

Supplementary Materials: Sample Preparation Strategies for the Effective Quantitation of Hydrophilic Metabolites in Serum by Multi-Targeted HILIC-MS/MS

Elisavet Tsakelidou, Christina Virgiliou, Lemonia Valianou, Helen G. Gika, Nikolaos Raikos and Georgios Theodoridis

Table S1. Metabolites determined in serum by the applied LC-MS/MS method divided in three groups based on their intrinsic concentration in blood.

| Group A | Group B | Group C |
|-----------------|---------------------------|---------------|
| Hypoxanthine | Glycine | Lactic |
| Theobromine | Xanthine | Aspartic acid |
| Caffeine | Taurine | Lysine |
| Benzoic acid | Galactose | Uracil |
| Thiamine | 2-Hydroxy-isovaleric acid | Glucose |
| Thymidine | 2-Hydroxy-isobutyric acid | |
| Nicotinamide | Leucine | |
| Creatinine | Isoleucine | |
| Acetylcarnitine | Tyrosine | |
| Ribose | Valine | |
| Choline | Phenylalanine | |
| Betaine | Methionine | |
| Putrescine | Inosine | |
| Creatine | Hypotaurine | |
| TMAO | Pyruvic acid | |
| Pyridoxine | Proline | |
| Cotinine | Uridine | |
| Lactose | Pyroglutamic acid | |
| | Alanine | |
| | Asparagine | |
| | Threonine | |
| | Glutamine | |
| | Glutamic acid | |
| | Serine | |
| | Ornithine | |
| | Tryptophan | |
| | 3-Methylhistidine | |
| | Sarcosine | |
| | Arginine | |
| | Hippuric acid | |

Table S2. Spiking concentrations for the standard addition calibration curves and for the assessment of recovery.

| | | Concentrations in serum | | |
|----------------|--------------------|------------------------------|------------------------------|------------------------------|
| | spiking | Group A ($\mu\text{g/mL}$) | Group B ($\mu\text{g/mL}$) | Group C ($\mu\text{g/mL}$) |
| 5 point curves | Level 1 | 0.08 | 0.72 | 3.80 |
| | Level 2 | 0.20 | 1.80 | 9.50 |
| | Level 3 | 0.50 | 4.50 | 23.80 |
| | Level 4 | 1.00 | 9.00 | 47.50 |
| | Level 5 | 1.20 | 10.80 | 57.00 |
| LQC | low level spiking | 0.10 | 0.90 | 4.75 |
| HQC | high level spiking | 1.00 | 9.00 | 47.50 |