Sample preparation and extraction

Unconventional samples

  Take out the sample from the -80°C refrigerator and thaw it on ice.Mix 500 mg of the sample and 1000 uL of 70% methanol water internal standard extractant, vortex for 3 minutes, then sonicate for 10 minutes in ice water bath, and stand still at -20°C for 30 minutes. Centrifuge (12000 rpm, 4°C) for 10 min, and transfer 300 uL of the supernatant to a new centrifugal tube. Finally centrifuge (12000 rpm, 4°C) for 3 min and take the supernatant for analysis.

HPLC Conditions (T3)

  All samples were acquired by the LC-MS system followed machine orders.The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 µm, 2.1 mm\*100 mm); column temperature, 40 ∘∘C; flow rate, 0.4 mL/min; injection volume, 2 μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); The column was eluted with 5% mobile phase B (0.1% formic acid in acetonitrile) at 0 minute followed by a linear gradient to 90% mobile phase B (0.1% formic acid in acetonitrile) over 11 minutes, held for 1 minute, and then come back to 5% mobile phase B within 0.1 minute, held for 1.9 minutes.