Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

A targeted neurotransmitters quantification and nontargeted metabolic profiling method for pharmacometabolomics analysis of olanzapine by using UPLC-HRMS

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CONTENTS

Methods

1.1 Non-targeted metabolomic analytical and data preprocessing methods

The parameters for peak finding, filtering, alignment, scaling and identification using R software. (based on data of plasm samples using LC-ESI (+)-MS, for example)

```
rm(list=ls(all=TRUE)）
library(Biobase)
library(xcms)
library(multtest)
library(CAMERA)
sessionInfo()
xs<-xcmsSet(profmethod = "binlin",method="centWave",ppm = 2.5, peakwidth=c(5,35), snthresh =20,prefilter=c(10,5000),integrate=1, mzdiff =0.005)
xs<-group(xs,bw=5,minfrac=0.3,mzwid=0.025)
save(xs,file="xs.Rda")
ret.xs.obiwarp<-retcor(xs,method="obiwarp",plottype="deviation")
ret.xs.obiwarp<-group(ret.xs.obiwarp, bw = 5,minfrac=0.3,mzwid=0.025)
ret.xs.obiwarp
fill.ret.xs.obiwarp<-fillPeaks(ret.xs.obiwarp)
fill.ret.xs.obiwarp
save(fill.ret.xs.obiwarp, file="fill.ret.xs.obiwarp.Rda")
an.C<
annotate(fill.ret.xs.obiwarp,sigma=6,perfwhm=0.3,cor_eic_th=0.75,maxcharge=3,maxiso=3,mzabs=0.03,multiplier=3,polarity="positive/negative")
peaklist.C<-getPeaklist(an.C)
write.csv(peaklist.C,file='annotated.C.csv')
an.M<-
annotate(fill.ret.xs.obiwarp,sigma=6,perfwhm=0.3,cor_eic_th=0.75,maxcharge=3,maxiso=3,mzabs=0.03,multiplier=3,polarity="positive/negative ")
peaklist.M<-getPeaklist(an.M)
write.csv(peaklist.M,file='annotated.M.csv')
an.QC<-
annotate(fill.ret.xs.obiwarp,sigma=6,perfwhm=0.3,cor_eic_th=0.75,maxcharge=3,maxiso=3,mzabs=0.03,multiplier=3,polarity="positive/negative ")
```

```
peaklist.QC<-getPeaklist(an.QC)
write.csv(peaklist.QC,file='annotated.QC.csv')
report.fill.ret.xs.obiwarp<-
diffreport(fill.ret.xs.obiwarp,"C","M","QC",eicmax=8000,file="POS-after
conversion")
save(report.fill.ret.xs.obiwarp,file="report.fill.ret.xs.obiwarp.Rad")
```

1.2 Clinical sample collection

Healthy volunteers took olanzapine orally disintegrating tablets (5 mg/tablet) produced by Changzhou Siyao Pharmaceuticals CO.,LTD. as test preparation, and olanzapine orally disintegrating tablets (5 mg/tablet) produced by Eli Lilly company (5 mg/tablet) as reference preparation. After fasting overnight for at least 10 hours, the subjects began to eat high calorie and high fat meal 30 minutes before administration in the morning of the test day, and finished eating within 30 minutes. They took 5 mg of the test preparation or reference preparation orally 30 minutes after the start of meal. The drug was placed on the surface of the tongue, swallowed after complete disintegration in the mouth, without water. Drinking water was forbidden from 1 h before taking medicine to 1 h after taking medicine, and standard meals were taken after 4 h and 10 h. At the end of the first cycle, the washing period was 14 days, and the second cycle was carried out alternately. Subjects must meet all of the following criteria for inclusion: Healthy subjects over the age of 18 (including the age of 18) were both male and female. Weight: not less than 50 kg for men and 45 kg for women; BMI is within the range of 19-26 kg/m2 (BMI = weight (kg) / height 2 (M2)). Health status: no history of heart, liver, kidney, digestive tract, nervous system, mental disorders and metabolic abnormalities, physical examination, vital signs examination, laboratory examination (blood routine, urine routine, blood biochemistry, coagulation function), 12 lead electrocardiogram, chest X-ray examination results are normal or abnormal without clinical significance. The subjects must have informed consent to the test and signed the written informed consent voluntarily. The subjects were able to communicate well with the researchers and complete the experiment according to the protocol. Subjects can opt out of the clinical trial at any time, and their further treatment will not be affected. Blood samples were collected from each subject in each week period. Before administration (within 30 minutes) and 0.25 h, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5

h, 6 h, 8 h, 12 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h, blood samples were collected from forearm vein for about 4 mL, put into heparin lithium anticoagulant tube, centrifuged at 4 ◦C for 10 min, transferred upper plasma, frozen in refrigerator at - 60 to – 80 ◦C for preservation and experiment.

