Supplementary Information (SI) for Analyst.


Supporting Table S1. Method validation and metabolite identification levels.

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Cytosine | Positive | y=4998659.16528+369670.61499x | 0.999 | 2.5-250 | 3.4 | 6.1 | 8.3 | Absolute | Level 1 |
| Spermidine | Positive | y=188549.71848+39175.1024x | 0.996 | 10-1000 | 8.7 | 5.3 | 12.6 | Absolute | Level 1 |
| Purine | Positive | y=185708.20634+115.26714x | 0.991 | 150-2000 | 1.4 | 9.3 | 12.5 | Absolute | Level 1 |
| Adenosine | Positive | y=976331.93727+209001.73862x | 0.995 | 10-2000 | 3.6 | 6.5 | 8.1 | Absolute | Level 1 |
| Putrescine | Positive | y=2543452.9865+19365.70759x | 0.997 | 150-800 | 1.3 | 11.1 | 16 | Absolute | Level 1 |
| Methionine | Positive | y=2.14767E7+14424.51009x | 0.993 | 25-800 | 1.9 | 4.2 | 4.7 | Absolute | Level 1 |
| Spermine | Positive | y=-825752.06855+25198.93879x | 1 | 250-2000 | 5.9 | 8 | 9.3 | Absolute | Level 1 |
| SAM | Positive | y=986250.94562+96028.96715x | 0.999 | 2.5-500 | 2.5 | 16.8 | 11.8 | Absolute | Level 1 |
| SAH | Positive | y=280242.50825+16528.30909x | 0.997 | 2.5-500 | 4.9 | 13.6 | 19.2 | Absolute | Level 1 |
| DHF | Positive | y=-142.84932+1653.30538x | 0.998 | 2.5-500 | 6.5 | 9.8 | 15.1 | Absolute | Level 1 |
| Folinic acid | Positive | y=42226.03727+52509.01898x | 0.998 | 2.5-500 | 8.2 | 11.3 | 13.5 | Absolute | Level 1 |
| Thymine | Positive | y=8736771.41603+101463.96643x | 0.995 | 10-400 | 0.9 | 1.3 | 2.6 | Absolute | Level 1 |
| Adenine | Positive | y=5452230.28398+14329.77004x | 0.992 | 150-1000 | 0.8 | 6.8 | 6 | Absolute | Level 1 |
| N,N-Dimethylglycine | Positive | y=360008.18923+97.06685x | 0.995 | 200-4000 | 4 | 11.6 | 6.8 | Absolute | Level 1 |
| Valine | Positive | y=174046.47511+0.47091x | 0.956 | 10000-40000 | 0.7 | 4.2 | 3.1 | Absolute | Level 1 |
| 4-Hydroxystyrene | Positive | y=111930.39315+91.82407x | 0.983 | 150-2000 | 0.5 | 8.6 | 6.1 | Absolute | Level 1 |
| Leucine | Positive | y=1162414.1623+89.75245x | 0.966 | 500-10000 | 0.5 | 2.7 | 4.5 | Absolute | Level 1 |
| Isoleucine | Positive | y=1361768.17982+192.93907x | 0.993 | 3000-20000 | 1.3 | 2.7 | 4.9 | Absolute | Level 1 |
| Asparagine | Positive | y=21134.87712+52.37244x | 0.998 | 150-800 | 0.3 | 2.4 | 5.2 | Absolute | Level 1 |
| ornithine | Positive | y=1796797.0736+52.37244x | 0.996 | 3000-20000 | 1.3 | 2.3 | 4.5 | Absolute | Level 1 |
| Stachydrine | Positive | y=516446.10552+76.57705x | 0.982 | 3000-16000 | 0.7 | 7.8 | 4.7 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Glutamate | Positive | y=54765.00774+6.43096x | 0.996 | 500-16000 | 1.9 | 2.9 | 4.4 | Absolute | Level 1 |
| Xanthine | Positive | y=575945.7401+273.03039x | 0.984 | 500-20000 | 1.3 | 8.6 | 2.5 | Absolute | Level 1 |
| 3-Methylxanthine | Positive | y=10325.35353+410.59364x | 0.992 | 2.5-800 | 6.5 | 2.3 | 8.2 | Absolute | Level 1 |
| LPC 14:0 | Positive | y=140957.79116+358.42456x | 0.992 | 100-6000 | 5.5 | 11.3 | 11.2 | Absolute | Level 1 |
| nicotinic acid | Positive | y=2172.39071+174.69202x | 0.994 | 2.5-2000 | 7.1 | 10.7 | 13.9 | Absolute | Level 1 |
