**Supplemental Information for**

Single-step extraction coupled with targeted HILIC-MS/MS approach for comprehensive analysis of human plasma lipidome and polar metabolome

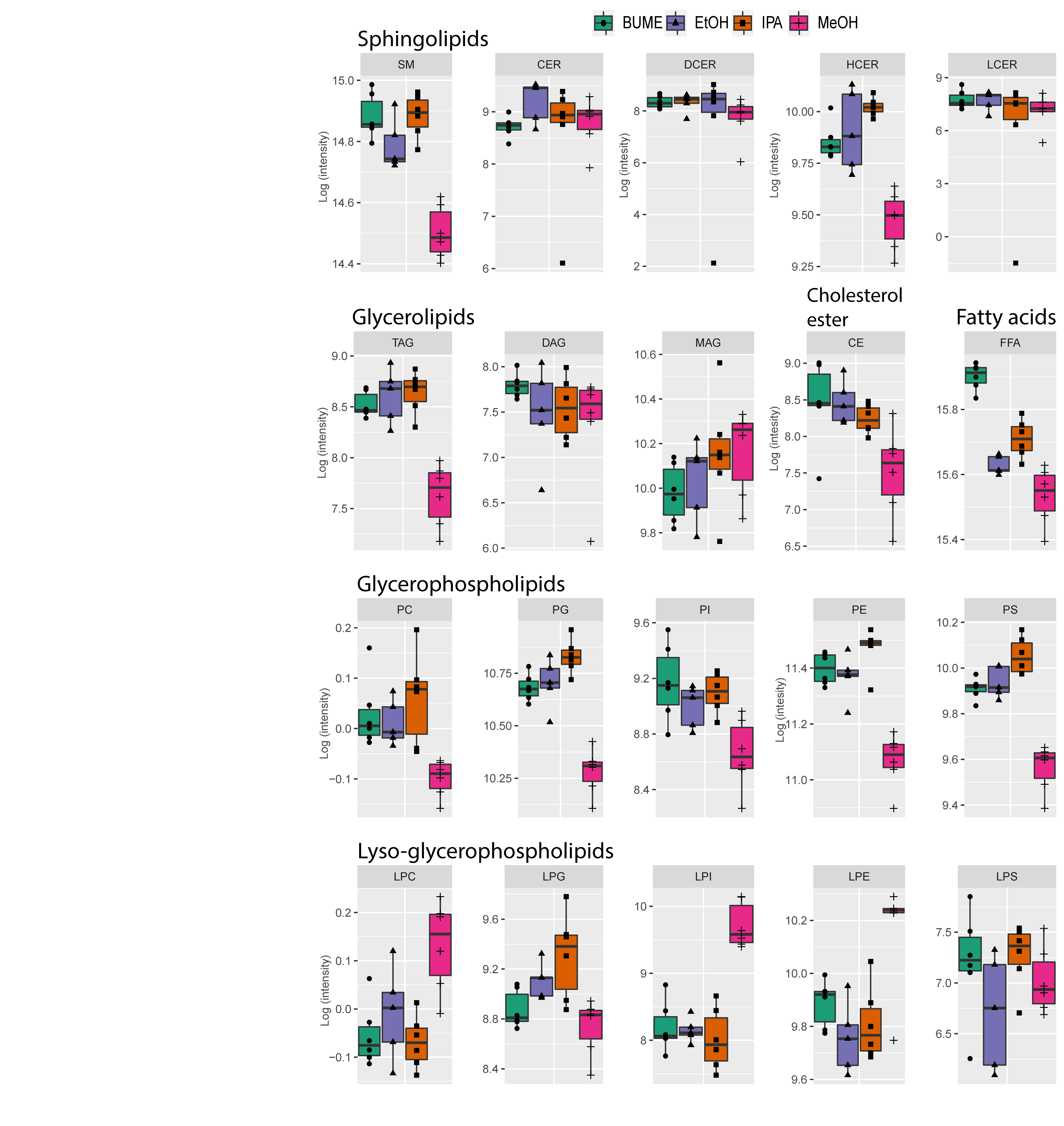
