

LC data acquisition method

LC-MS/MS was performed as described by Mels *et al.* (2011) with slight modifications. The autosampler was set at 4 °C and a sample injection volume of 2 µL was used. For separation, a C18 Zorbax SB-Aq reverse phase column (2.1mm x 150 mm x 3.5 µm, Agilent©) fitted with a Phenomenex guard column, was used. The column was kept at 30 °C during the entire run. The mobile phases consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The analytical LC gradient started with 5% B at 0 min followed by a linear gradient to 20% B at six min and another increase to 100% B at 13 min. The gradient was kept at 100% B for five min and then decreased to 5% within two min followed by re-equilibration for eight min to maintain reproducibility. The flow rate was 0.3 mL/min for the first 13 min after which it was increased to 0.35 mL/min for the rest of the run. The total run time for each sample was approximately 28 min. The samples were delivered to the QQQ-MS via electrospray ionisation (ESI) in positive mode with the source conditions set as follows: nitrogen drying gas temperature of 300 °C and flow of 7.5 L/min, a nebuliser pressure of 30 psi and capillary voltage of 3500 V. The compounds were analysed in multiple reaction monitoring (MRM) mode using enhanced sensitivity with the multiplier voltage set at 300 Delta EMV and a dwell time of 45 milliseconds for all metabolites.