| Tryptamine | Positive | y=1393.62654+792.63039x | 0.994 | 2.5-500 | 4.9 | 8.6 | 13.6 | Absolute | Level 1 |
| 4-hydroxycinnamic acid | Positive | y=77393.39337+211.4045x | 0.991 | 150-2000 | 9.1 | 11.7 | 6.4 | Absolute | Level 1 |
| IAA | Positive | y=396741.1254+807.4704x | 0.99 | 10-1000 | 2.8 | 4.2 | 5.1 | Absolute | Level 1 |
| xanthurenic acid | Positive | y=86779.01858+2299.0701x | 0.992 | 2.5-500 | 1.8 | 6.4 | 18.4 | Absolute | Level 1 |
| (R)-(Indol-3-yl)lactate | Positive | y=20309.66466+118.47489x | 0.993 | 2.5-500 | 3.4 | 2.6 | 8.1 | Absolute | Level 1 |
| 4-Aminophenol | Positive | y=3036.62088+3.95638x | 0.992 | 10-800 | 3.3 | 3 | 7.1 | Absolute | Level 1 |
| 5-HT | Positive | y=1930.14727+43.54929x | 0.996 | 2.5-2000 | 3.9 | 6.2 | 4 | Absolute | Level 1 |
| Kynurenine | Positive | y=87910.08682+115.63348x | 0.992 | 10-1000 | 2.3 | 9.1 | 13.6 | Absolute | Level 1 |
| LPC 15:0 | Positive | y=630072.39015+2934.04808x | 0.994 | 2.5-500 | 3 | 16.5 | 12.5 | Absolute | Level 1 |
| N-Methylnicotinamide | Positive | y=29454.15405+2722.30738x | 0.996 | 2.5-1000 | 2.1 | 5.9 | 7.8 | Absolute | Level 1 |
| N-Methylnicotinic acid | Positive | y=93498.92823+121.58726x | 0.992 | 150-800 | 4.4 | 9.3 | 5.4 | Absolute | Level 1 |
| Try | Positive | y=147331.3857+52.14089x | 0.995 | 10-1000 | 1.1 | 4.5 | 5.2 | Absolute | Level 1 |
| kynurenic acid | Positive | y=5964.61698+4.32385x | 0.996 | 2.5-2000 | 1.9 | 9.3 | 9.3 | Absolute | Level 1 |
| Melatonin | Positive | y=28451.45065+2118.30389x | 1 | 25-1000 | 2.3 | 2.4 | 3.8 | Absolute | Level 1 |
| MIAA | Positive | y=54464.74+53.67283x | 0.994 | 10-1000 | 1.1 | 4.1 | 5.5 | Absolute | Level 1 |
| N-Acetyl-5-HT | Positive | y=5030.79256+2673.80507x | 0.992 | 2.5-300 | 4.6 | 1.7 | 10.7 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Guanine | Positive | y=-69201.30506+3690440.45677x | 0.997 | 0.01P-1P | 9.1 | 13.4 | 13.8 | Absolute | Level 1 |
| Uracil | Positive | y=-88278.11191 +6417315.54076x | 0.999 | 0.01P-1P | 4.7 | 2.5 | 10.4 | Absolute | Level 1 |
| Homocysteine | Positive | y=41168.30051+275502.0232x | 0.993 | 0.01P-5P | 3.5 | 1.4 | 1.7 | Absolute | Level 1 |
| 10-formyl-THF | Positive | y=734.55767 +4779.1687 x | 0.986 | 0.01P-5P | 7.8 | 14.8 | 9.4 | Absolute | Level 1 |
| LPC O-16:0 | Positive | y=3550031.56862+6.42245E7x | 0.995 | 0.01P-5P | 3.5 | 17.6 | 11.2 | Absolute | Level 1 |
| Hypoxanthine | Positive | y=4262633.60072 +1.00655E8 x | 0.999 | 0.01P-5P | 1.5 | 10 | 4.7 | Absolute | Level 1 |
| LPC P-18:0 | Positive | y=224313.07432+2.07956E7x | 0.999 | 0.01P-5P | 9.6 | 14.1 | 11.6 | Absolute | Level 1 |
| LPC 17:0 | Positive | y=4589264.26306+9.7439E7x | 0.998 | 0.01P-5P | 2.5 | 4.9 | 10.2 | Absolute | Level 1 |
| LPC 22:6 | Positive | y=-4612350.90073+2.01388E8x | 0.997 | 0.01P-0.5P | 4.3 | 4.3 | 13.3 | Absolute | Level 1 |
| LPC 20:5 | Positive | y=1113470.01809+5.6132E7x | 0.999 | 0.01P-5P | 1.4 | 9.6 | 11.9 | Absolute | Level 1 |
| LPC 15:1 | Positive | y=746317.81598+3.92777E7x | 0.995 | 0.01P-5P | 1.3 | 12.9 | 11.7 | Absolute | Level 1 |
| LPC 16:3 | Positive | y=-565535.38503+2.03243E7x | 0.999 | 0.01P-5P | 1.6 | 7.5 | 13.8 | Absolute | Level 1 |