1.3 Method validation procedure

The results show that limits of quantification ranged from 1 to 100 000 ng mL^{-1}. The method exhibited excellent linear calibration curves for the assay of analytes, which were greater than 0.99. The inter- and intra-day precision and accuracy of analytes were lower than 15%. The recovery rates of analytes are in the range of 71.9~114.8%. The samples of all analytes placed in the autosampler at room temperature for 24 h, and 48 h were stable.

1.3.1 Linearity

The determination of linearity was evaluated by using 8 calibration concentration levels and calibration curves were established using weighted ($w = 1/x^2$) linear regression analysis. While correlation coefficient (r) were served as a standard to linearity, good Linearity with correlation coefficients were 0.99 was considered to be acceptable.

1.3.2 Accuracy and precision

The intra- and inter-day accuracy and precision of the method were evaluated by analyzing QC samples at three different concentration levels (Low, Medium, High) of mixed standards: QC samples were prepared in six replicates on the same day and three independent days, respectively.

1.3.3 Extraction recovery

The extraction recovery was determined at three concentrations levels (low, medium and high) by calculated from the ratio of peak area (A/B) × 100% obtained by two different sample processing methods at each concentration. A and B are mean at the same concentration of analytes in samples, A obtained from spiked before homogenization and extraction, B obtained from spiked after homogenization and extraction, and then prepare 6 samples in parallel for each concentration.

1.3.4 Stability studies

Stability study tested six replicates of QC samples at three concentration levels (low, medium and high) under various conditions, including freeze–thaw stability and

autosampler stability. Freeze–thaw stability was assessed QC samples after three freeze-thaw cycles from –80 °C to room temperature; Autosampler stability was investigated QC samples placed in the autosampler at 4 °C temperature for 24 h, and 48 h and then analyzed immediately by LC-MS.

1.3.5. Validation for non-targeted metabolomics

For non-targeted metabolomics analysis, to control data quality and obtain reliable data from metabolomics analysis, we were used QC samples to monitor the stability of the system and appraise data quality. The data matrix obtained by LC - (+) ESI-MS and (-) ESI-MS analysis of QC samples was analyzed by PCA. The projection results of each sample on the first principal component are shown in Supporting Information Fig. S5A, B. It can be seen from the figure that the relative deviations of the peak areas of QC samples are controlled within the 2 SD range. The results show that the stability of the analysis system is good during the mass analysis. Additionally, unsupervised principal component analysis (PCA) was performed based on all samples. All QC samples during batch analysis were clustered in the center of the PCA scores plot, also indicating that the stability of the analysis system is good. Results showed in Fig. S5 A and B.

