

A Waters Acquity ultra performance liquid chromatography (UPLC) system consisting of a binary solvent manager and a sample manager, a Waters SYNAPT HDMS quadrupole time-of-flight (TOF) mass spectrometer and an analytical workstation with Waters MassLynx 4.1 software were used. The injection volume was 8 μ L. Separations were carried out with a Waters Acquity HSS T3 column (100mm \times 2.1mm I.D., 1.8 μ m) coupled with Vanguard HSS T3 guard column. The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The eluting conditions were: isocratic 2% B (0–1 min), linear gradient from 2% to 5% B (1–2 min), 5% to 12% B (2–5 min), 12% to 20% B (5–10 min), 20% to 30% B (10–12 min), 30% to 50% B (12–13 min), 50% to 100% B (13–15 min), isocratic 100% B for 1min, then back to 2% B in 1 min and isocratic 2% B for 3min before next run. The flow rate was 0.5 ml/min. The column were maintained at 24 $^{\circ}$ C. Mass spectrometry was performed with ESI source operating in both positive (ES⁺) and negative (ES⁻) ion mode. The lock mass compound was leucine enkephalin (ES⁺ m/z 556.2771 and ES⁻ m/z 554.2615). The lock spray reference scan frequency was 20s with reference cone voltage of 30V. The source temperature was set at 100 $^{\circ}$ C and the desolvation temperature was set at 450 $^{\circ}$ C with desolvation gas flow of 900 L/h. The capillary voltage and cone voltage were set to 3kV and 40V, respectively. The collision energies were set as 6V (trap)/4V (transfer) with 2.00ml/min trap gas flow.