 contested metabolic profiling characterized by complementary platforms, multiplexing and low volume consumption are increasingly used for studies using biobank material. Using liquid-liquid extraction, we developed a sample workup suitable for quantification of 6 fat- and 26 water-soluble biomarkers. 50μL of serum/plasma was mixed with dithioerythritol, ethanol and isooctane/chloroform. The organic layer was used for analysis of the fat-soluble vitamins all-trans retinol (A), 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, α-tocopherol (E), γ-tocopherol (E) and phylloquinone (K1) by LC-MS/MS. The remaining aqueous fraction was mixed with ethanol, water, pyridine and methylchloroformate (in toluene) to derivatize the water-soluble biomarkers. The resulting toluene layer was used for GC-MS/MS analysis of alanine, α-ketoglutarate, asparagine, aspartic acid, cystathionine, total cysteine, glutamic acid, glutamine, glycine, histidine, total homocysteine, isoleucine, kynurenine, leucine, lysine, methionine, methylmalonic acid, ornithine, phenylalanine, proline, sarcosine, serine, threonine, tryptophan, tyrosine and valine. Isotope-labelled internal standards were used for all analytes.

Chromatographic run times for the LC-MS/MS and GC-MS/MS were 4.5 and 11 minutes, respectively. The limits of detection (LOD) for the low-concentration analytes (25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and phylloquinone) were 25, 17 and 0.33 nM, respectively, while all other analytes demonstrated sensitivity significantly lower than endogenous concentrations. Recoveries ranged from 85.5 - 109.9% and within- and between-day coefficients of variance (CVs) were 0.7 - 9.4% and 1.1 - 17.5% respectively. This low volume, high-throughput multianalyte assay is currently in use in our laboratory for quantification of 32 serum/plasma biomarkers in epidemiological studies.