6.1.1 Sample preparation and extraction

6.1.1.1 Cell sample

Sample was thawed on ice, then added 500 uL pre-cooled extractant (80% methanol aqueous solution),and whirl for 2 min. Freeze the mixture for 5 min in liquid nitrogen after remove ice for 5 min, it will be whirledfor 2 min, circulate this at 3 times. Centrifuge the mixture again with 15000 r/min at 4 ◦C for 20 min.Finally take the supernatant into the sample bottle for LC-MS/MS analysis.

6.1.2 HPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, Nexera UHPLC LC-30A system, https://www.shimadzu.com.cn/an/hplc/Nexera-UHPLC-hplc-system/3381.html; MS, QTRAP®System, https://sciex.com/). The analytical conditions were as follows, UPLC: column, SeQuant ZIC-pHILIC 5 μm(2.1 × 100 mm); column temperature,40◦C; flow rate, 0.4 mL/min; injection volume, 2μL; solvent system, 10 mmol/L ammonium acetate
+0.3% ammonia solution, 90% acetonitrile water; gradient program, 5:95 V/V at 0 min, 50:50 V/V at 9.5 min, 5:95 V/V at 11.1 min, 5:95 V/V at 14.0 min.

6.1.3 ESI-QTRAP-MS/MS

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer(QTRAP), QTRAP® LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operatingin positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 450 ◦C; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 40, 55, and 35.0 psi, respectively; the collision gas (CAD) was medium. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.