

Supplementary Information for the paper “Optimizing nanoLC-TIMS-TOF and lipidomics sample preparation for low volume of plasma”

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Table S1. Volume scaling of starting material and extraction solvents for the 2-propanol/water (IPA:H₂O) setup

Plasma, μL	Water, μL	2-Propanol, μL	EquiSPLASH™ internal standard, μL	Supernatant, μL	Resuspension solvent, μL
50	50	400	5.0	200	500
25	25	200	2.5	120	250
10	10	80	1.0	40	100
5	5	40	0.5	20	50
1	1	8	0.1	4	10

Table S2. Volume scaling of starting material and extraction solvents for the methanol/methyl *tert* butyl ether (MeOH:MTBE) setup

Plasma, μL	MeOH, μL	MTBE, μL	EquiSPLASH™ internal standard, μL	Supernatant, μL	Resuspension solvent, μL
50	175.0	175.0	5.0	200	500
25	87.5	87.5	2.5	100	250
10	35.0	35.0	1.0	40	100
5	17.5	17.5	0.5	20	50
1	3.5	3.5	0.1	4	10

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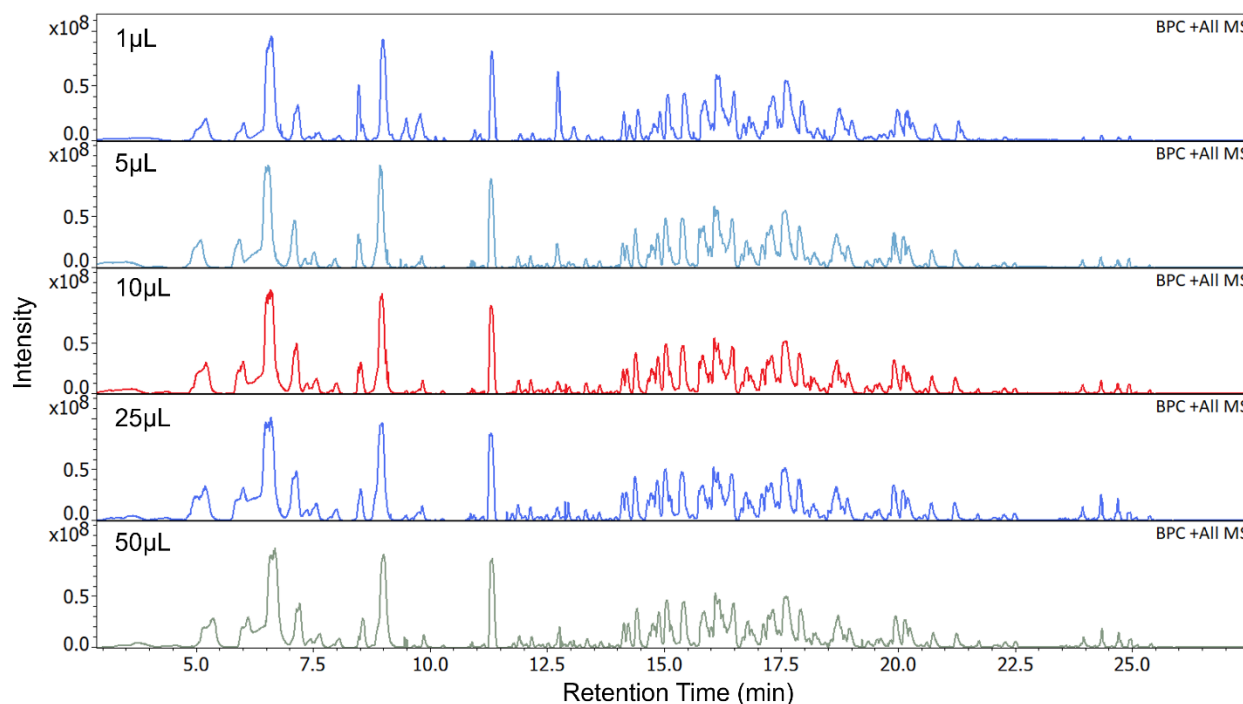


Figure S1. Stacked and normalized base peak chromatograms (BPCs) of different starting volumes of NIST® SRM® 1950 human plasma extracted using the IPA:H₂O protocol. The traces are representative qualitative examples. Differences in signal intensity and in several retention-time regions are visible at lower plasma input volumes. These chromatograms were not used to establish analytical reproducibility or preservation of the complete lipid profile.

