

Supporting information

Comprehensive evaluation of blood plasma and serum sample preparations for HRMS-based chemical exposomics: overlaps and specificities

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1. Solvents and chemicals

Native and isotopically labeled standard compounds were purchased from suppliers Bertin, LGC, Sigma Aldrich and VWR and were stored at -20°C. Details can be found in Supporting Information (SI, Table A1). Ultrapure water was generated using a Millipore Milli-Q Gradient system. UPLC-MS-grade acetonitrile and formic acid were purchased from Biosolve (Dieuze, France). UPLC-MS-grade methanol was purchased from Carlo Erba (Val-de-Reuil, France). HPLC-MS-grade methyl tert-butyl ether (MTBE) and ethyl acetate were purchased from Fisher Scientific (Illkirch-Graffenstaden, France). Aqueous ammonia was purchased from VWR (Strasbourg, France).

2. Data acquisition

Samples were analyzed on QTOF-MS (AB Sciex X500R) interfaced with an AB SCIEX ExionLC AD UPLC. Compound chromatographic separation was achieved using an Acquity UPLC HSS T3 C18 column (1.8µm, 1.0 × 150mm) maintained at 40°C. Injection volume was set at 2 µL. Flow rate was set at 100 µL/min with mobile phases of ultrapure water (A) and acetonitrile (B) both modified with 0.01% formic acid. The gradient was set as: 0-2.5 min, 10-20% B; 2.5-20 min, 20-30% B; 20-38 min, 30-45% B; 38-45 min, 45-100% B; 45-55 min, 100% B; 55-60 min, 10% B. Full-scan mass spectra was acquired in both – and + electrospray ionization (ESI) modes between 50-1100 m/z using ESI source settings: temperature 550°C, ionspray voltage 4,5kV (-4,5kV in negative mode), declustering potential 80V (-80V in negative mode), accumulation time 300 ms, spray N2 gas 35 arbitrary units, heat conduction gas 35 arbitrary units; curtain gas 7 arbitrary units, collisionally activated dissociation gas 7 arbitrary units, run time 60min. MS/MS fragmentation was performed on selected samples using sequential window acquisition of the theoretical mass spectrum (SWATH) or data dependent

acquisition (DDA). SWATH experiments were performed in both – and + ESI modes, using the following source settings: MS1 accumulation time 80ms, MS2 accumulation time 30 ms, collision energy 35eV, collision energy spread 15eV, cycle time 469ms, mass range 50-1100 m/z. Acquisition windows were established for each matrix and mode using an vendor-provided automated SWATH window calculator based on results from full scan injections. DDA experiments were performed in both – and + ESI modes, using the following source settings: MS1 accumulation time 250ms, MS2 accumulation time 100ms, collision energy 35eV, cycle time 2.35s, mass range 50-1100 m/z. Precursor ion selection parameters were as follows: a maximum of 20 candidate ions per cycle, intensity threshold 1cps, and dynamic background subtraction was enabled (candidate ions only includes ions increasing in intensity).

3. Quality control procedures

A solvent blank (i.e. acetonitrile/ultrapure water 90:10 (v/v)) and an extracted ultrapure water blank (i.e. extraction performed with ultrapure water in place of sample) were systematically injected with each batch to respectively ensure lack of carryover in the UPLC system and monitor contamination linked to the sample preparation process. Composite QC samples were injected after the blanks to equilibrate the analytical system, and repeatedly throughout the batch (every 5 samples). Samples were injected randomly. IS peak areas were monitored to assess analytical drift.

4. Sample preparation procedures

The twelve sample preparation methods used for this work are described below. As the spiking level, sample volume and recovery volume vary between experiments; they are not specified in each procedure and are recapitulated in Table B1.

Experiment	Spiking level (ng/mL)	Sample volume (μL)	Recovery volume (μL)
Preselection	40	200	100
Comparison to protein precipitation	10	100	20
Method detection limit	0.1, 0.5, 1, 5, 10, 20, 40	100	20

Table B1 – Spiking levels, sample volumes and recovery volumes used for all sample preparation procedures for three spiking experiments.

