Text S1. Metabolite extraction method

After slowly thawing the samples at 4°C, an appropriate amount of each sample was added to a pre-cooled methanol/acetonitrile/water solution (2:2:1, v/v). The mixture was vortexed and subjected to low-temperature ultrasonication for 30 minutes, followed by standing at -20°C for 10 minutes. The samples were then centrifuged at 14,000 g for 20 minutes at 4°C. The supernatant was collected and vacuum-dried. For mass spectrometry analysis, the dried samples were reconstituted in 100 μL of acetonitrile-water solution (acetonitrile: water = 1:1, v/v), vortexed, and centrifuged at 14,000 g for 15 minutes at 4°C. The resulting supernatant was used for subsequent analysis.

Text S2. UHPLC-Q-Exactive Orbitrap MS

Analysis was performed using an UHPLC (Vanquish UHPLC, Thermo) coupled to a Orbitrap. For HILIC separation, samples were analyzed using a 2.1 mm × 100 mm ACQUIY UPLC BEH Amide 1.7 μm column (waters, Ireland). In both ESI positive and negative modes, the mobile phase contained A=25 mM ammonium acetate and 25 mM ammonium hydroxide in water and B= acetonitrile. The gradient was 98% B for 1.5 min and was linearly reduced to 2% in 10.5 min, and then kept for 2 min, and then increased to 98% in 0.1 min, with a 3 min re-equilibration period employed.

The ESI source conditions were set as follows: Ion Source Gas1 (Gas1) as 60, Ion Source Gas2 (Gas2) as 60, curtain gas (CUR) as 30, source temperature: 600℃, IonSpray Voltage Floating (ISVF) ± 5500 V. In MS only acquisition, the instrument was set to acquire over the m/z range 80-1200 Da, the resolution was set at 60000 and the accumulation time was set at 100ms. In auto MS/MS acquisition, the instrument was set to acquire over the m/z range 70-1200 Da, the resolution was set at 30000 and the accumulation time was set at 50ms, exclude time within 4 s.

Text S3. Quality evaluation of metabolomics experimental data

This experiment provides a comprehensive assessment of the stability of the instrument, the repeatability of the experiment and the reliability of the data quality. Overall, the stability of the instrumental analysis system of this experiment was excellent, and the experimental data were stable and reliable.

1. Comparison of QC sample total ion flow diagrams (TIC)

The total ion chromatograms (TIC) of the QC samples were compared by overlapping the spectra, as shown in the Fig. S1. The experimental results indicate that the response intensities and retention times of the chromatographic peaks were essentially overlapping, suggesting that the variation caused by instrumental error was minimal throughout the experiment.

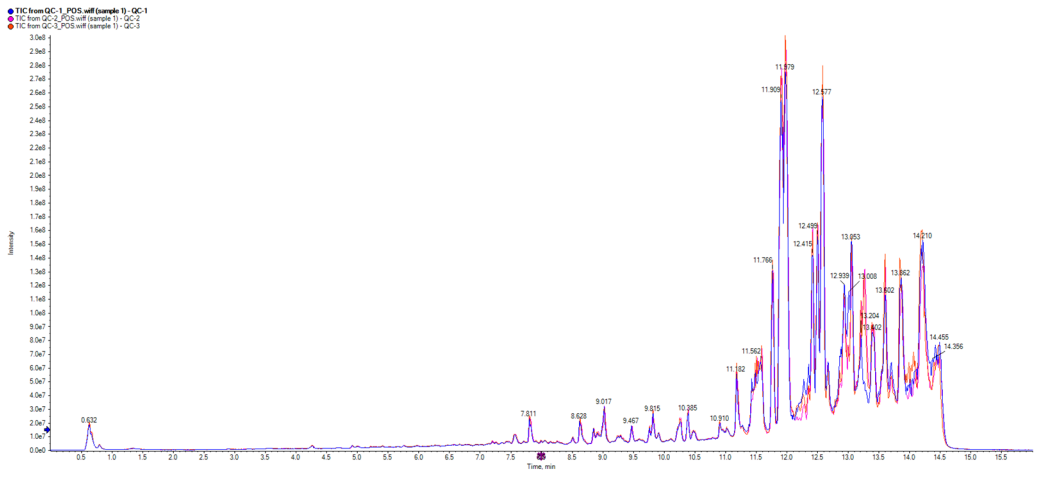

Figure S1. Positive ion mode QC sample total ion flow map overlay spectrum. The horizontal coordinate indicates the retention time of each peak and the vertical coordinate indicates the intensity value of the peak.

