

LC sample preparation protocol

For LC-MS/MS, brain region extracts (57 μL) containing internal standards and isotopes were evaporated under a stream of nitrogen gas at 37 °C. To derivatise the samples, N-butanol:acetyl chloride (BR: 300 μL ; OB: 200 μL) was added and the samples were incubated at 50 °C for 1 h. Thereafter the butylated samples were evaporated to dryness under a stream of nitrogen gas at 37 °C. Finally, the dried residue was reconstituted in a final volume of water:acetonitrile (50:50) (v/v) containing 0.1 % formic acid (BR: 50 μL ; OB: 25 μL). The samples were thoroughly vortex mixed to dissolve the dried compounds. The final volume of the BR samples was then transferred to 250 μL pulled point glass inserts. Due to the small volumes used during derivatisation, OB samples were derivatised in micro-inserts to maximise recovery for small sample volumes. Finally, each vial was loaded onto an Agilent© 1200 series auto sampler for LC-MS/MS analysis.