Figures and Tables

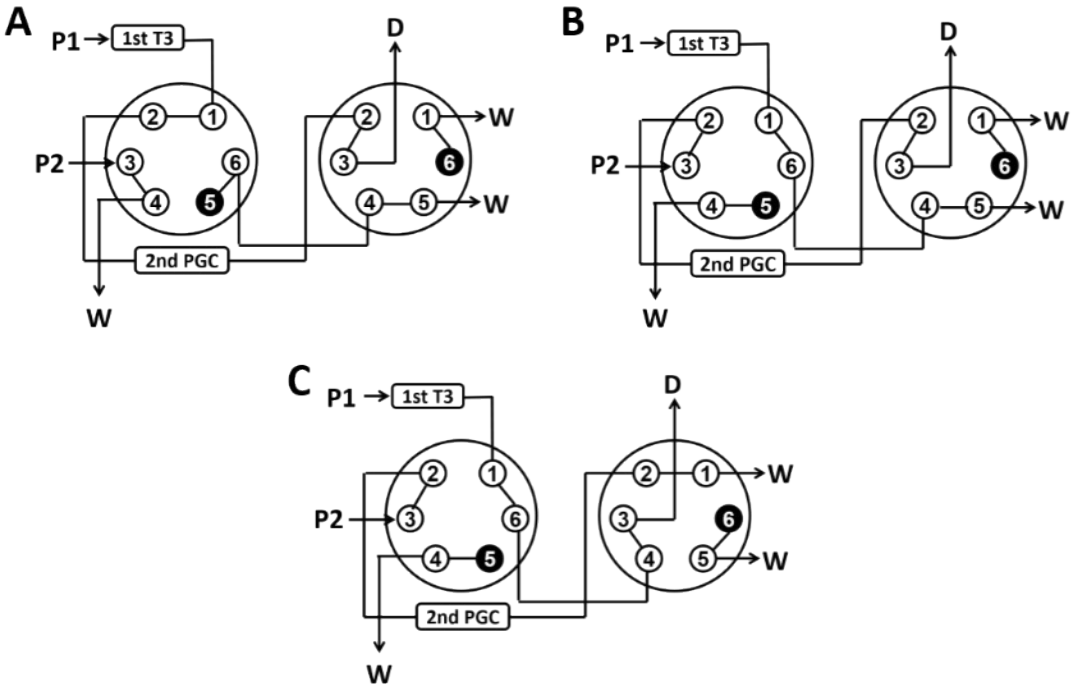

Fig. S1 The instrumental LC separation system setup (P: pump, D: detector, W: waste)

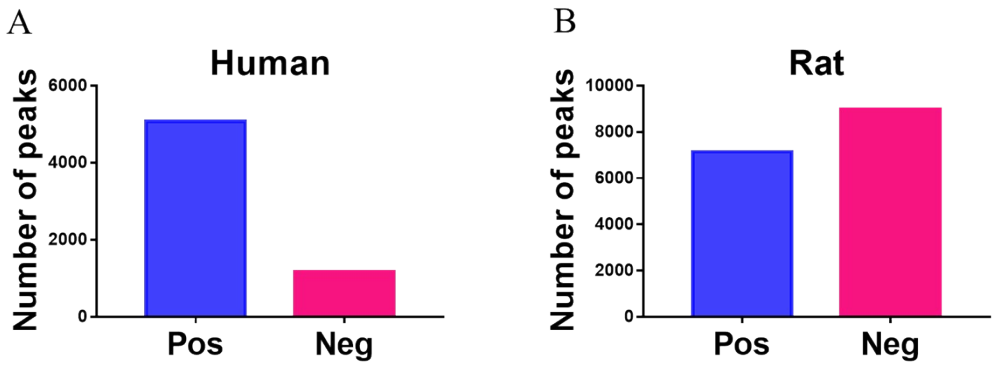

Fig. S2 A, B. Number of features detected by LC- (+/-) ESI- MS using different mobile phase gradient from human and rat sample.

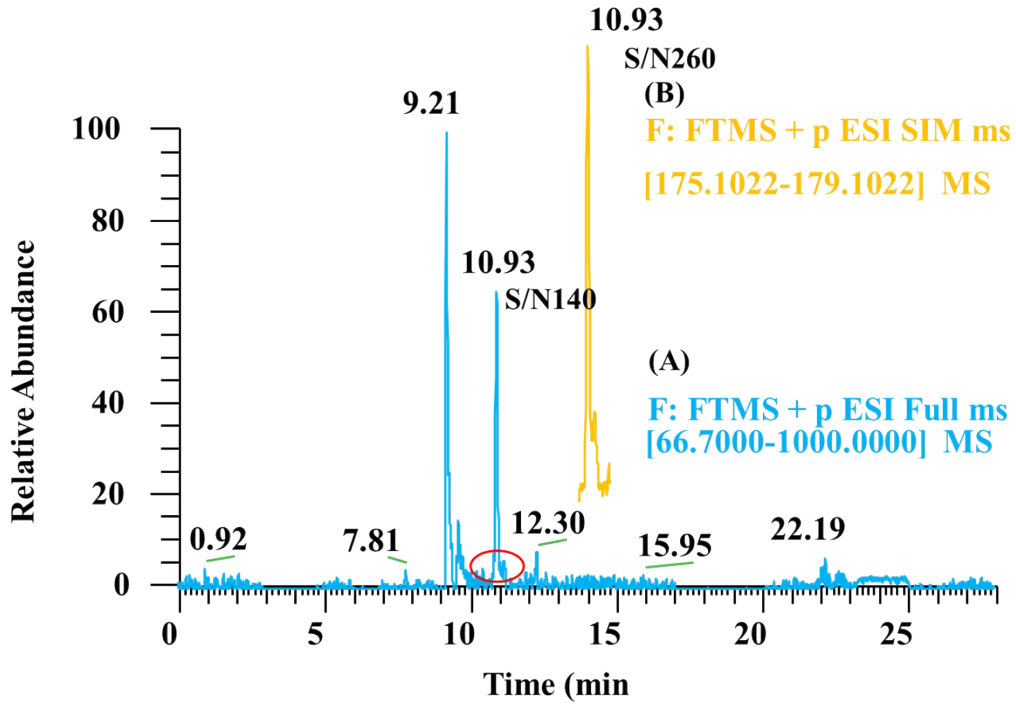

Fig. S3 The typical XICs of the serotonin obtained from full MS scan (A) and from tSIM (B)