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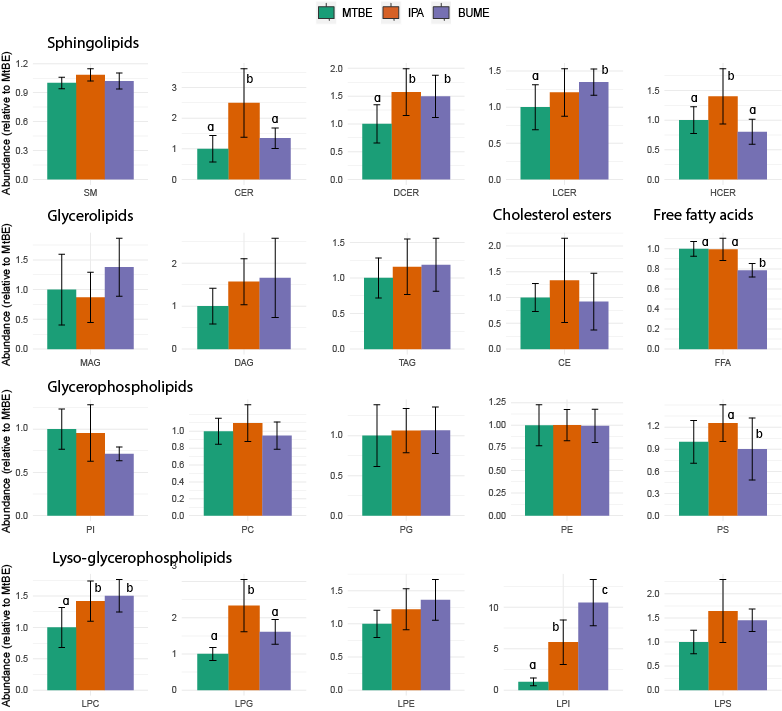
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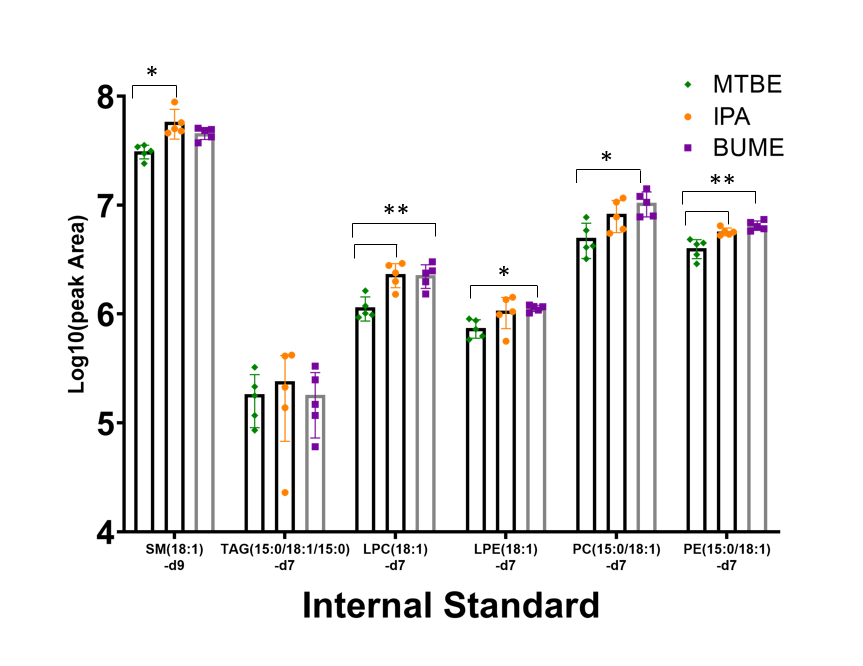
**Supplementary Materials**