4.1. Protein precipitation

Protein precipitation was carried out using a 4:1 (v/v) cold methanol to matrix ratio. Samples were left at -20°C for 1h to improve protein removal. Centrifugation was performed at 4°C and 17,000g for 20 min, after which supernatants were collected and evaporated to dryness under vacuum. Samples were recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.2. Phospholipid and protein removal

4.2.1. Ostro (Waters), Phree (Phenomenex)- Acetonitrile, PL (Supelco), PL Ultra (Supelco)

A 99:1 (v/v) acetonitrile to formic acid mixture was added to the matrix using a 3:1 (v/v) ratio. Samples were vortexed then placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 100 μ L of the 99:1 (v/v) acetonitrile to formic acid mixture was drawn through the plate for rinsing. The resulting solutions were evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.2.2. Phree (Phenomenex)- Methanol

A 99:1 (v/v) methanol to formic acid mixture was added to the matrix using a 4:1 (v/v) ratio. Samples were vortexed then placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 100 μ L of the 99:1 (v/v) methanol to formic acid mixture was

drawn through the plate for rinsing. The resulting solutions were evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.2.3. PLD (Biotage)

A 99:1 (v/v) acetonitrile to formic acid mixture was added to the matrix using a 4:1 (v/v) ratio. Samples were vortexed then placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 100 μ L of the 99:1 (v/v) acetonitrile to formic acid mixture was drawn through the plate for rinsing. The resulting solutions were evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.2.4. Prime HLB (Waters)

Samples were placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 2 mL of a 95:5 (v/v) ultrapure water to methanol mixture was drawn through the plate for rinsing. Elution was performed with 2 mL of a 90:10 (v/v) acetonitrile to methanol mixture. The resulting solutions were evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.3. Solid phase extraction

4.3.1. HLB Oasis, Strata X (Phenomenex)

A 98:2 (v/v) ultrapure water to formic acid mixture was added to the matrix using a 1:1 (v/v) ratio. Solid phase was conditioned with 1 mL of methanol followed by 1 mL of ultrapure water. Samples were placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 2 mL of a 95:5 (v/v) ultrapure water to methanol mixture was drawn through the plate for rinsing. After drying, elution was performed using 1 mL of methanol (first extract), then 1 mL of ethyl acetate (second extract). Extracts were separately evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.3.2. Strata XC (Phenomenex)

A 98:2 (v/v) ultrapure water to formic acid mixture was added to the matrix using a 1:1 (v/v) ratio. Solid phase was conditioned with 1 mL of methanol followed by 1 mL of ultrapure water. Samples were placed on the plate and drawn through it drop by drop under vacuum. An additional volume of 2 mL of a 95:5 (v/v) ultrapure water to methanol mixture was drawn through the plate for rinsing. After drying, elution was performed using 1 mL of a 95:5 (v/v) methanol to aqueous ammonia ratio (first extract), then 1 mL of methanol (second extract). Extracts were separately evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

4.4. Supported liquid extraction

Samples were placed on the plate and drawn through it drop by drop under vacuum. Elution was performed with twice 900 μ L of methyl tert-butyl ether (MTBE). The resulting solutions were evaporated to dryness under vacuum, and recovered in 90:10 (v/v) ultrapure water to acetonitrile mixture as to obtain the desired sample concentration factor.

5. Application of PPT and Phree to cohort samples

Sample preparation methods PPT and Phree (acetonitrile) were applied to serum samples from the Pelagie cohort and plasma samples from a Danish birth cohort. Quality control was performed on the injected batches, both at the targeted and non-targeted scales. Results are presented in Figure S1.