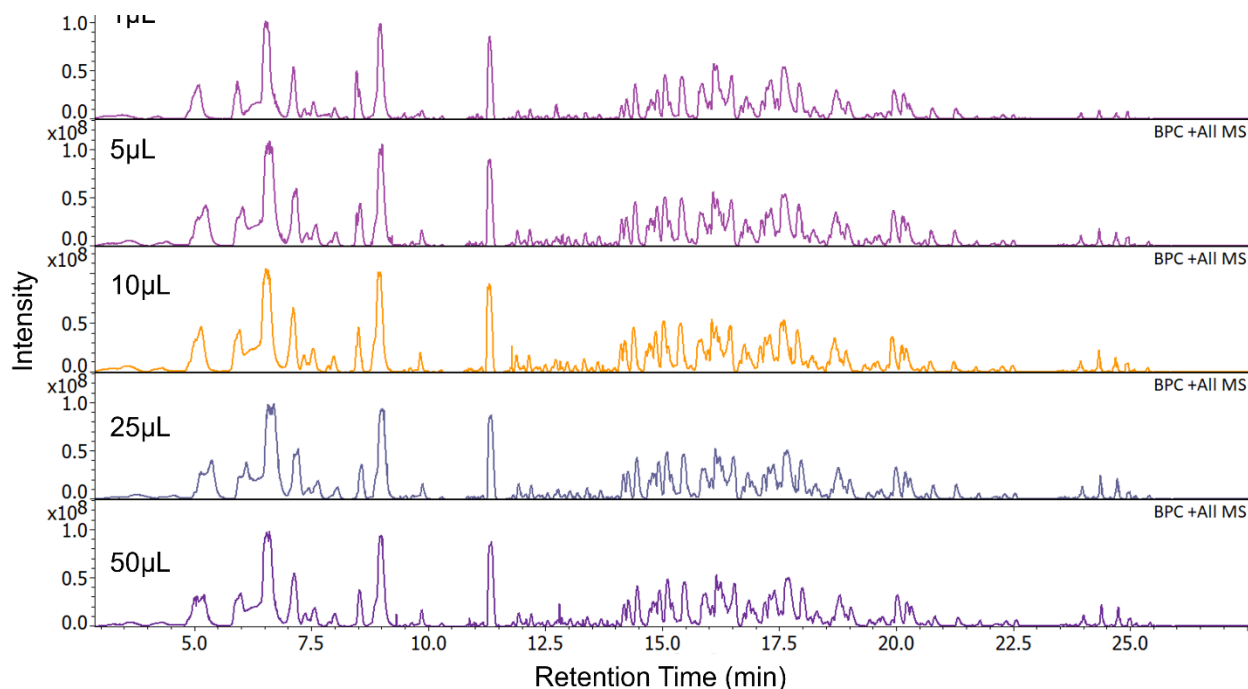


Figure S2. Stacked and normalized BPCs of different starting volumes of NIST® SRM® 1950 human plasma extracted using the MeOH:MTBE protocol. The traces are representative qualitative examples. Differences in signal intensity and in several retention-time regions are visible at lower plasma input volumes. These chromatograms were not used to establish analytical reproducibility or preservation of the complete lipid profile.

