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Spot and Cumulative Urine Samples Are Suitable Replacements for 24-Hour Urine Collections for Objective Measures of Dietary Exposure in Adults Using Metabolite Biomarkers

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Supplementary data

Supplemental Section 1 Methods for biomarker quantification using LC-QqQ-MS

Quantification was performed on a Thermo-Scientific ESI triple quadrupole mass spectrometer (TSQ Quantum Ultra) coupled to an Accela autosampler HPLC system (Thermo-Scientific). Chromatographic separations were performed on Merck ZIC®-pHILIC (polymeric 5 μm, 150 x 4.6 mm) and Thermo-Scientific Hypersil Gold (1.9 μm, 200 x 2.1 mm) columns (both maintained at 60 °C) for HILIC and reverse-phase analyses, respectively. For HILIC analysis the mobile phase consisted of 10 mM ammonium acetate in water : acetonitrile (95:5, v/v) (solvent A) and 10 mM ammonium acetate in water : acetonitrile (5:95, v/v) (solvent B), The gradient program used for HILIC analysis was 0 min, 95 % B (400 μL/min); 15 min, 20 % B (400 μL/min); 15.01 min, 20 % B (500 μL/min); 20 min, 20 % B (500 μL/min); 20.01 min, 95 % B (500 μL/min); 25 min, 95 % B (500 μL/min). Mobile phase for reverse-phase analysis was 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in methanol (solvent B). The gradient program used for reverse-phase analysis was 0 min, 0 % B; 0.5 min, 0 % B; 5 min, 60 % B; 11 min, 100 % B; 13 min, 100 % B; 13.01 min, 0 % B; 19 min, 0 % B. A flow rate of 400 μL/min was used for the reverse phase analysis. A 20 μL of injection volume was used for both analyses.

All metabolites were tuned by direct infusion into the respective mobile phase for sheath, aux, and ion sweep gas pressures, spray voltage, vaporizer and capillary temperatures, skimmer offset, collision energy (CE), and tube lens values for both positive and negative ionization polarities using metabolite standard solutions. The optimised ion source values for HILIC analysis were, aux gas 60 (arbitrary units), spray voltage 1000 V, and capillary temperature 270 °C, whereas for reverse phase analysis were, aux gas 50 (arbitrary units), spray voltage 4000 V, and capillary temperature

215 °C. The optimized values of sheath gas 70 psi, ion sweep gas 0, and vaporizer temperature 250 °C were used for both HILIC and reverse phase analyses. The mass spectrometric analysis was performed in multiple reaction monitoring (MRM) mode in both positive and negative ionization modes using these optimized settings and optimized values of shimmer offset, collision energy, and tube lens for each target compounds. The scan times of 0.010 s and 0.003 s were used for HILIC and reverse-phase analysis, respectively, whereas a scan width of 0.010 u, and a peak width (both Q1 and Q3) of 0.7 FWHM were used for both analyses. Argon was used as collision gas for tandem mass spectrometric analysis at a pressure of 1.5 mTorr. The Xcalibur software (Thermo-Finnigan) was used for data acquisition and control of all system components. Peak identity was established by both the characteristic parent and product ion pairs (minimum of 3 qualifying product ions) and retention time.

Quantifications were performed by using eight (0.0219-100 µg/mL) and eleven (0.0002-30 µg/mL) points calibration curves of standards mixtures for HILIC and reverse-phase analysis, respectively. Syringic acid (5 µg/mL) was used as an internal standard for the quantification. Each batch of analysis (maximum 24-Hour) was run with a new calibration curve, and three (for HILIC) and four (for reverse-phase) quality control (QC) samples with different quantities of standards across entire calibration range to calculate the quantification accuracies.

The median relative standard deviation (RSD) for measured biomarkers was 4.9 % and 7.4 %; in quality control standards and urine respectively.