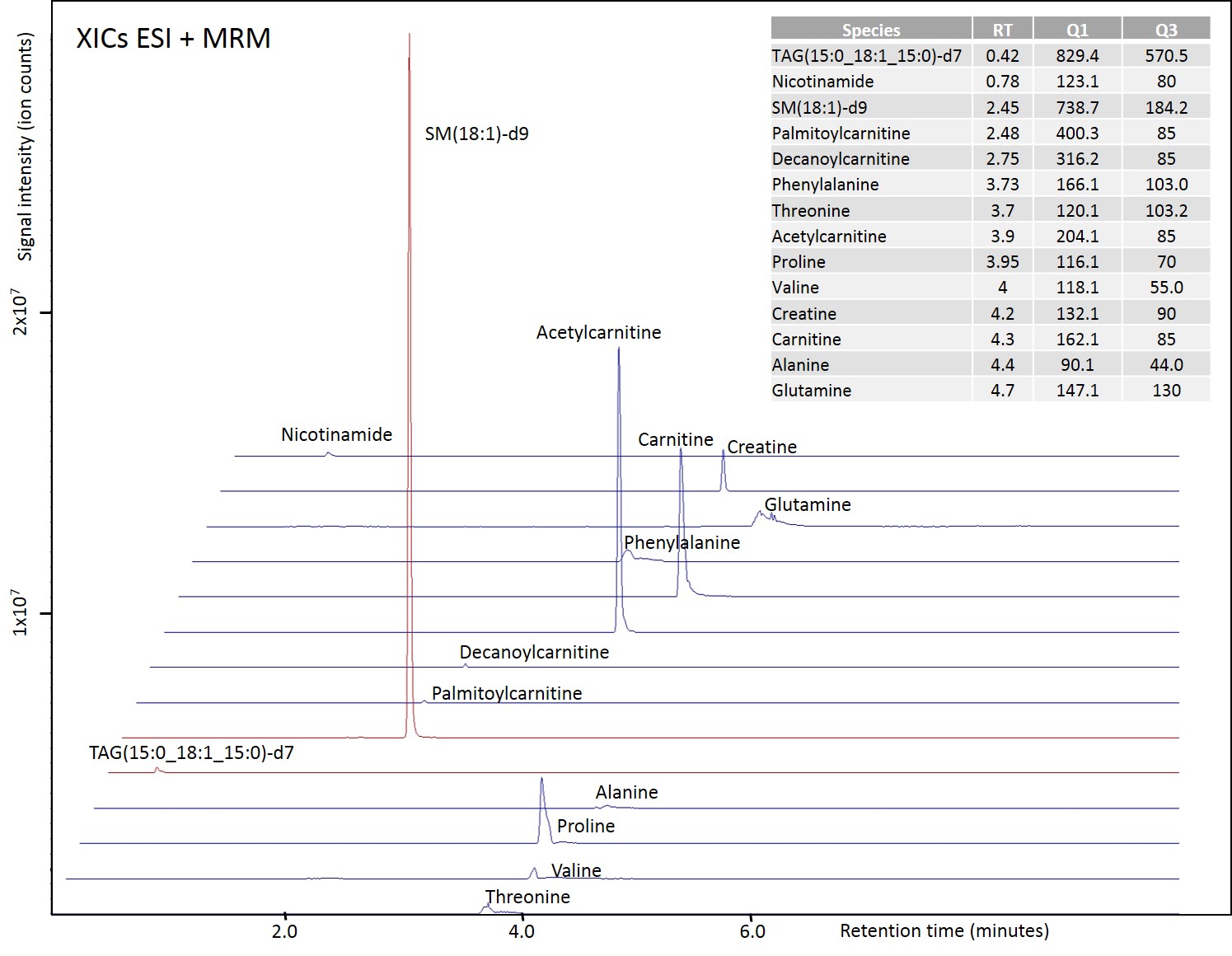
**Figure S1.** Relative signal abundance of different lipid classes in MeOH, EtOH, IPA and BUME extracts of human plasma