| LPC 20:3 | Positive | y=26503.56876+2458197.78638x | 0.998 | 0.01P-5P | 8 | 11.7 | 16.5 | Absolute | Level 1 |
| C 18:1 | Positive | y=115.3118+6273.14543x | 1 | 0.01P-5P | 9.8 | 16.4 | 13.5 | Absolute | Level 1 |
| LPC 18-OH_1 | Positive | y=-559.12591+22513.2604x | 0.989 | 0.01P-1P | 1.2 | 15.1 | 18.2 | Absolute | Level 1 |
| C 18:1-OH | Positive | y=4.48337+2980.2719x | 1 | 0.01P-5P | 7 | 16.1 | 13.9 | Absolute | Level 1 |
| C 20:5 | Positive | y=53.40996+1986.42511x | 0.999 | 0.05P-5P | 2.6 | 8 | 11.5 | Absolute | Level 1 |
| C 18:2 | Positive | y=-6540.14941+533557.25434x | 0.998 | 0.02P-2.5P | 1.6 | 5 | 10.6 | Absolute | Level 1 |
| LPC 15:1_1 | Positive | y=3643.0095+327047.29274x | 0.997 | 0.01P-2.5P | 2.3 | 12.9 | 11.7 | Absolute | Level 1 |
| C 16-OH | Positive | y=120.1127+28681.70385 x | 0.999 | 0.01P-0.5P | 4.2 | 12.8 | 14.2 | Absolute | Level 1 |
| C 18 | Positive | y=754.20522+113484.78863x | 0.998 | 0.01P-2.5P | 4.3 | 13.8 | 12.6 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| LPC 16:3_1 | Positive | y=-9322.20034+346167.02343x | 0.999 | 0.01P-2.5P | 1.6 | 17.5 | 13.8 | Absolute | Level 1 |
| C 18-OH | Positive | y=-82.6504+5588.89371x | 0.997 | 0.01P-0.5P | 1.9 | 15.8 | 13.3 | Absolute | Level 1 |
| LPC 20:3_1 | Positive | y=723.00331+21410.9807x | 0.999 | 0.01P-5P | 9.5 | 11.7 | 16.5 | Absolute | Level 1 |
| C 14:3 | Positive | y=-164.89312+34396.16561x | 0.999 | 0.01P-0.5P | 3.4 | 4.6 | 11 | Absolute | Level 1 |
| w49-UK | Positive | y=22937.87699+15401.11434x | 0.994 | 0.01P-2.5P | 8.7 | 9 | 13.8 | Semi-quantitative | Level 4 |
| w52-UK | Positive | y=1160.21102 +8858.97212x | 0.931 | 0.01P-0.5P | 2.3 | 10.3 | 5.4 | Semi-quantitative | Level 4 |
| w54-UK | Positive | y=328.9804+12099.1758x | 0.97 | 0.01P-0.5P | 4.8 | 8.5 | 5.6 | Semi-quantitative | Level 4 |
| w55-UK | Positive | y=10791.14274+660269.84764 x | 0.998 | 0.01P-0.5P | 1.9 | 17.2 | 8.8 | Semi-quantitative | Level 4 |
| w56-UK | Positive | y=112.68593+1267.81048x | 1 | 0.01P-5P | 3.1 | 12.7 | 11.2 | Semi-quantitative | Level 4 |
| w57-UK | Positive | y=24.98419 +32485.91081x | 0.999 | 0.01P-0.5P | 1.9 | 7.7 | 6.7 | Semi-quantitative | Level 4 |
| w58-UK | Positive | y=14828.37258+643021.99468x | 1 | 0.01P-5P | 6 | 8.4 | 12 | Semi-quantitative | Level 4 |
| w59-UK | Positive | y=-1455.39412+169922.12155 x | 0.997 | 0.01P-0.5P | 6 | 17.1 | 12.6 | Semi-quantitative | Level 4 |
| 4.3 | 13.3 | y=4472.27733 +6008.38664x | 0.999 | 0.01P-5P | 1.9 | 7.6 | 14 | Absolute | Level 1 |
| w62-UK | Positive | y=75912.39442+125436.29832x | 1 | 0.01P-5P | 9 | 15 | 13.2 | Semi-quantitative | Level 4 |
| w63 -UK | Positive | y=131.81452+1048.10702 x | 0.993 | 0.01P-1P | 1.3 | 16.2 | 32.3 | Semi-quantitative | Level 4 |
| w64 -UK | Positive | y=-2398.3161+223905.33566x | 0.997 | 0.01P-0.5P | 8.9 | 4.5 | 13.2 | Semi-quantitative | Level 4 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| w65-UK | Positive | y=38757.18893+10439.12945x | 0.998 | 0.01P-5P | 3.2 | 7.8 | 7.4 | Semi-quantitative | Level 4 |
| w66-UK | Positive | y=-2406.64374+576229.31286 x | 0.999 | 0.01P-0.5P | 2.6 | 15.3 | 12.4 | Semi-quantitative | Level 4 |
