LC-MS/MS Analysis

LC-MS analysis for lipidomics was performed using a Q Exactive™ Hybrid Quadrupole-Orbitrap MS (Thermo Fisher Scientific, MA, USA) coupled to a 1290 Infinity UHPLC (Agilent, CA, USA) with heated electrospray ionization (HESI). Lipids mixtures were loaded using a e Hypersil GOLD™ C18 HPLC column(2.1 × 100 mm; 1.9 μm particle size; Thermo Fischer Scientific). The mobile phase solvents consisted of (A) Acetonitrile:Methanol:Water (19:19:2, v/v/v) + 20 mmol/L ammonium formate + 0.1% formic acid (v/v) and (B) 2-propanol + 20 mmol/L ammonium formate + 0.1% formic acid (v/v), and the flow rate was fixed at 0.2 mL/min. The gradient of mobile phase was as follows: 0–5 min, 5% B; 5–15 min, 5–30% B; 15–22 min, 30–90% B; 22–25 min, 90% B; 25–26 min, 90–5% B; 26–30 min, 5% B. Parameters were set as follows: positive mode, spray voltage; 3.8 kV, capillary temperature; and 320℃, and S-lens radio frequency(RF); 60%. 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, 1×10^6 of AGC target, and maximum IT of 60 ms. For MS2 scans, the following parameters were used: 35,000 of resolution, 1×10^5 of AGC target, maximum IT was 250 ms, ±1 m/z of isolation width, and NCE for dd-MS2 of 30%.