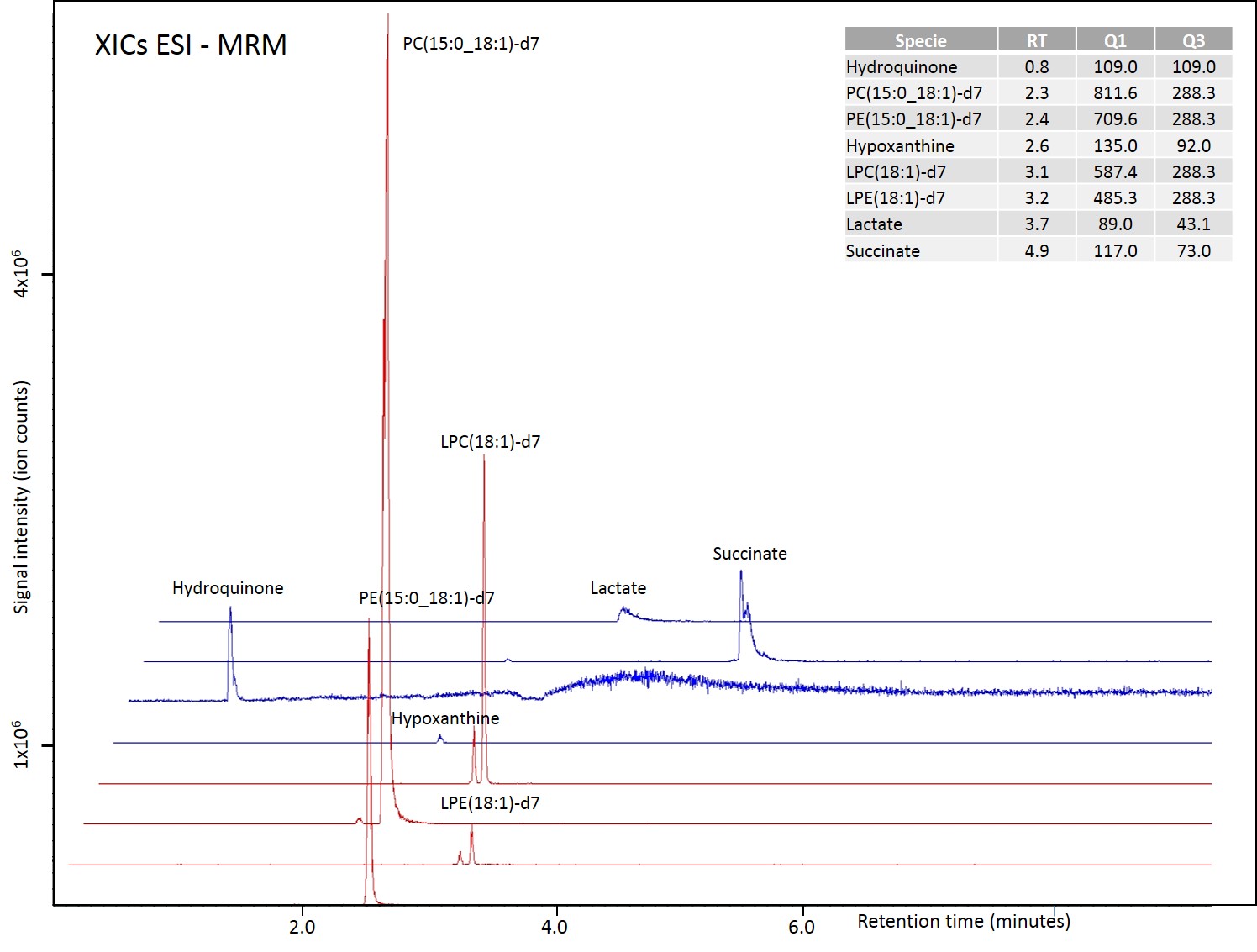
**Figure S2.** **Relative signal abundance (per lipid class) to signal recovered with biphasic extraction using MTBE.** Relative signal abundance is represented for each single-step method, using IPA or BUME, to the reference MTBE extract, and per lipid class. Error bars indicate ± SD and a, b, c labels indicate statistically different groups, no label means not significantly difference using the Tukey post hoc test after ANOVA with the significance level p < 0.05. Lipid classification abbreviations: SM-sphingomyelin, CER –ceramides, DCER-dihydroceramides, LCER-lactosylceramiedes, HCER-hexosylceramides, MAG-monoacylglycerol, DAG-diacylglycerol, TAG-triacylglycerol, CE-cholesterol esters, FFA- free fatty acids, PI- phosphatidylinositol, PC- phosphatidylcholine, PG-phosphatidylglycerol, PE phosphatidylethanolamine, PS- phosphatidylserine, LPC- lysophosphatidylcholine, LPG-lyso phosphatidylglycerol, LPE- lysophosphatidylethanolamine, LPI- lysophosphatidylinositol, LPS- lysophosphatidylserine.