| w67-UK | Positive | y=10884.10336+24101.81461x | 1 | 0.01P-2.5P | 4.8 | 16.1 | 13 | Semi-quantitative | Level 4 |
| w68-UK | Positive | y=-964.57876+154994.82737x | 0.992 | 0.01P-0.5P | 8.7 | 8.9 | 12.5 | Semi-quantitative | Level 4 |
| w69-UK | Positive | y=1562.60877+159559.72377x | 0.999 | 0.01P-0.5P | 2.6 | 6.2 | 8 | Semi-quantitative | Level 4 |
| w70-UK | Positive | y=493.41562+8643.72246x | 1 | 0.01P-2.5P | 6.7 | 15.7 | 10.5 | Semi-quantitative | Level 4 |
| w71-UK | Positive | y=24193.10939+167904.7486x | 0.999 | 0.01P-0.5P | 4.3 | 10.9 | 18.2 | Semi-quantitative | Level 4 |
| w72-UK | Positive | y=-2897.52261+315894.31872x | 1 | 0.01P-2.5P | 9 | 17.4 | 12.9 | Semi-quantitative | Level 4 |
| w74-UK | Positive | y=2477.95298+24141.43327x | 0.996 | 0.01P-5P | 5.5 | 10.4 | 5 | Semi-quantitative | Level 4 |
| w75-UK | Positive | y=8656.8634+908691.06533x | 0.997 | 0.01P-2.5P | 2.8 | 2.5 | 10.4 | Semi-quantitative | Level 4 |
| LPC 16:2 | Positive | y=2290.63877+118508.49252x | 0.998 | 0.01P-5P | 9 | 11.8 | 10.6 | Absolute | Level 1 |
| w53-UK | Positive | y=-634.85937+42490.3211x | 1 | 0.01P-2.5P | 8.6 | 10.5 | 14.4 | Semi-quantitative | Level 4 |
| w61-UK | Positive | y=-2540.45394+482836.03395x | 0.998 | 0.01P-0.5P | 5 | 6.8 | 12.9 | Semi-quantitative | Level 4 |
| Creatinine | Positive | y=195202.76525+2193589.37124x | 0.964 | 0.01P-0.5P | 0.5 | 1 | 5.5 | Absolute | Level 1 |
| Acetylcholine | Positive | y=17585.73881+750622.60847x | 0.994 | 0.01P-0.5P | 9.6 | 14.8 | 6.4 | Absolute | Level 1 |
| LPC 16:0 | Positive | y=+1.43316E7x | 1 | 0.01P-1P | 7.2 | 4.6 | 9.9 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| N,N-dimethyl-C20 Phytosphingosine | Positive | y=633.30565+9229.22392x | 1 | 0.01P-2.5P | 1.3 | 8.9 | 11.1 | Absolute | Level 1 |
| C22 Phytosphingosine | Positive | y=391.731+3630.69868x | 1 | 0.01P-2.5P | 8.7 | 16.1 | 11.9 | Absolute | Level 1 |
| Sarmentogenin | Positive | y=-521.46778+302542.28224x | 1 | 0.01P-0.5P | 1.7 | 13.8 | 17.1 | Absolute | Level 1 |
| LPC O-16:0 | Positive | y=-10195.24735+445212.49213x | 0.994 | 0.01P-0.5P | 2.4 | 17.6 | 11.2 | Absolute | Level 1 |
| LPC 15:0 | Positive | y=-2869.03063+358043.98541x | 1 | 0.01P-2.5P | 2.6 | 15.2 | 11 | Absolute | Level 1 |
| Hypoxanthine | Positive | y=3400.77059+246162.63591x | 0.998 | 0.01P-0.5P | 1.8 | 10 | 4.7 | Absolute | Level 1 |
| LPC 16:1 | Positive | y=-186642.56645+7748920x | 0.996 | 0.01P-0.5P | 1.5 | 16.4 | 12.1 | Absolute | Level 1 |
| LPC 18:0 | Positive | y=21703.35378+3119180x | 0.997 | 0.01P-0.5P | 6.1 | 9.6 | 8.2 | Absolute | Level 1 |
| LPC P-18:0 | Positive | y=12216.63428+364512.75963x | 0.994 | 0.01P-5P | 9.2 | 14.1 | 11.6 | Absolute | Level 1 |
| LPC 17:1 | Positive | y=-2463.40875+129194.24468x | 0.997 | 0.01P-0.5P | 9.1 | 2.9 | 9.9 | Absolute | Level 1 |
| LPC 17:0 | Positive | y=2521.06448+658302.92466x | 0.998 | 0.01P-2.5P | 1.9 | 4.9 | 10.2 | Absolute | Level 1 |
| LPC(18:0e) | Positive | y=48768.8868+4726150x | 0.997 | 0.01P-2.5P | 3 | 5.7 | 10.1 | Absolute | Level 1 |
