Serum/Plasma Metabolomic Analyses.

PlasmaPretreatment1,2,3,5 will be used for each sample prior to derivitization for GC-MS analysis. A 100-μL aliquot of sample is spiked with two internal standard solutions (10μL heptadecanoic acid in methanol, 1 mg/mL) and vortexed for 10 seconds. For urine samples, urease (30 units) will be added prior to metabolite extraction with 300 μL methanol. For plasma samples, metabolite extraction is performed using 300 μL methanol-chloroform (3:1) followed by 10 minutes storage at –20°C. After centrifugation at 10,000 rpm for 10 minutes, a 300 μL aliquot of the supernatant is transferred to a glass sampling vial for vacuum drying at room temperature.

Plasma samples will be pretreated differently prior to LC-MS analysis. Before analysis, samples are thawed and centrifuged at 13,000 rpm for 5 min. For urine samples, a 300 μL of supernatant was diluted with 600 μL of ultrapure water containing internal standard of 4-chlorophenylalanine (10 μg/mL), vortexed and then centrifuged at 13000 rpm for 20 min, and the supernatant is used for LC-MS analysis. For plasma samples, a volume of 100 μL supernatant is mixed with 10 μL of aqueous 4-chlorophenylalanine (100 μg/mL, used as the internal standard) and 400 µL of a mixture of methanol and acetonitrile (5:3). The mixture is vortexed for 2 min, allowed to stand for 10 min, then centrifuged at 13000 rpm for 20 min, and the supernatant is used for LC-MS analysis.

***Chemical Derivatization.*** Each sample will be chemically derivatized using developed protocols 1,2 prior to GC-MS analysis.

***GC-TOF-MS Data Acquisition.*** An Agilent 6890N gas chromatography coupled with a Pegasus HT time-of-flight mass spectrometry (Leco Co., St. Joseph, MI, USA) were used. The mass spectra are obtained with electron impact ionization (70 eV) at full scan mode (m/z 40-600) with a Rxi-5ms capillary column (30 m × 250 µm i.d., 0.25-µm film thickness, Restek, PA, USA) using helium as the carrier gas, at a constant flow rate of 1.0 mL/min. The acquired data files from GC-TOF-MS are analyzed by ChromaTOF software (Leco Co., CA, USA).

***LC-TOF-MS Data Acquisition.*** An Agilent HPLC 1200 system coupled with an 6220 MSD Time-of-flight mass spectrometer (Agilent Corporation, Santa Clara, CA) were used. For Agilent LC-TOFMS, spectral data conversion is performed using using Agilent MassHunter Qualitative Analysis Program (vB.05.00) (Agilent) and XCMS package, respectively.

***Metabolite Annotation and Marker Selection1-5.*** Compound annotation is performed using an in-house library containing ~800 mammalian metabolites (reference standards). On-line database such as NIST library 2011, HMDB, LECO/Fiehn Metabolomics Library, etc, are also used for compound annotation and verification.

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3. Xie G, Plumb R, Su M, et al. Ultra-performance LC/TOF MS analysis of medicinal Panax herbs for metabolomic research. J Sep Sci 2008;31:1015-26.

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