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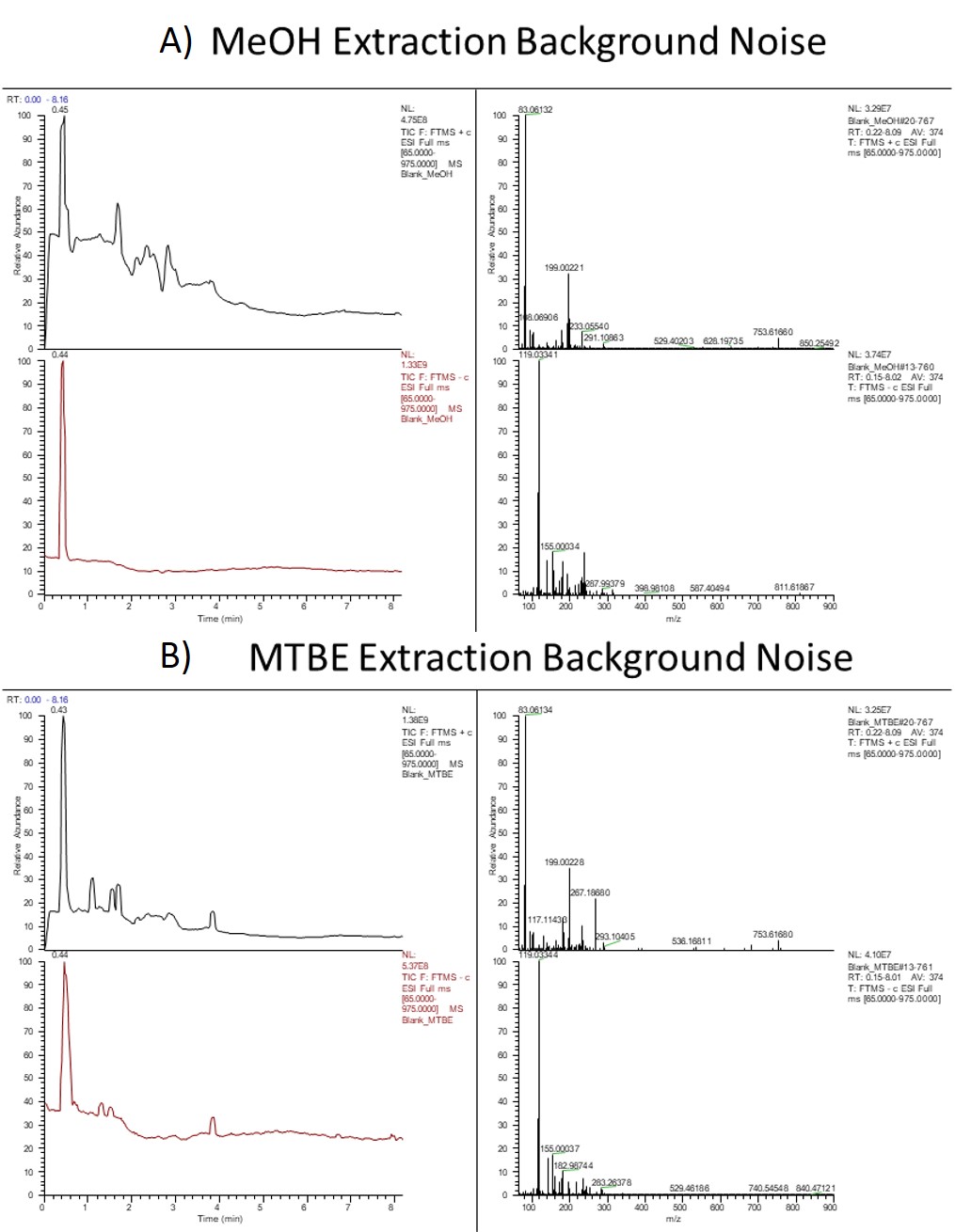
**Figure S3.** Signal abundance of internal standards (representing six lipid classes) spiked into plasma samples during single step IPA, BUME and biphasic Matyash extraction (organic MTBE phase).