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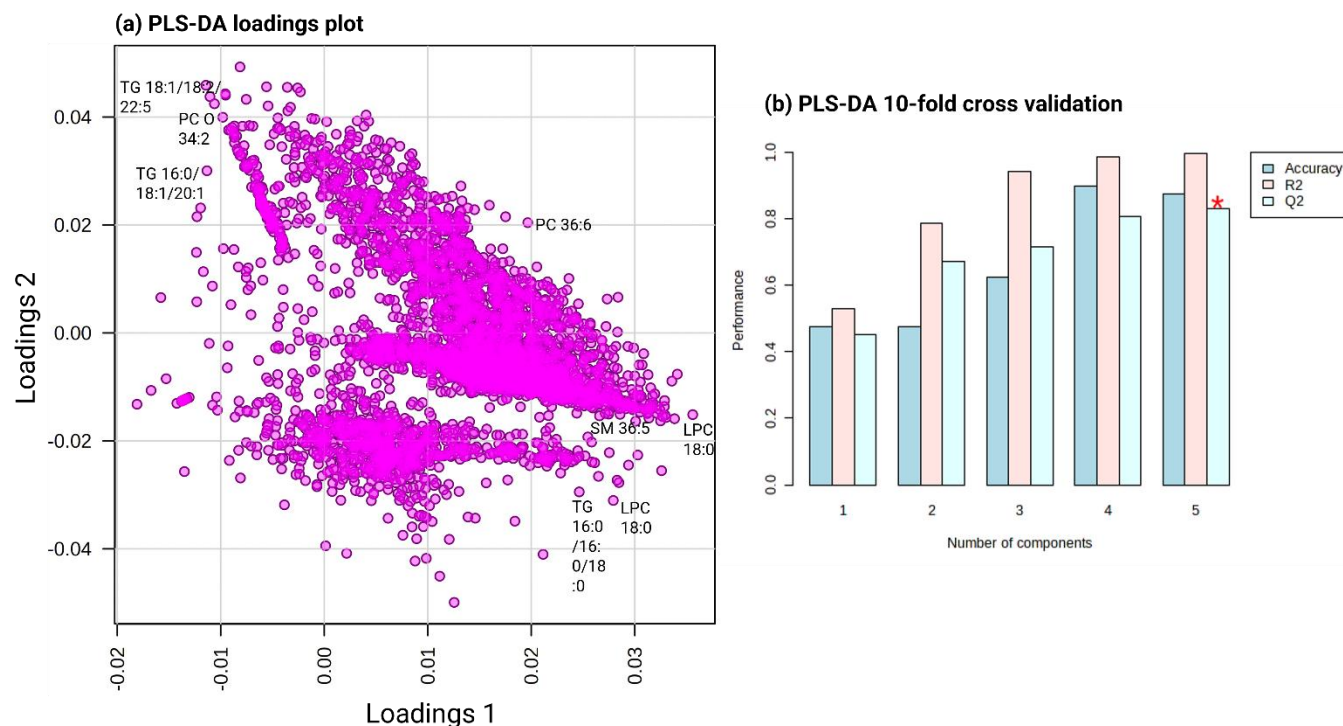


Figure S3. Exploratory PLS-DA analysis of the LC-MS lipidomics data. (a) PLS-DA loading plot generated from log-transformed, Pareto-scaled data. Each point represents a detected feature, and selected putatively annotated features are labelled for visualization. The labels do not constitute a complete ranking of feature contributions or identify statistically established discriminant markers. The corresponding scores plot is shown in Figure 2c. Blanks were retained in the model to visualize background-related contributions; consequently, the model was not used as inferential evidence for differences among the experimental plasma groups. (b) Ten-fold cross-validation output showing classification accuracy, R^2 , and Q^2 for models containing one to five components.

Column pressure traces at different buffer concentrations before and after 12h (~overnight) continuous runs