| LPC 20:4 | Positive | y=-81227.6788+3008540x | 0.992 | 0.01P-0.5P | 8.3 | 12 | 9.8 | Absolute | Level 1 |
| LPC 20:2 | Positive | y=-221.1471+33564.71719x | 1 | 0.01P-2.5P | 7.7 | 14.1 | 11.8 | Absolute | Level 1 |
| LPC 20:1 | Positive | y=-2069.13621+84135.5648 | 1 | 0.01P-2.5P | 8 | 14.3 | 13.3 | Absolute | Level 1 |
| LPC 22:6 | Positive | y=-81801.00697+3847150x | 0.997 | 0.01P-0.5P | 6.7 | 9.3 | 10.5 | Absolute | Level 1 |
| C14 sphingosine | Positive | y=6245.21033+2867.52413x | 0.993 | 0.01P-5P | 8 | 15.7 | 15.7 | Absolute | Level 1 |
| α-Amino octanoic acid | Positive | y=5536.38355+106347.90167x | 0.997 | 0.01P-2.5P | 2.6 | 2.4 | 5.9 | Absolute | Level 1 |
| C16 Sphinganine | Positive | y=2296.90303+127475.66117x | 0.998 | 0.01P-0.5P | 8.7 | 11.4 | 12 | Absolute | Level 1 |
| C16 Phytosphingosine | Positive | y=480.08355+7008.99857x | 1 | 0.01P-2.5P | 7.4 | 18.5 | 12.7 | Absolute | Level 1 |
| Carnitine C10:1 | Positive | y=5221.18184+1960900x | 0.999 | 0.01P-0.5P | 1.9 | 4.3 | 10.6 | Absolute | Level 1 |

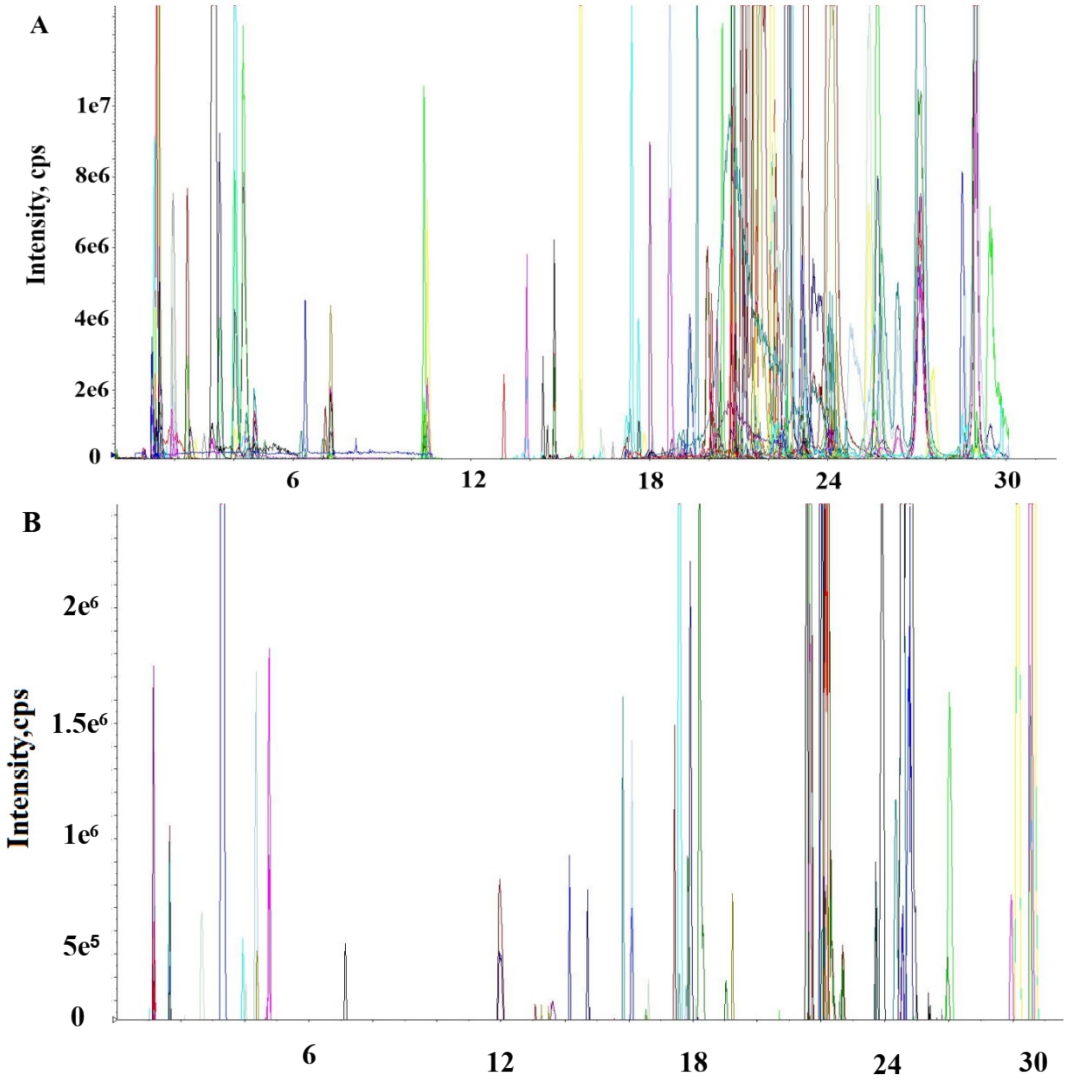
| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Carnitine C10:0-OH | Positive | y=-73.27684+108048.99838x | 0.999 | 0.01P-0.5P | 3 | 3.6 | 10.9 | Absolute | Level 1 |
| Carnitine C12:0-OH | Positive | y=820.1964+23432.5093x | 0.997 | 0.01P-2.5P | 3.1 | 3.2 | 11.2 | Absolute | Level 1 |
| Carnitine C14:1 | Positive | y=-1426.56948+297644.3217x | 1 | 0.01P-2.5P | 1.9 | 17.4 | 12.9 | Absolute | Level 1 |
| Carnitine C14:2-OH | Positive | y=907.85519+19779.67412x | 0.998 | 0.01P-5P | 3.5 | 2.8 | 12.2 | Absolute | Level 1 |
| Palmitoyl-Carnitine | Positive | y=-51.83132+225326.55139x | 0.998 | 0.01P-2.5P | 1.2 | 15.7 | 12.2 | Absolute | Level 1 |
| Carnitine C16:2-OH | Positive | y=-127.31249+16061.30876x | 0.999 | 0.01P-0.5P | 9.2 | 12.4 | 12.4 | Absolute | Level 1 |
| LPC(16:0)-OH | Positive | y=-422.33246+20914.14024x | 0.996 | 0.01P-0.5P | 9.2 | 16.5 | 12.2 | Absolute | Level 1 |
| LPC 20:5 | Positive | y=-11928.65939+1310930x | 0.999 | 0.01P-2.5P | 9.4 | 19.6 | 11.9 | Absolute | Level 1 |
| Carnitine C8-OH | Positive | y=433.73537+238012.64779x | 1 | 0.01P-0.5P | 2.8 | 5.3 | 9.4 | Absolute | Level 1 |
| Indoleacetonitrile | Positive | y=687.76456+11457.96525x | 0.996 | 0.01P-0.5P | 2.7 | 10 | 9.9 | Absolute | Level 1 |
| Tyr | Positive | y=653.94153+106360.69464x | 1 | 0.01P-0.5P | 2.5 | 8.9 | 5.7 | Absolute | Level 1 |
| 5-HTP | Positive | y=223.3375+4655.28659x | 0.996 | 0.01P-1P | 9.9 | 15 | 9.2 | Absolute | Level 1 |
| Phytosphingosine | Positive | y=56.23497+2144.33687x | 1 | 0.01P-2.5P | 2.7 | 17.6 | 12 | Absolute | Level 1 |
| 5-HIAA | Positive | y=252.82292+158151.20736x | 1 | 0.01P-0.5P | 1.4 | 6.1 | 7 | Absolute | Level 1 |
| indoleacrylic acid | Positive | y=974.06094+143029.12993x | 1 | 0.01P-0.5P | 0.4 | 3.5 | 5.9 | Absolute | Level 1 |
| IPA | Positive | y=820.16481+368839.78536x | 1 | 0.01P-0.5P | 0.5 | 12 | 8.5 | Absolute | Level 1 |
| Indole | Positive | y=-1841.7944+977651.40694x | 0.999 | 0.01P-0.5P | 3 | 37.7 | 9.5 | Absolute | Level 1 |
| nicotinamide | Positive | y=1800.03782+125554.39686x | 0.995 | 0.01P-1P | 0.4 | 1.3 | 5.6 | Absolute | Level 1 |
