

Metabolites Extraction

The metabolites were extracted. As the following, 3.2 mm one punch, was collected from each DBS sample and transferred into a 96-well plate for metabolite extraction. Metabolite was extracted with 250 ul of extraction solvent as percentage (20:40:40) (H₂O: ACN: MeOH) to each well with 2 hours incitation at room temperature. Subsequently, sample extracts were dried through SpeedVac (Thermo Fischer, Christ, Germany). The dried samples were reconstituted in 100 ul of 50% A: B mobile phase, were (A: 0.1% Formic acid in H₂O, B: 0.1% FA in 50% ACN: MeOH). Additional punches were taken for quality controls (QCs) from the project samples to maintain the instrument performance.