Samples were separated on an amide column, using mobile phase A consists of water mixed with 25 mM ammonium acetate and 25 mM Ammonium hydroxide and mobile phase B ACN. The injection volume was 4 µL and flow rate was 0.4 ml/min.

1. The generic HPLC gradient was listed in Table 1:

Table 1: HPLC gradient

Time	A	B
0.0 min	10%	90%
1.0 min	10%	90%
11.0 min	13%	87%
14.0 min	20%	80%
16.5 min	30%	70%
18.5 min	50%	50%
20.5 min	80%	20%
25.0 min	80%	20%
25.1 min	10%	90%
34.0 min	10%	90%

2. MS analysis was carried out on the Q-Exactive MS/MS in both positive and negative ion modes.

1) Set the relevant tuning parameters for the probe as listed: aux gas heater temperature, 400 °C; sheath gas, 40; auxiliary gas, 13; spray voltage, 3.5 kV for positive mode and negative mode. Set the capillary temperature at 350 °C, and S-lens at 55.

2) Build a DDA method as follows: Full scan range: 60 to 900 (m/z); resolution for MS1 and ddMS2: 70,000 and 17,500 respectively; maximum injection time for MS1 and ddMS2: 100 ms and 45 ms; automatic gain control (AGC) for MS1 and ddMS2: 3e6 and 2e5; isolation window: 1.6 m/z; normalized collision energies (NCE): 10, 17, 25 or 30, 40, 50.

3) Build a full scan method as follows: Full scan range: 60 to 900 (m/z); resolution: 140,000; maximum injection time: 100ms; automatic gain control (AGC): 3e6 ions.

Raw files were submitted to Thermo Compound Discover 2.1, (CD), and processed with Untargeted Metabolomics workflow with minor modification to find and identify the differences between samples:
Performs retention time alignment, unknown compound detection, and compound grouping across all samples. Predicts elemental compositions for all compounds, fills gaps across all samples, and hides chemical background (using Blank samples). Identifies compounds using mzCloud (ddMS2) and ChemSpider (formula or exact mass). Also performs similarity search for all compounds with ddMS2 data using mzCloud. Maps compounds to biological pathways using KEGG database

For retention time alignment, the max time shift was 2 mins, and a tolerance of 0.5 min was used for grouping unknown compounds. Mass tolerance were set as 10 ppm for feature detection and 5 ppm for compound annotation. The exact mass of each feature was submitted to ChemSpider with 4 databases selected (BioCyc; Human Metabolome Database; KEGG; LipidMAPS). Results from Compound Discover, the compound table, was exported as .xsls file, and then analysed with R.