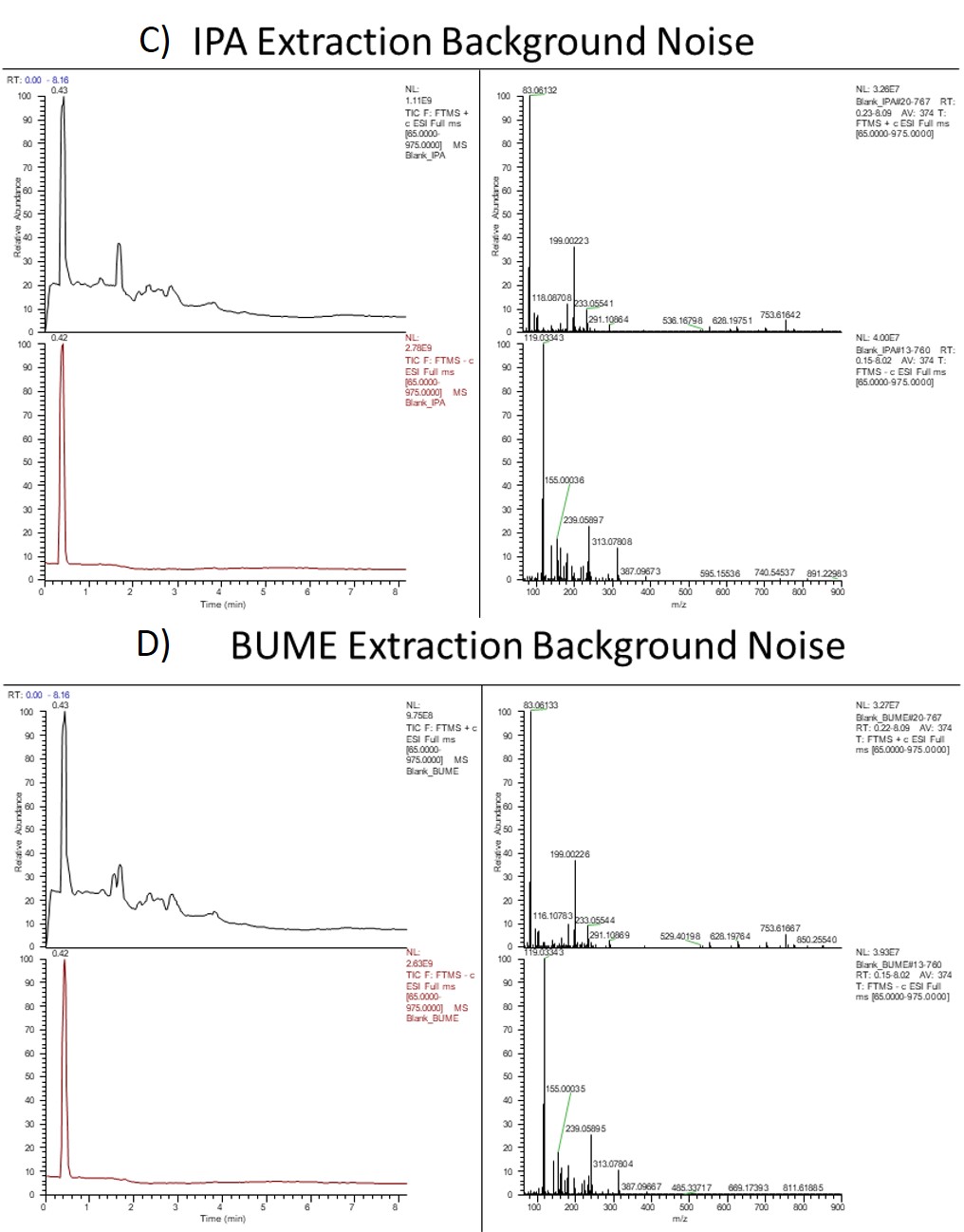


**Figure S4**. Retention of polar metabolites and complex lipids throughout the chromatographic gradient applied for complex lipid analysis using HILIC-MS/MS in positive ionization mode (see Materials and Methods for method details).

