Supplementary Material

1 Methods

1.1 LC-MS analysis of ZSD

Liquid chromatographic separations were performed utilizing a Waters ACQUITY™ Ultra-High-Performance Liquid Chromatography (UHPLC) system (Waters Corporation, Manchester, UK). Metabolite separation was achieved using an ACQUITY BEH C18 column (dimensions: 50 mm × 2.1 mm, particle size: 1.7 μm). The liquid chromatography analysis employed two mobile phases: Phase A, consisting of purified water with 0.1% formic acid, and Phase B, composed of acetonitrile. The sample injection volume was 5 μL, and the gradient elution program was structured as follows: 10%-30% B (0-5 min); 30%-80% B (5-10 min); 80%-85% B (10-12 min); 85%-95% B (12-15 min); 95%-95% B (15-18 min); 95%-10% B (18-25 min). The flow rate was maintained at 0.4 mL/min.

Post chromatographic separation, the eluate was directed into an SYNAPT G2Si Quadrupole Time-of-Flight Mass Spectrometer (Q-TOF MS) (Waters Corp., Manchester, UK) equipped with an electrospray ionization (ESI) source. Mass spectrometry data for ZSD extracts were collected in both positive and negative ion modes. The critical parameters for the ESI source were configured as follows: capillary voltage set to +3.0 kV or -2.5 kV; source temperature at 120 °C; sample cone voltage at 40 V; desolvation gas temperature at 500 °C; nitrogen flow rate at 900 L/h; and cone gas flow rate at 50 L/h. The mass spectrometry analysis covered an m/z range from 100 to 1500. The MS/MS information was obtained under fast-DDA mode, with the Top 15 most intense ions selected for fragmentation. Compound annotation was performed by comparing the acquired MS and MS/MS data against database records. The MSP format databases, including MSMS_Public_EXP_NEG_VS17 and MSMS_Public_EXP_Pos_VS17, were obtained from https://systemsomicslab.github.io/compms/msdial/main.html#MSP and subsequently imported into the MS-DIAL software for database searching.

1.2 sersum sample analysis

Serum chromatographic separation was performed on an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm) at 50 °C. The mobile phase comprised 0.1% formic acid in water (phase A) and acetonitrile (phase B). The mobile phase comprised 0.1%

formic acid in water (phase A) and acetonitrile (phase B). The gradient program was set as follows: 3%-70% B (1-8 min); 70% B (8-10 min); 70%-90% B (10-17 min); 90%-100% B (17-18 min); 100% B (18- 21 min); 100%-3% B (21-22 min); 3% B (22-27 min). The flow rate was set to 0.4 mL/min.

MS detection was conducted on a SYNAPT G2Si Q-TOF MS system (Waters Corp., Manchester, UK) equipped with an ESI source. Data acquisition was performed in positive and negative ion modes, respectively. The mass range was set to m/z 100-1500 for full-scan analysis. Tandem mass spectrometry data was collected using the fast-DDA (Top 15) method. Both full scan and fast-DDA were conducted under resolution mode. The ESI source parameters were set to a capillary voltage setting of 3.0 kV or -2.5 kV; source temperature was 150 °C; sample cone voltage was 40 V; desolvation temperature was 500 °C; nitrogen gas flow was 900 L/h; and cone gas flow was 50 L/h. Before commencing sample analysis, the precision of the MS instrument was calibrated with sodium formate at a concentration of 0.5 mM. Real-time calibration was conducted concurrently with sample analysis employing leucine-enkephalin at a concentration of 200 ng/mL.

1.3 Data analysis

The data are expressed as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was conducted using GraphPad Prism software. A P-value of less than 0.05 was considered to indicate statistical significance.