| LPC 18:3 | Positive | y=-2216.7264+63660.52682x | 0.999 | 0.01P-2.5P | 2.1 | 7 | 10.1 | Absolute | Level 1 |
| Taurine | Negative | y=2620887.57126+1785.29802x | 0.996 | 10-2000 | 2.2 | 9.4 | 6.8 | Absolute | Level 1 |
| Phenylalanine | Negative | y=4954.96978+3.99642x | 0.997 | 250-2000 | 2.5 | 2.5 | 1.5 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Glutamine | Negative | y=1594.82508+6.64784x | 1 | 2.5-400 | 3.7 | 3.4 | 8.2 | Absolute | Level 1 |
| LPE 18:1 | Negative | y=76553.10488+87.23141x | 0.985 | 2.5-1000 | 2.8 | 2.8 | 3.3 | Absolute | Level 1 |
| Succinic acid | Negative | y=358.07577+0.44446x | 0.899 | 25-1000 | 2 | 14.6 | 5.9 | Absolute | Level 1 |
| Hydroxyisocaproic acid | Negative | y=2365.13484+62.46671x | 1 | 2.5-1000 | 2.9 | 11.8 | 3.3 | Absolute | Level 1 |
| N-Methyl-aspartic acid | Negative | y=20107.73481+29.12907x | 1 | 10-1000 | 3.3 | 3 | 3.1 | Absolute | Level 1 |
| Tauroursodeoxycholic acid | Negative | y=-1916.27521+26.10003x | 0.999 | 250-1000 | 8.3 | 16.7 | 3.4 | Absolute | Level 1 |
| Oxoglutaric acid | Negative | y=86806.51451+33.91172x | 0.932 | 150-1000 | 9.9 | 9.1 | 5.5 | Absolute | Level 1 |
| Pyruvic acid | Negative | y=1016.29733+1.14511x | 0.998 | 150-2000 | 3.4 | 3.3 | 3.8 | Absolute | Level 1 |
| Aspartate | Negative | y=949.18721+2.18541x | 0.995 | 25-500 | 4.2 | 5.3 | 5 | Absolute | Level 1 |
| phenyllactate | Negative | y=-14856.14371+1271.38842x | 0.995 | 2.5-2000 | 5.2 | 14.3 | 2.7 | Absolute | Level 1 |
| indole-3-carboxylic acid | Negative | y=-10608.58336+651.39281x | 0.996 | 2.5-1000 | 5.9 | 17.3 | 1.9 | Absolute | Level 1 |
| indolepropionic acid | Negative | y=5037.29288+17.97507x | 0.993 | 2.5-800 | 2.4 | 9.3 | 15.5 | Absolute | Level 1 |
| Indoxyl sulfate | Negative | y=79433.0004+155.67734x | 0.994 | 2.5-1000 | 2.3 | 2.3 | 2.5 | Absolute | Level 1 |
| LPC 18:0 | Negative | y=-2257644.11652+2.18669E8x | 0.999 | 0.01P-0.5P | 3.9 | 9.6 | 8.2 | Absolute | Level 1 |
| Gluconic acid | Negative | y=90.22338+506.6143x | 0.996 | 0.01P-2P | 3.4 | 3.3 | 1.6 | Absolute | Level 1 |
| LPE 18:3 | Negative | y=87.15073+2512.30641x | 1 | 0.01P-4P | 3.9 | 8.8 | 3.7 | Absolute | Level 1 |
| LPE 20:2 | Negative | y=-8307.69945+357471.13505x | 0.996 | 0.01P-0.5P | 9.8 | 9.5 | 3.5 | Absolute | Level 1 |
| LPE 18:2 | Negative | y=43209.97393+816122.43664x | 1 | 0.01P-4P | 6.8 | 6.1 | 1.3 | Absolute | Level 1 |
| LPC P-18:1 | Negative | y=-9437.36618+388363.79445x | 0.995 | 0.01P-0.5P | 5.6 | 5.6 | 5.9 | Absolute | Level 1 |
| LPC 22:4 | Negative | y=449.3903+14715.36812x | 0.998 | 0.01P-4P | 2.8 | 2.7 | 1.1 | Absolute | Level 1 |
| LPE 18:0 | Negative | y=1179.05785+37456.79814x | 1 | 0.01P-4P | 2.9 | 3.7 | 2.3 | Absolute | Level 1 |

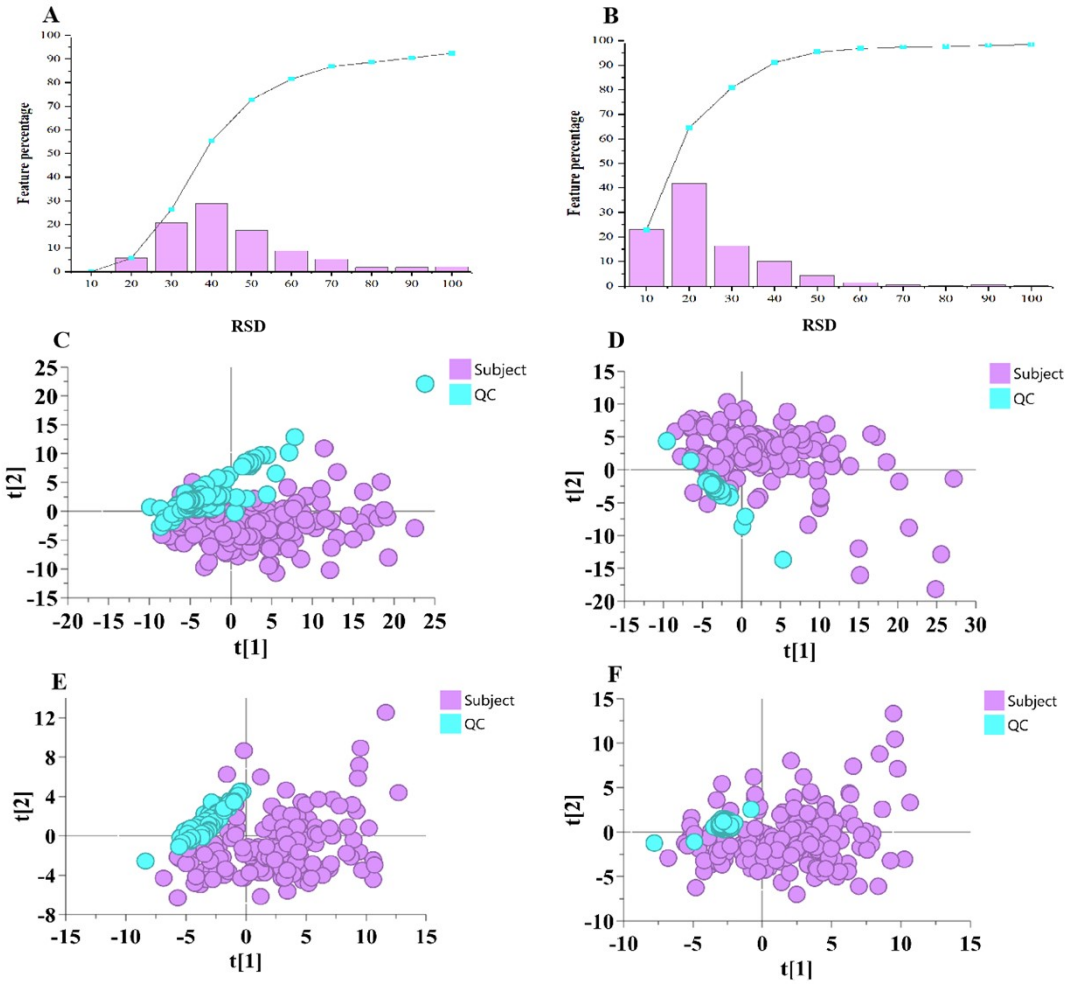
| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| FA 16:0 | Negative | y=1326.85301+33509.02409x | 0.993 | 0.01P-1P | 2.5 | 6.6 | 5.7 | Absolute | Level 1 |
| Pyrazole-1-alanine | Negative | y=2391.21312+22932.1068x | 0.997 | 0.02P-2P | 8.4 | 7.7 | 7 | Absolute | Level 1 |
| C 18:1-OH | Negative | y=-157.57061+2249.84555x | 1 | 0.05P-4P | 7.3 | 6.9 | 2 | Absolute | Level 1 |