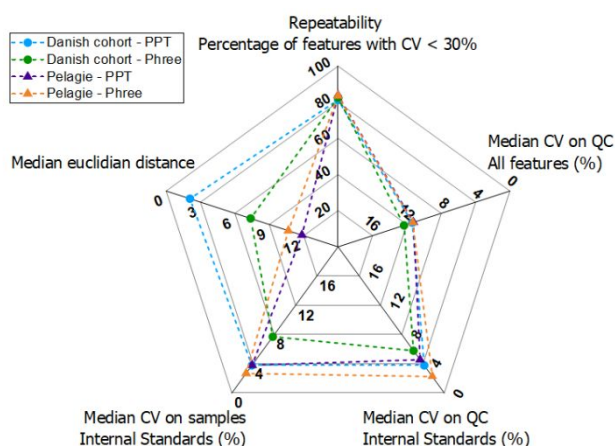


Figure S1 - Quality control parameters for the application of two sample preparation methods to two sets of cohort samples ($n=8$ plasma samples from the Danish cohort, and $n=10$ serum samples for Pelagie). Outer edges identify best performances.

Suspect screening was performed on the associated datasets, resulting in 44 xenobiotic annotations in serum and 41 xenobiotic annotations in plasma. For each annotated compound, fold changes (i.e. area ratio of features in samples prepared with Phree and protein precipitation) were computed for annotated compounds. Fold change values were also computed at the non-targeted level on quality control samples. Results are presented in Figure S2.

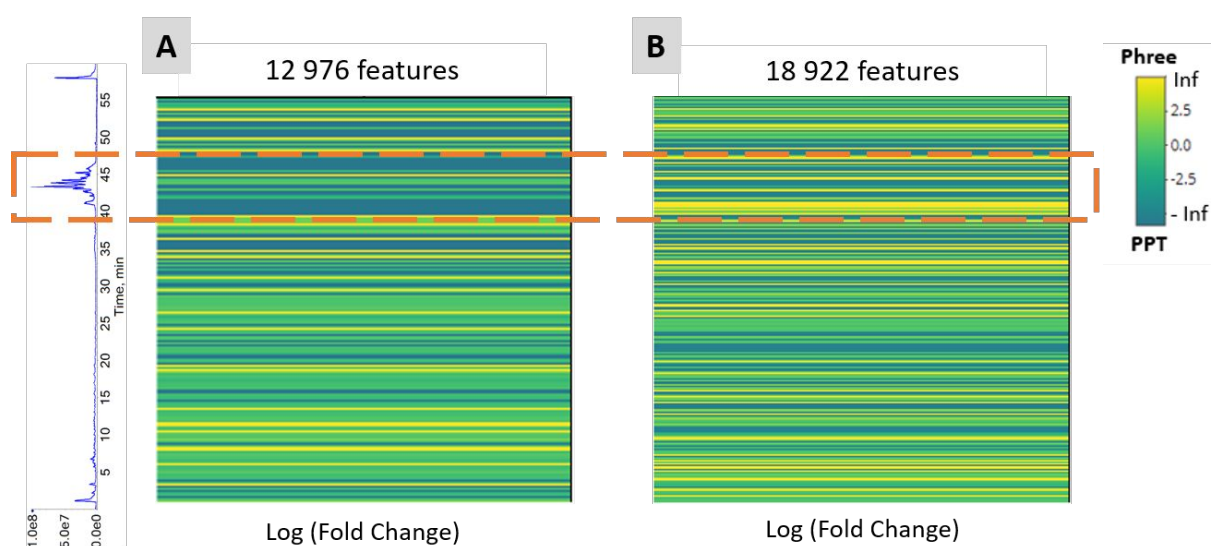


Figure S2 – Comparison of fold change values (i.e. area ratio of features in samples prepared with Phree and protein precipitation) for quality control samples in Pelagie serum samples (A) and Danish plasma samples (B). Yellow indicates features only visible in Phree-prepared samples and blue indicates features only visible in protein-precipitated samples. Features are

organized by retention time value (from bottom to top). The orange dashed rectangle indicates the range where lysophospholipids and peptides are mostly observed.