Untargeted LC-MS method

Dried LC-MS samples were re-suspended in H2O (27µL) containing injection standards (tBoc-L-Alanine, tBoc-L-Asparagine, tBoc-L-Phenylalanine). Two solvent blanks (SB, 100% H2O with t-Boc standards) and 2 water blanks (BB, 100% H2O) were added at the end of the sample prep. All samples were then capped, vortexed at 2000 RPM for 2 minutes, and transferred to the instrument for analysis.

Samples were analyzed on an Agilent 1200 LC / 6530 qTOF LC-MS system using a Waters Acquity HSS T3 reversed-phase column (1.8 µ, 50 mm x 2.1 mm ID). For positive ion detection, mobile phase A was 100% water with 0.1% formic acid and mobile phase B was 100% methanol with 0.1% formic acid. For negative ion detection, formic acid was replaced with 0.1% ammonium bicarbonate. The gradient was as follows: 0-0.5 min 1% B, 0.5-2 min 1-99% B, 2-6 min 99% B, 6-9 min 1% B. The flow rate was 0.35 mL/min and column temperature kept at 40°C. The injection volumes for positive and negative mode were 7 µL and 12 µL respectively. Jetstream ESI source gas temperature was set to 350°C, drying gas flow rate - 10 L/min, nebulizer pressure - 30psig, sheath gas temperature - 350°C and flow rate - 11 L/min. Capillary current was set to 10.0 µA and VCap voltage - to 3500V. Inline mass calibration was performed by delivering reference masses through a dedicated inlet on mass spectrometer. Reference masses were 99.09 and 922.009 m/z (Positive mode) and 62.0009 and 981.9956 m/z (Negative mode).