Sample Preparation for LC-MS/MS

To 40 µg of total protein from each FF sample (UI-and control-group), five volumes of pre□chilled (-20°C) chloroform-methanol solution (2:1 v/v) was added. The mixture was vigorously vortexed for 30 sec to ensure proper mixing and then incubated overnight at 4°C to allow for complete protein precipitation and metabolite partitioning. After overnight incubation, the samples were centrifuged at 12,000 x g for 10 min at 4°C. The upper aqueous fraction, enriched with hydrophilic metabolites, and the bottom chloroform fraction, containing the hydrophobic metabolites, were carefully separated, vacuum dried, and stored at −80°C until use (Nakayasu et al., 2016). Prior to LC-MS/MS analysis, the dried samples were reconstituted in 20µL of a methanol-water solution (1:1 v/v). Each reconstituted sample was then spiked with 2 ppm reserpine (internal standard).

Reference:

- Nakayasu ES, Nicora CD, Sims AC, Burnum-Johnson KE, Kim YM, Kyle JE, Matzke MM, Shukla AK, Chu RK, Schepmoes AA, Jacobs JM, Baric RS, Webb-Robertson BJ, Smith RD, Metz TO. MPLEx: a Robust and Universal Protocol for Single-Sample Integrative Proteomic, Metabolomic, and Lipidomic Analyses. mSystems. 2016 May 10;1(3): e00043-16. doi: 10.1128/mSystems.00043-16. PMID: 27822525; PMCID: PMC5069757.