| Gln-Glu | Negative | y=723.37942+3333.00864x | 0.999 | 0.01P-4P | 2.2 | 2 | 4.5 | Absolute | Level 1 |
| w30-UK | Negative | y=11669.38608+555435.34816x | 1 | 0.01P-2P | 2.3 | 9.3 | 8.4 | Semi-quantitative | Level 4 |
| w31-UK | Negative | y=219.60501+7317.82028x | 0.999 | 0.01P-1P | 3.7 | 13.1 | 3.2 | Semi-quantitative | Level 4 |
| w33-UK | Negative | y=6041.16201+1668646.00797x | 0.998 | 0.01P-2P | 9.6 | 9.1 | 2.7 | Semi-quantitative | Level 4 |
| w34-UK | Negative | y=119.3166+11573.52629x | 1 | 0.01P-1P | 6.6 | 4.8 | 6.7 | Semi-quantitative | Level 4 |
| w35-UK | Negative | y=-788.88438+59980.21938x | 1 | 0.01P-2P | 5.8 | 14.7 | 1.8 | Semi-quantitative | Level 4 |
| w36-UK | Negative | y=2148.21159+50061.35029 x | 1 | 0.01P-4P | 0.9 | 0.8 | 2.1 | Semi-quantitative | Level 4 |
| w38-UK | Negative | y=2225.07653+167173.35555x | 0.993 | 0.01P-1P | 6.5 | 6.8 | 3.4 | Semi-quantitative | Level 4 |
| w39-UK | Negative | y=8.70128+4163.51978x | 1 | 0.01P-1P | 4.4 | 12.4 | 12.6 | Semi-quantitative | Level 4 |
| 4-Hydroxyglutamate semialdehyde | Negative | y=767.27825+1868.43629x | 0.998 | 0.01P-2P | 7.2 | 6.6 | 7.7 | Absolute | Level 1 |
| w40-UK | Negative | y=-86.16151+21935.62142x | 1 | 0.02P-1P | 4.4 | 2.6 | 5.4 | Semi-quantitative | Level 4 |
| w41-UK | Negative | y=2327.34812+57409.06487x | 0.999 | 0.02P-1P | 7.6 | 6.3 | 14.7 | Semi-quantitative | Level 4 |
| w42-UK | Negative | y=2562.45895+45879.88385x | 0.997 | 0.01P-4P | 9.5 | 8.2 | 5.3 | Semi-quantitative | Level 4 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| w43-UK | Negative | y=-448.37522+62101.87221x | 0.997 | 0.01P-0.5P | 5 | 3.7 | 2.4 | Semi-quantitative | Level 4 |
| w44-UK | Negative | y=-1426.26524+101720.37712x | 0.996 | 0.01P-0.5P | 8.7 | 8.4 | 4.1 | Semi-quantitative | Level 4 |
| w45-UK | Negative | y=320.77604+9434.1914x | 0.998 | 0.01P-0.5P | 3.4 | 13 | 5.5 | Semi-quantitative | Level 4 |
| w46-UK | Negative | y=682.2643+22025.80035x | 0.999 | 0.01P-4P | 6.4 | 5.9 | 5 | Semi-quantitative | Level 4 |
| w46-UK | Negative | y=14037.68296+337614.27376x | 0.998 | 0.01P-4P | 8.7 | 16.7 | 5.2 | Semi-quantitative | Level 4 |
| w47-UK | Negative | y=513.17882+994.19407x | 0.988 | 0.02P-2P | 3.5 | 15.1 | 19.5 | Semi-quantitative | Level 4 |
| Glu-Gly | Negative | y=312.16047+500.74671x | 0.991 | 0.05P-4P | 6.7 | 16 | 6.7 | Absolute | Level 1 |
| Hydroxybutyric acid | Negative | y=22875.83558+285797.59953x | 0.996 | 0.01P-2P | 7.8 | 7.2 | 2.4 | Absolute | Level 1 |
| Glu-Leu | Negative | y=167.47116+13727.1456x | 1 | 0.01P-1P | 5.7 | 4.1 | 3.6 | Absolute | Level 1 |
| Tyrosol 4-sulfate | Negative | y=129.53242+2781.18272x | 0.998 | 0.01P-4P | 8.4 | 7.1 | 6.8 | Absolute | Level 1 |
| Lactate | Negative | y=2911.95286+51386.38845x | 0.976 | 0.01P-0.5P | 2 | 1.8 | 1.5 | Absolute | Level 1 |
| Glycochenodeoxycholate sulfate | Negative | y=653.67344+23552.90424x | 0.995 | 0.01P-2P | 7.9 | 7 | 12.9 | Absolute | Level 1 |
| Nonanedioic acid | Negative | y=9677.62178+16487.85374x | 0.995 | 0.05P-4P | 3.3 | 3.2 | 4.5 | Absolute | Level 1 |
