeatability. The evaluation gave knowlegde of the normal variation in lipidomes in different blood matrices typically used in a clinical setting, illustrated in **Figure 3**.

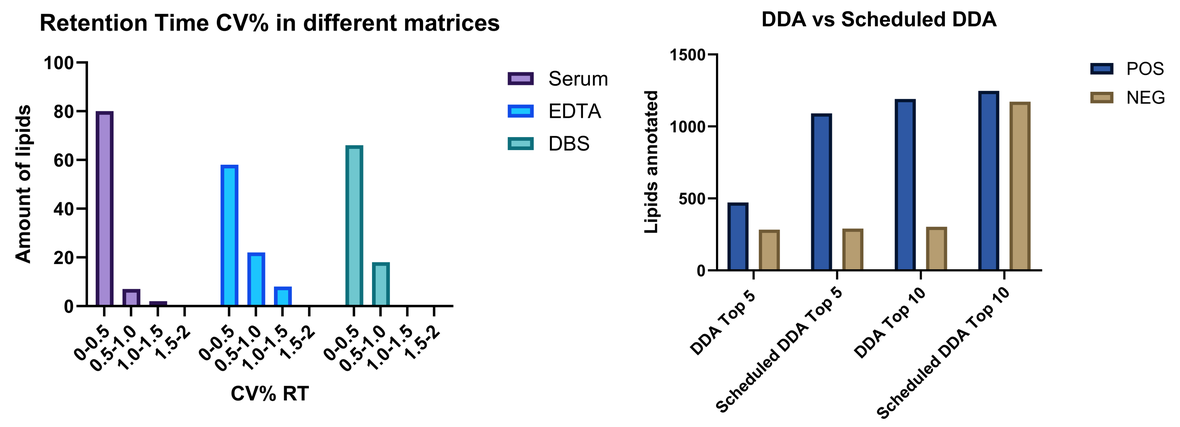

Figure 2: Left, retention time reproducibility in different matrices (N = 50 per matrix). Right, lipids annotated using regular DDA (Top 5 and 10) and scheduled DDA (Top 5 and 10) in positive and negative ionization mode.

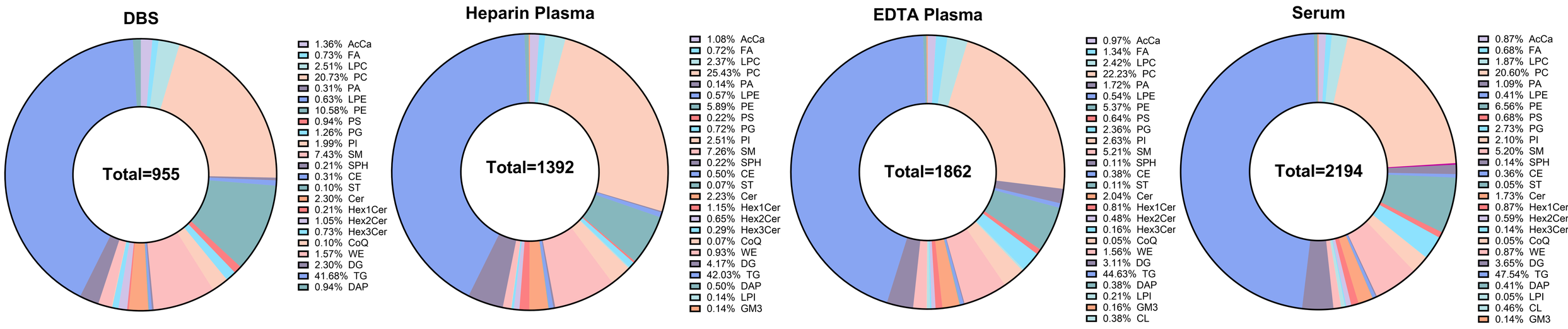

Figure 3: The observed lipidome in DBS, heparin plasma, EDTA plasma and serum, using the scheduled DDA (Top 5) method. N = 50 for DBS, EDTA plasma and serum, N = 20 for heparin plasma. The total number of lipids annotated is a combination of lipids annotated in positive and negative ionization mode. Lipids were annotated using LipidSearch (5.1) and the grade needed to be C or higher to be included.

Proof of Concept

To illustrate the methods usefulness in a clinical setting, we conducted a proof of concept study (manuscript in preparation) aimed at identifying postprandial lipid markers for the consumption of Calogen, a long chain triglyceride fat emulsion. This study included 8 healthy volunteers, EDTA plasma and DBS samples were taken at selected time points as seen in **Figure 4**. A differential analysis comparing samples taken before, and 4 hours after consumption of Calogen revealed a total of 342 significant features differentiating the two groups.

