1.1 LC-MS Metabolomics

Waters Acquity UPLC system coupled with a Xevo G2-S QTOF mass spectrometer with an electrospray ionization source (ESI) was used to explore the metabolic profile. The extracted metabolites were separated using an ACQUITY UPLC using an XSelect column (100×2.1mm 2.5 μm) (Waters Ltd., Elstree, UK). Mobile phase solvent A was 0.1% formic acid in dH2O, while solvent B consisted of 0.1% formic acid in 50% ACN: MeOH. A gradient elution program was run: 0-16 min with 95-5% A, 16-19 min at 5% A, 19-20 min 5-95% A, and 20-22 min maintaining 5-95% A, all at a flow rate of 300 μL/min. MS spectra were obtained in both positive (ESI+) and negative (ESI-) electrospray ionization modes. The MS parameters were as follows: source temperature at 150°C, desolvation temperature at 500°C (ESI+) or 140°C (ESI-), capillary voltage at 3.20 kV (ESI+) or 3 kV (ESI-), cone voltage at 40 V, desolvation gas flow at 800.0 L/h, and cone gas flow at 50 L/h. Collision energies for low and high functions were set at off and 10 V to 50 V, respectively, in MSE mode. The mass spectrometer was calibrated using sodium formate in the 100–1200 Da range. Data were collected using Masslynx™ V4.1 workstation in continuum mode (Waters Inc., Milford, Massachusetts, USA).