Chromatography

Chromatographic separation was performed using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm *i.e.*, 1.7 μm particle size) and protected using a C18 precolumn, both from Waters Corporation (Wexford, Ireland). The mobile phases were water (phase A) and acetonitrile:2-propanol (1:1, *v/v*) (phase B), both containing 1% 1M ammonium acetate and 0.1% (*v/v*) formic acid ammonium acetate as ionization agents. The LC pump was programmed at a flow rate of 0.4 mL min^{-1} and the elution gradient was as follows: from min 0–2, the percentage of phase B was modified from 35% to 80%, from min 2-7, the percentage of phase B was modified from 80% to 100% and this final percentage held for 7 min. A post-time of 7 min was used to regain the initial conditions for the next analysis. Thus, the total analysis time per sample was 21 min (including postprocessing). The settings of the dual ESI ionization source were as follows: capillary voltage 3.6 kV, nozzle voltage 1500 V, N_2 pressure in the nebulizer 21 psi, N_2 flow rate and temperature as heat gas 11 L min^{-1} and 379 °C, respectively. Accurate mass spectra in MS scan were acquired in the m/z range 100 – 1700 in positive ion mode.