

Methods

Plasma Sample Preparation

A modified liquid-liquid extraction protocol was used to extract hydrophobic and hydrophilic compounds from the plasma samples (73). Briefly, 50 μL of plasma spiked with internal standards underwent a protein crash with 250 μL ice cold methanol. 750 μL methyl tert-butyl ether (MTBE) and 650 μL 25% methanol in water were added to extract the hydrophobic and hydrophilic compounds, respectively. 500 μL of the upper hydrophobic layer and 400 μL of the lower hydrophilic layer were transferred to separate autosampler vials and dried under nitrogen. The hydrophobic layer was reconstituted with 100 μL of methanol and the hydrophilic layer was reconstituted with 50 μL 5% acetonitrile in water. Both fractions were stored at $-80\text{ }^{\circ}\text{C}$ until LC/MS analysis.

73. Yang Y, Cruickshank C, Armstrong M, Mahaffey S, Reisdorph R, Reisdorph N. 2013. New sample preparation approach for mass spectrometry-based profiling of plasma results in improved coverage of metabolome. *J Chromatogr A* 1300:217-26.