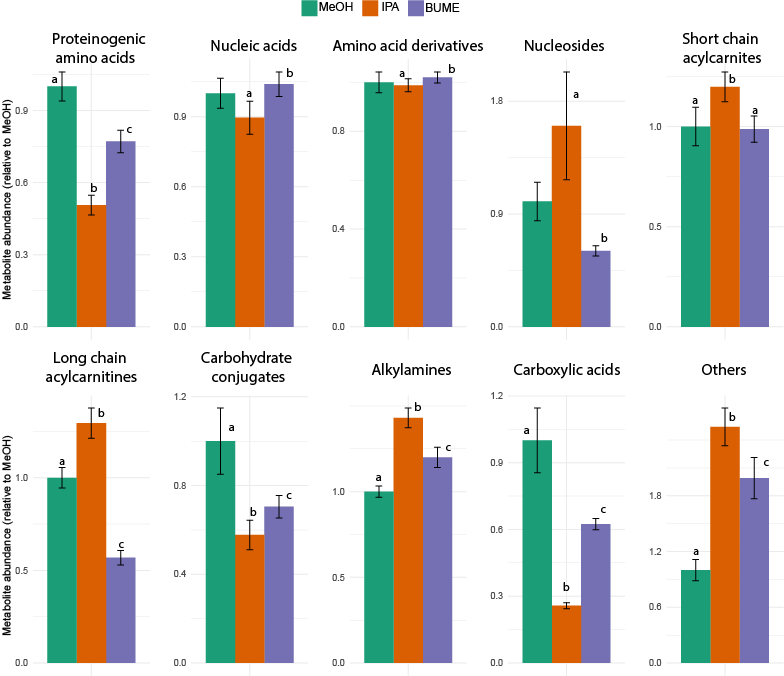


**Figure S5**. Retention of polar metabolites and complex lipids throughout the chromatographic gradient applied for complex lipid analysis using HILIC-MS/MS in negative ionization mode (see Materials and Methods for method details).

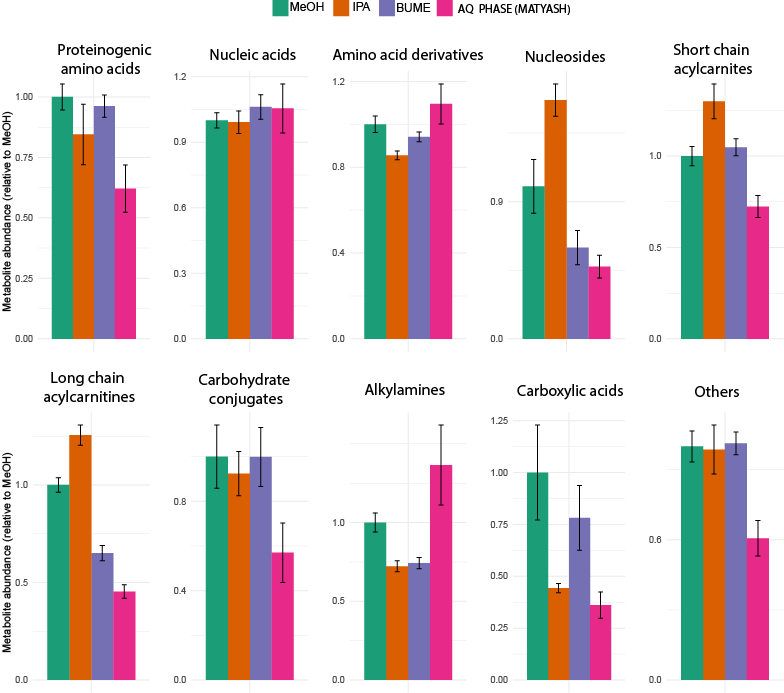




**Figure S6. Background noise from blank extractions performed with A) methanol, B) Matyash extraction (organic MTBE phase), C) IPA and D) BUME.** Blank samples were extracted following the same protocols as plasma samples and analysed by HILIC coupled to a high resolution mass spectrometry (HILIC-HRMS) instrument (QExactive Focus - Thermo Fisher Scientific) in full scan mode ( m/z 65 – 900 ) at 70,0000 FWHM mass resolving power in both positive and negative ionization mode using the ESI source conditions: heated ESI temperature 300 °C, capillary temperature 250°C, sheath gas 60 a.u., auxiliary gas 10 a.u. and capillary voltage -/+ 3500 V.



