LC-MS/MS Analysis

LC-MS analysis for metabolomics was performed using a Q Exactive™ Hybrid Quadrupole-Orbitrap MS (Thermo Fisher Scientific, MA, USA) coupled with a 1290 Infinity UHPLC (Agilent, CA, USA). Metabolite mixtures were loaded using a ZORBAX Eclipse Plus C18 Rapid Resolution High Definition (RRHD) column (2.1 × 50 mm, 1.8 μm particles). The mobile phase solvents consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in 80% acetonitrile, and the flow rate was fixed at 0.2 mL/min. The gradient of mobile phase was as follows: 2.5% solvent B in 5 min, 2.5–12.5% solvent B in 29 min, 12.5–25% solvent B in 11 min, 25–37.5% solvent B in 11 min, 37.5-80% solvent B in 0.1 min, holding at 80% of solvent B in 13.9 min, 80–2.5% solvent B in 0.1 min, 2.5% solvent B for 19.9 min. The electrospray source was equipped with a Heated Electrospray ionization (HESI-II) Probe combined with the standard Thermo Scientific™ Ion Max source. Parameters were set as follows: positive mode, spray voltage; 3.8 kV, capillary temperature; and 320°C. Properties of full MS/dd-MS2 were set up as follows: full-MS scans, 150 to 2000 m/z of scan range, 70,000 of resolution at 400 m/z, 1×10^6 of AGC target, and maximum IT of 60 ms. For MS2 scans, the following parameters were used: 17,500 of resolution at 400 m/z, 2×10^5 of AGC target, maximum IT was 250 ms, ±2 m/z of isolation width, and NCE for dd-MS2 of 30%