Chromatography method

Hydrophilic Liquid Interaction Chromatography (HILIC) was used for the chromatographic separation for metabolites. The separation was achieved using a Waters Acquity UPLC BEH amide column (2.1 x 150mm with particle size of 1.7µm, part no. 186004802), operating at 45°C with a flow rate of 200µL/min. The LC gradient consists of a binary buffer system, namely buffer "A" (LC/MS grade water) and buffer "B" (LC/MS grade ACN) both containing 10 mM ammonium formate. Independent buffer systems were used for positive and negative electrospray ionisation (ESI) acquisition respectively, for ESI+ the pH of buffers was adjusted using 0.1% formic acid and for negative using 0.1% ammonia solution. The LC gradient was the same for both polarities, namely 95% "B" at T0 hold for 1.5 min and a linear decrease to 50% "B" at 11 min, followed by hold for 4 mins, return to starting condition and hold for further 4.5 mins (column stabilization).

Standard reverse phase chromatography was used for the chromatographic separation of lipids. The separation was achieved using a Waters Acquity UPLC CSH C18 column (2.1 x 150mm with particle size of 1.7µm, part no. 186005298), operating at 55 °C with a flow rate of 200µL/min. The LC gradient consists of a binary buffer system, namely buffer "A" (LC/MS grade water:ACN, 40:60 % V/V) and buffer "B" (IPA:ACN, 90:10 % V/V) both containing 10mM ammonium formate. Independent buffers systems were used for positive and negative ESI modes respectively, for ESI+ the pH of buffers was adjusted using 0.1% formic acid and for negative using 0.1% ammonia solution. The LC gradient was the same for both polarities, namely 60% "B" at T0 hold for 1.5 min, linear increase to 85% "B" at 7 min, increase to 95% "B" at 12.5 min and hold for 4.5 min before returning to starting conditions and holding for further 4.5 min (column stabilization).