LC-MS Metabolomics

Metabolomics analysis was explored using the Waters Acquity UPLC system coupled with a Xevo G2-S QTOF mass spectrometer equipped with an electrospray ionization source (ESI). In detail, the extracted metabolites were chromatographed by an ACQUITY UPLC using XSelect (100 ×2.1mm 2.5 µm) column (Waters Ltd., Elstree, UK), the mobile phase composed of 0.1% formic acid in dH_2O as solvent A and solvent B consists of 0.1% formic acid in 50% ACN: MeOH. A gradient elution was run as follows: 0-16 min 95- 5% A, 16-19 min 5% A, 19-20 min 5-95% A, 20-22 min 95-95% A, at 300 µL/min flow rate. MS spectra were acquired under positive and negative electrospray ionization modes (ESI±). MS conditions were as follows: source temperature was 150◦C, the desolvation temperature were 500/ 140 ◦C for ESI+/ ESI− in sequence, the capillary voltage was 3.20 kV (ESI+) or 3 kV (ESI−), cone voltage was 40 V, desolvation gas flow was 800.0 L/h, cone gas flow was 50 L/h. The collision energies of low and high functions were set at 0 and 10-50 V, respectively, in MSE mode. The mass spectrometer was calibrated with sodium formate in 100–1200 Da. Data were collected in continuum mode with Masslynx™ V4.1 (Waters Technologies, Milford, MA., USA) workstation.