2. QC correlation analysis

Pearson correlation analysis was performed on the QC samples, as shown in the Fig. S2. Generally, a correlation coefficient greater than 0.9 indicates a strong correlation. The experimental results show that the correlation coefficients between the QC samples were all above 0.9, demonstrating good experimental reproducibility.

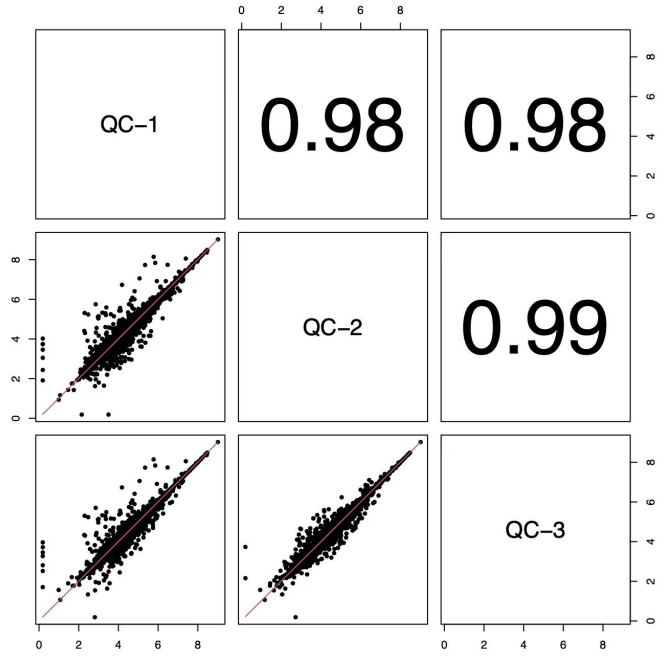

Figure S2. Correlation analysis plot of QC samples. The x-axis and y-axis represent the QC samples. Each dot in the grid represents an ion peak (metabolite) extracted from the QC samples, with the x-axis and y-axis representing the logarithmic values of the ion peak signal intensities. The values in the plot are the correlation coefficients, with a coefficient greater than 0.9 indicating a very strong correlation.

3. Relative Standard Deviation (RSD) of QC Samples.

To identify biomarkers, the relative standard deviation (RSD) of potential characteristic peaks in QC samples, also known as the coefficient of variation, must not exceed 30%. Characteristic peaks that do not meet this criterion should be excluded. Therefore, based on quality control (QC), quality assurance (QA) is typically performed to remove characteristic peaks with poor reproducibility in QC samples, thereby obtaining a higher quality dataset that is more conducive to biomarker detection. A smaller RSD of ion peak abundance in QC samples indicates better instrument stability and is an important indicator of data quality. In QC samples, the proportion of characteristic peaks with RSD < 30% can reach approximately 65%, suggesting good data quality and stable instrument analysis system performance.

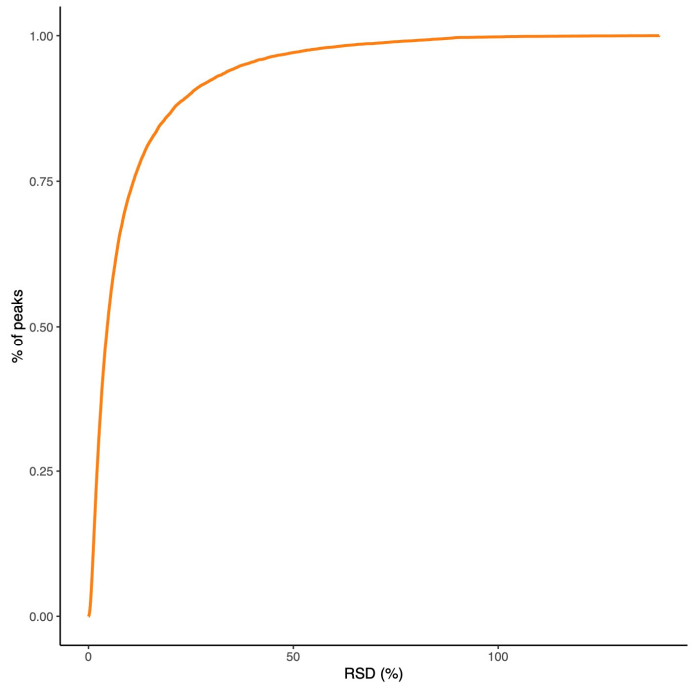

Figure S3. QC Relative Standard Deviation (RSD) Plot. The horizontal axis represents the RSD values, while the vertical axis represents the proportion of the number of peaks relative to the total number of peaks in the QC samples.