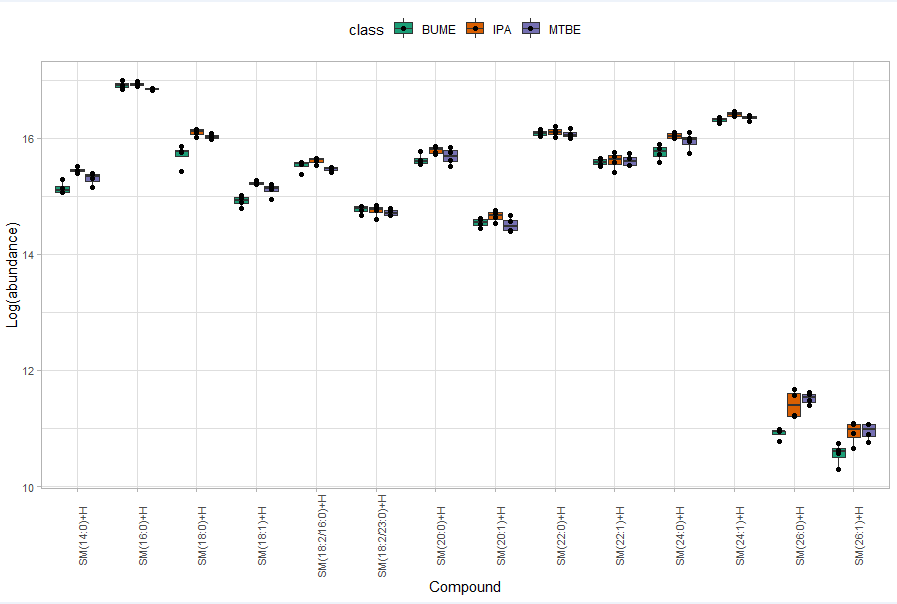
**Figure S7. Relative signal abundance (per polar metabolite class) to signal recovered with MeOH extract.** Relative signal abundance or fold change is represented for each single-step method, using IPA or BUME, to the reference MeOH extract, and per metabolite class. The class “Others”, i.e. other polar metabolites comprises glycocholate, hydroquinone, hydroxyphenyllactate, pyridoxine, salsolinol, trigonelline and tryptamine (Table S6). Carboxylic acids comprised mono-, di- and tri-carboxylic acids. Error bars indicate ± SD and a, b, c labels indicate statistically different groups no label means not significantly difference using the Tukey post hoc test with the significance level p < 0.05.



**Figure S8. Relative signal abundance of polar metabolites detected in the aqueous phase of Matyash extraction compared to other extraction protocols (including methanol extract as a reference).** Relative signal abundance or fold change is represented for each single-step method (using IPA or BUME) and aqueous phase of Matyash extraction to the reference MeOH extract. The class “Others”, i.e. other polar metabolites comprises glycocholate, hydroquinone, hydroxyphenyllactate, pyridoxine, salsolinol, trigonelline and tryptamine (Table S6). Carboxylic acids comprised mono-, di- and tri-carboxylic acids. Error bars indicate ± SD and a, b, c labels indicate statistically different groups no label means not significantly difference using the Tukey post hoc test with the significance level p < 0.05.

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**Figure S9.** **Diversity and size of measurable lipidome and polar metabolome from BUME extract.** Complex lipids (A) and polar metabolites (B) that were reproducibly measured from BUME extract (CV < 30 %, see Figure 5) are presented in the pie charts to show the broadest and most reproducible coverage.



**Figure S10**. Signal intensity of the sphingomyelins detected in the method. The boxplots show that the signal abundance is independent of the number of saturations and chain length.

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**Figure S11.** Signal intensity of specific hexosylceramides and lactosylceramides with the same alkyl chain composition in IPA, BUME and MTBE extracts