| Malic acid | Negative | y=4709.14917+70760.93443x | 0.999 | 0.01P-4P | 3.1 | 2.8 | 3 | Absolute | Level 1 |
| Threitol | Negative | y=2109.13853+43523.40463x | 0.998 | 0.01P-2P | 7.5 | 6.9 | 2.1 | Absolute | Level 1 |
| FA C10:0-OH | Negative | y=2198.9456+29666.17888x | 0.996 | 0.01P-4P | 5.5 | 5.3 | 5.4 | Absolute | Level 1 |
| FA 12:1-OH (3 位羟基) | Negative | y=628.19636+8700.15644x | 0.996 | 0.01P-4P | 6.6 | 14.4 | 4.7 | Absolute | Level 1 |
| FA 14:1-OH (3 位羟基) | Negative | y=510.86627+7436.44716x | 0.996 | 0.01P-4P | 6.9 | 15.6 | 1.4 | Absolute | Level 1 |

| Name | Ion mode | Regression Equation | $R^2$ | Dynamic range（ng/mL） | Precision(%) | Intra-day | Inter-day | Quantification Level | MSI Identification Level |
|---|---|---|---|---|---|---|---|---|---|
| Dihydrotestosterone | Negative | y=6048.944+103539.27716x | 0.996 | 0.01P-4P | 6 | 16.2 | 1.7 | Absolute | Level 1 |
| {[(2E)-3-phenylprop-2-en-1-yl]oxy}sulfonic acid | Negative | y=10793.9398+399240.42409x | 0.998 | 0.01P-4P | 9 | 15.2 | 2.1 | Absolute | Level 1 |
| FA C18:2-O | Negative | y=920.00866+7139.71092x | 0.992 | 0.01P-4P | 7.7 | 7.5 | 15 | Absolute | Level 1 |
| Testosterone | Negative | y=36780.47688+1445890x | 0.992 | 0.01P-2P | 3.6 | 16.2 | 4.7 | Absolute | Level 1 |
| Tetrahydrodeoxycorticosterone sulfate | Negative | y=1943.65942+34414.72909x | 0.996 | 0.01P-4P | 6.8 | 12.8 | 1.8 | Absolute | Level 1 |
| PS(2-OMe-19:0/0:0) | Negative | y=3950.1867+234806.69171x | 0.999 | 0.01P-4P | 2.5 | 2.2 | 3.6 | Absolute | Level 1 |
| Biliverdin | Negative | y=90.00709+5394.26984x | 0.999 | 0.01P-0.5P | 4 | 13.3 | 6.5 | Absolute | Level 1 |



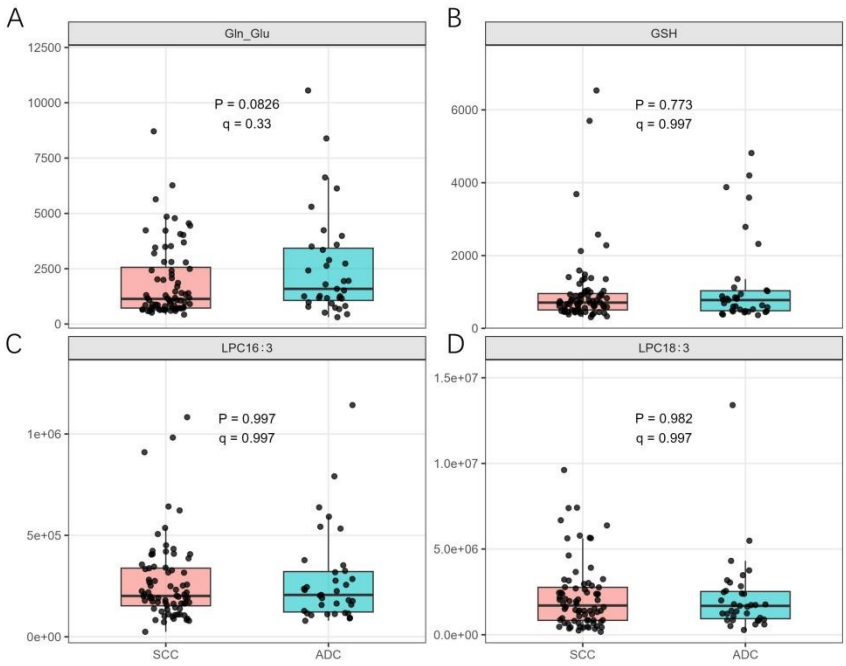

Supporting Figure S1 The total ion chromatograms (XICs) of QC samples in (A) positive ion mode and Negative (B)



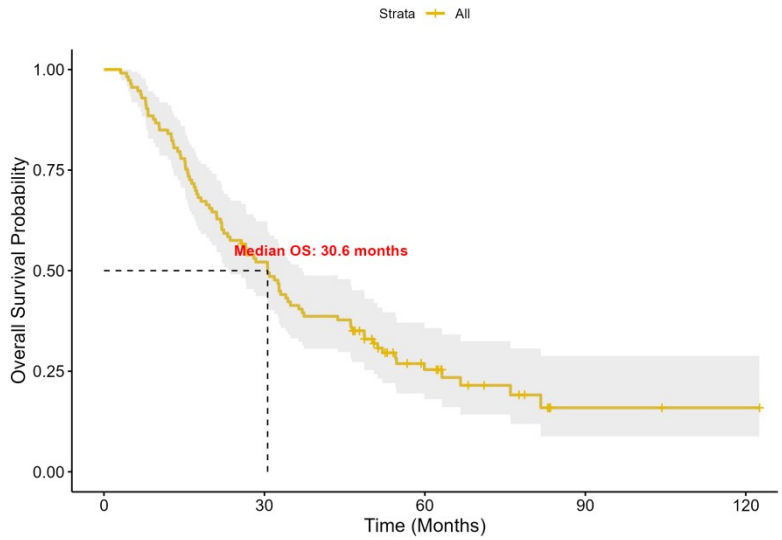

Supporting Figure S2. Principal Component Analysis (PCA) score plots of Quality Control (QC) samples demonstrating data stability before and after Norm ISWSVR correction. (A) PCA scores of QC samples in positive ion mode before (left) and after (right) correction. (B) PCA scores of QC samples in negative ion mode before (left) and after (right) correction. The tight clustering after correction indicates high data reproducibility.

Supporting Table S2.Comparison of the four key metabolites between squamous cell carcinoma and adenocarcinoma

| Metabolite | SCC, median (IQR) | ADC, median (IQR) | Fold change (SCC/ADC) | P value | FDR-adjusted q value |
|---|---|---|---|---|---|
| Gln_Glu | 1135.12 (720.42–2564.86) | 1588.91 (1064.97–3428.09) | 0.714 | 0.0826 | 0.33 |
| GSH | 707.56 (505.72–955.74) | 776.44 (484.02–1032.78) | 0.911 | 0.773 | 0.997 |
| LPC18:3 | 1704299.17 (839708.38–2765419.40) | 1682658.54 (936126.55–2521819.24) | 1.013 | 0.982 | 0.997 |
| LPC16:3 | 201171.47 (152518.85–338261.53) | 206149.23 (122077.92–321122.68) | 0.976 | 0.997 | 0.997 |




Supporting Figure S3. Box plot of the four key metabolites between squamous cell carcinoma and adenocarcinoma.(A) Gln_Glu; (B)GSH ; (C) LPC18:3; (D) LPC16:3.




Supporting Figure S4. Kaplan-Meier curve showing the median overall survival.