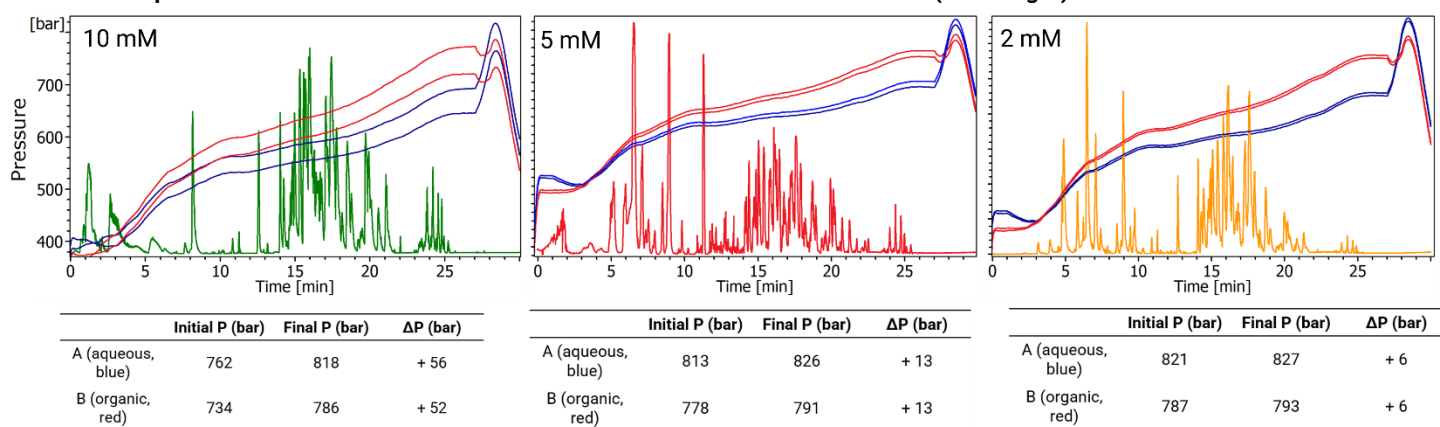


Figure S4. Qualitative comparison of nanoLC-MS pressure behavior under different buffer conditions. Pressure profiles obtained using (a) 10 mM, (b) 5 mM, and (c) 2 mM NH_4HCO_2 are shown before and after approximately 12 h of continuous operation. The buffer condition and acquisition time point corresponding to each trace are identified in the individual panels. The largest pressure observed occurred under the 10 mM condition, whereas smaller changes were observed under the 5 and 2 mM conditions. The traces are representative observations obtained using separate Aurora Ultimate C18 columns from the same manufacturing batch and were not used to establish quantitative sequence-level repeatability.

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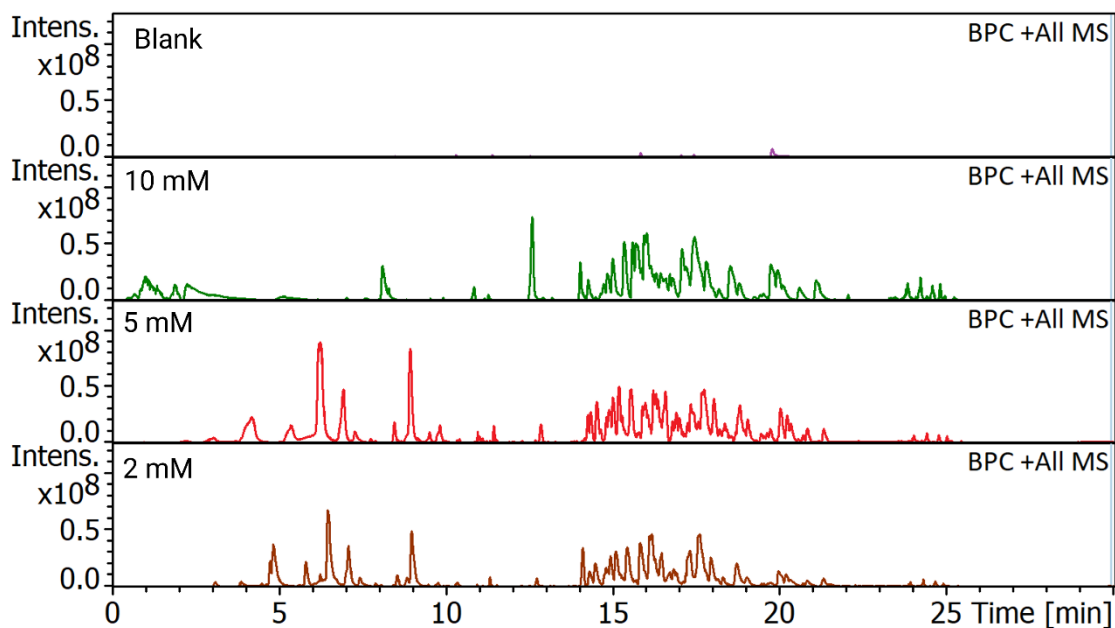


Figure S5. Stacked and normalized base-peak chromatograms from representative plasma samples analysed using 2, 5, and 10 mM ammonium formate. Differences in the overall signal intensity and earlier-eluting chromatographic profiles are presented as qualitative observations. Overall base-peak intensity was highest at 5 mM, intermediate at 10 mM, and lowest at 2 mM under the displayed conditions. These representative chromatograms were not used to quantify ion suppression, class abundance, or the appearance of additional lipid species.