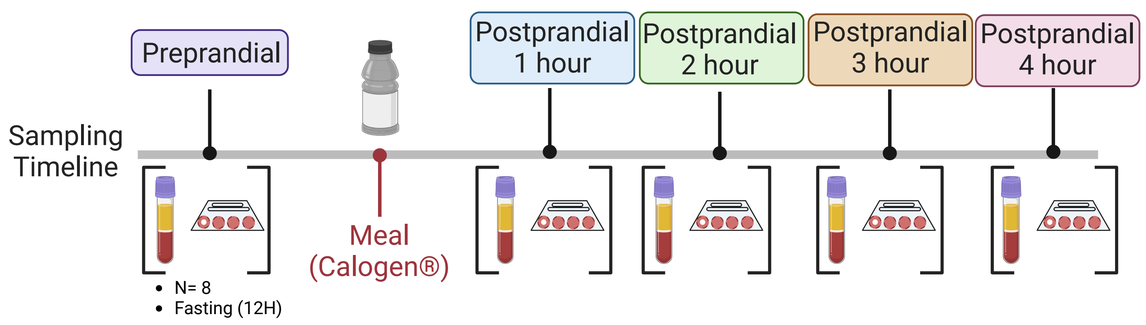

Figure 4: Study design for the Proof-of-Concept study, including sampling timeline and sampling timepoints.

The significant features were primarily from the triacylglycerol (TG), diacylglycerol (DG), fatty acid (FA), phosphoethanolamine (PE), and lyso-phosphoethanolamine (LPE) subclasses. The trends for TG(24:0_18:1_18:1) and LPE(18:1) is shown in **Figure 5**. The notable increase in TG and DG levels was expected as these lipids form part of the normal process of fat digestion, absorption, and storage. PE and LPE can increase due to cell membrane remodeling, as eating triggers insulin secretion which promotes nutrient storage and cell membrane remodeling.

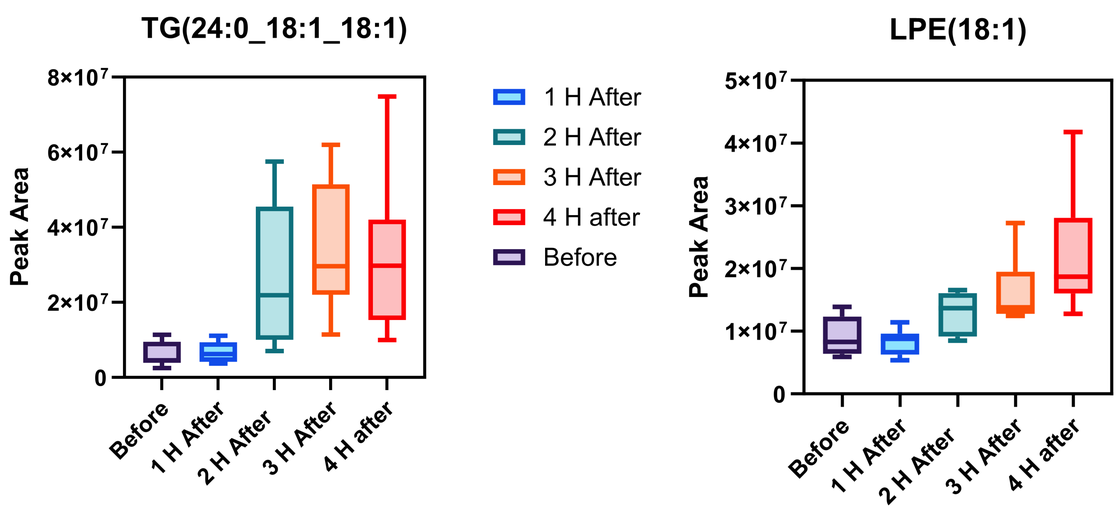

Figure 5: Boxplot of TG(24:0_18:1_18:1) (online match) and LPE(18:1)(match with analytical standard), N = 8 in each timepoint. These are only 2 of the many significant features in this study.

Limitations

Global lipidomics is a hypothesis generating approach. This method is only semi-quantitative, meaning that the results from the Proof-of-Concept study needs to be confirmed with a targeted method. Use of other organic solvents than IPA for lipid extraction could help increase the number of lipids detected in specific subclasses.

Conclusion

Scheduled DDA proved to outcompete regular DDA when it comes to amount of lipids annotated. A thorough evaluation of the methods reproducibility show satisfactory results for use in clinical setting. Twice as many lipids was annotated in serum compared to DBS. With the use of the scheduled DDA method the lipid features in the Proof-of-Concept study was annotated. These results showed significant alterations in TG, DG, PE, LPE and FA levels, highlighting the potential of our global lipidomics method in clinical diagnostics.

Referances

(1) Jankevics, A., Jenkins, A., Dunn, W.B., Najdekr, L,. An improved strategy for analysis of lipid molecules utilizing a reversed phase C30 UHPLC column and scheduled MS/MS acquisition. Talanta 2021, 229, 122262. https://doi.org/10.1016/j.talanta.2021.122262
(2) McDonald, J.G., Ejsing, C.S., Kopczynski, D. et al. Introducing the Lipidomics Minimal Reporting Checklist. Nat Metab **4**, 1086–1088 (2022). https://doi.org/10.1038/s42255-022-00628-3