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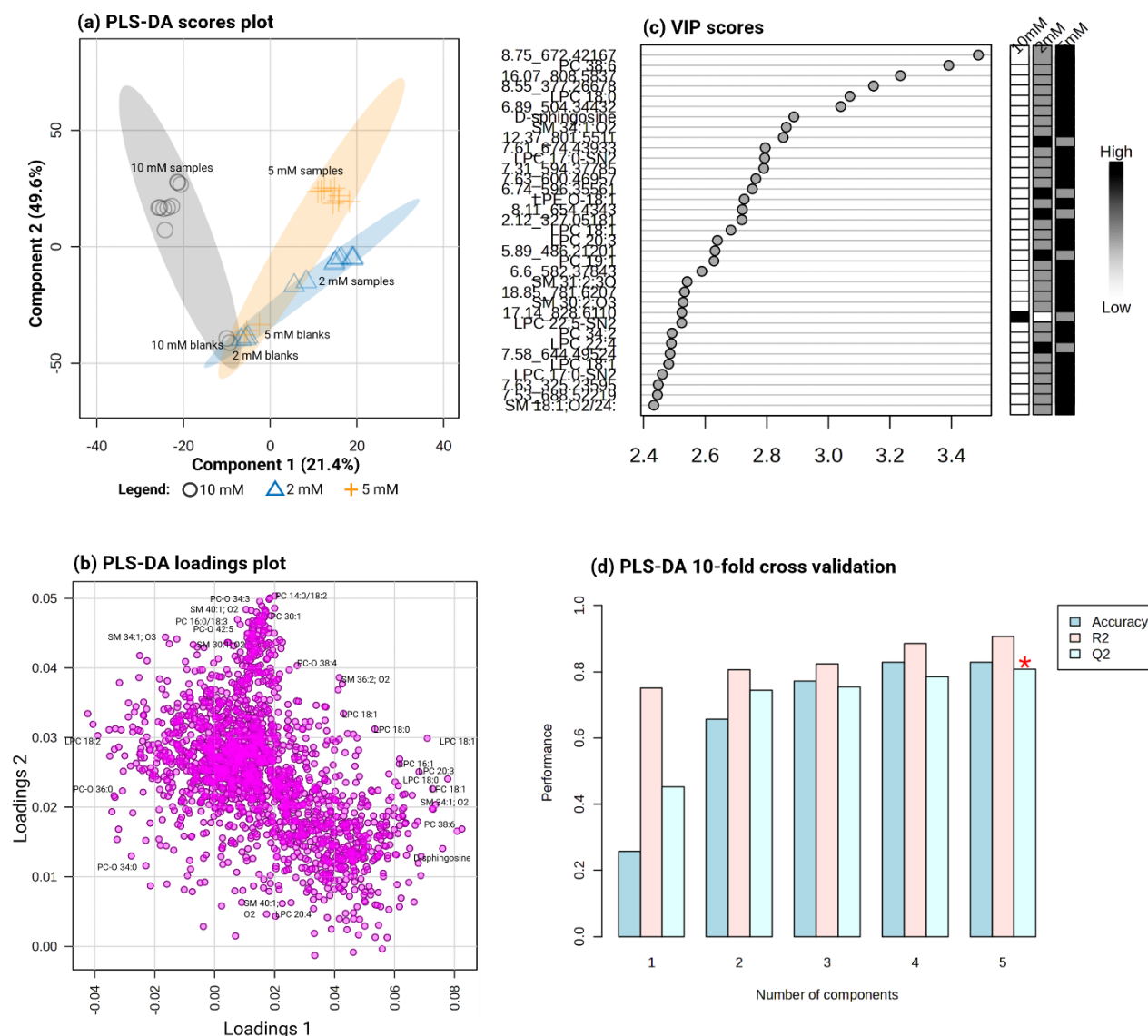


Figure S6. Exploratory multivariate visualization of nanoLC-MS lipidomics data obtained under different buffer conditions. (a) Log-transformed, Pareto-scaled PLS-DA scores plot containing the experimental samples and blanks. Ellipses show the displayed group regions. Because blank-versus-sample variation may influence the model structure, this plot was not used as inferential evidence for differences among buffer conditions. (b) VIP representation from the blank-containing model, with the adjacent heatmap showing relative feature intensities. The displayed features are descriptive contributors to this model and are not presented as statistically established markers. (c) Loading plot with selected putatively annotated features labelled for visualization. Structural details contained in the software-generated labels were not independently confirmed. (d) Ten-fold cross-validation output showing classification accuracy, R², and Q² for models containing one to five components.

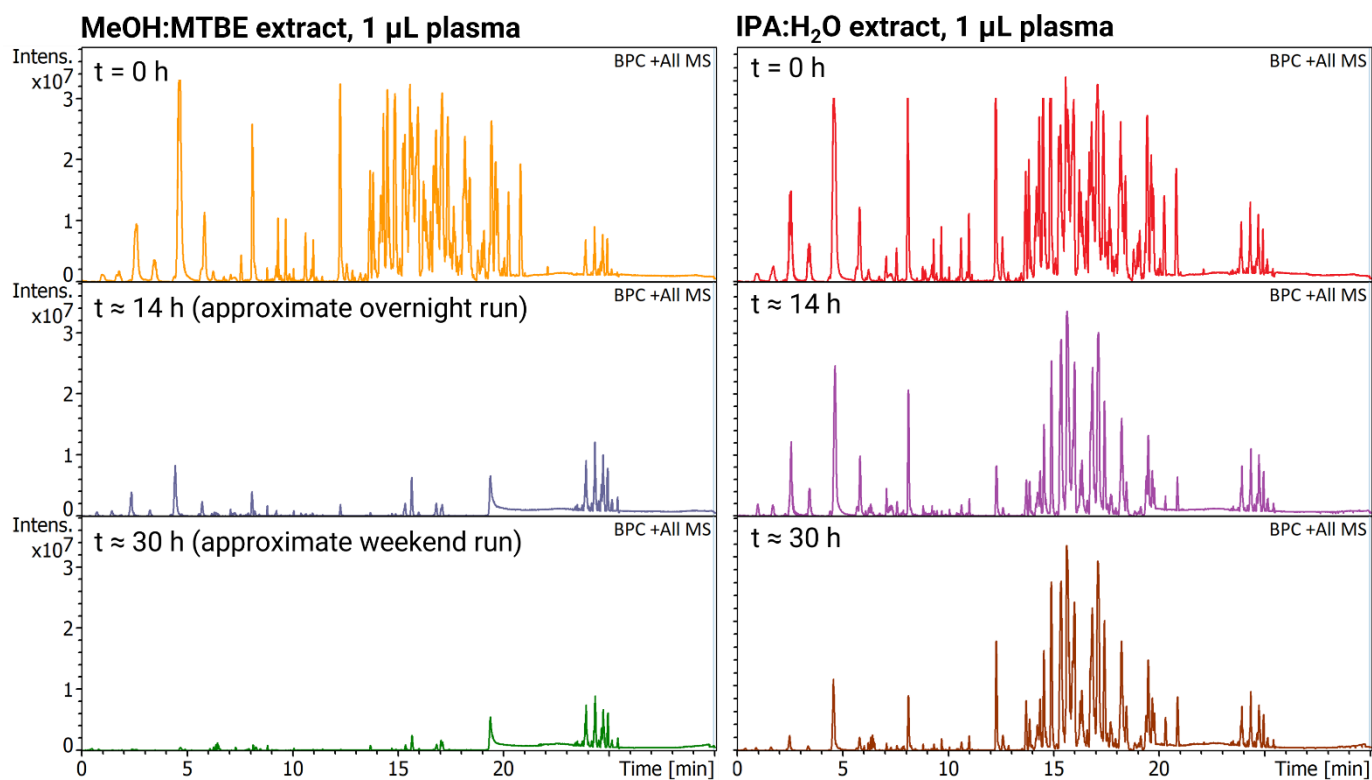


Figure S7. Qualitative stability assessment of representative 1 µL plasma extracts prepared using MeOH:MTBE and IPA:H₂O. BPCs are shown immediately after preparation and after approximately 14 and 30 h of autosampler storage. The MeOH:MTBE extracts showed pronounced changes in signal intensity and chromatographic profile over time. Changes were also visible in the IPA:H₂O extracts, but they were less pronounced under the tested conditions. These representative traces provide a qualitative comparison and were not used to establish analytical reproducibility or complete preservation of chromatographic complexity.