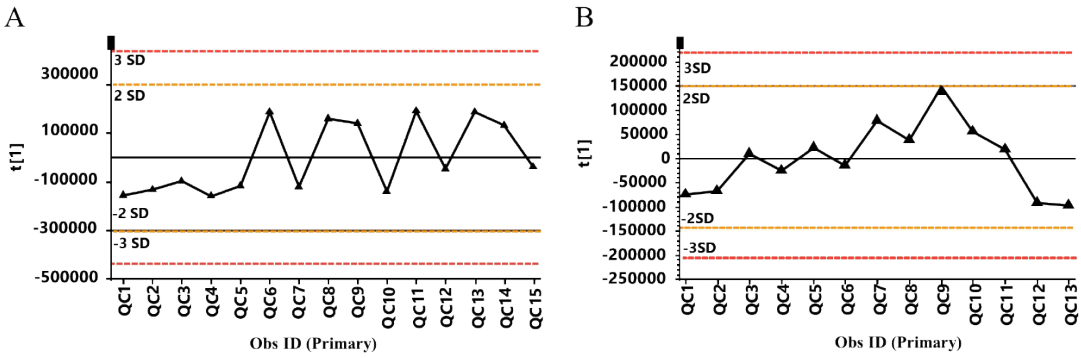

Fig. S4 A, B, Line plots of quality control (QC) samples generated by PCA using component 1 and 2. Peak area deviation could be evaluated by distribution of the runs. X-axis: run order; Y-axis: standard deviation. (A) QC plot for the first and second component from LC- (+) ESI-MS data; (B) from LC- (-) ESI-MS data.

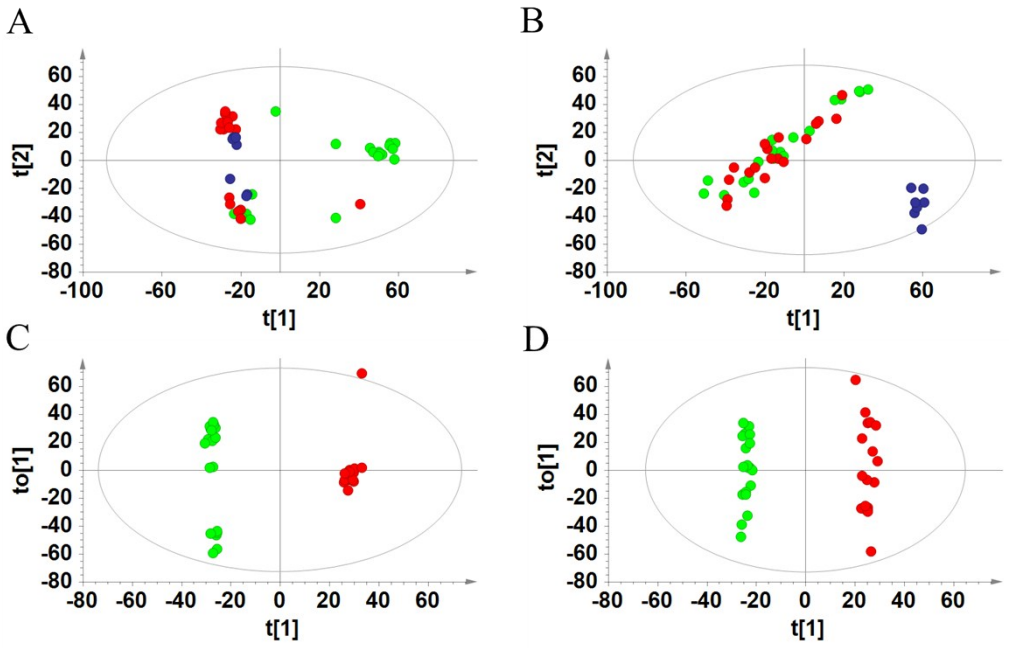

Fig. S5 PCA score plots based on the data from (A) LC-(+)ESI-MS, (B) LC-(-)ESI-MS.(●: before taking drug group ; ●: after taking drug group; ●:QC samples). OPLS-DA score plots based on the data from (C) LC-(+)ESI-MS, (D) LC-(-)ESI-MS.

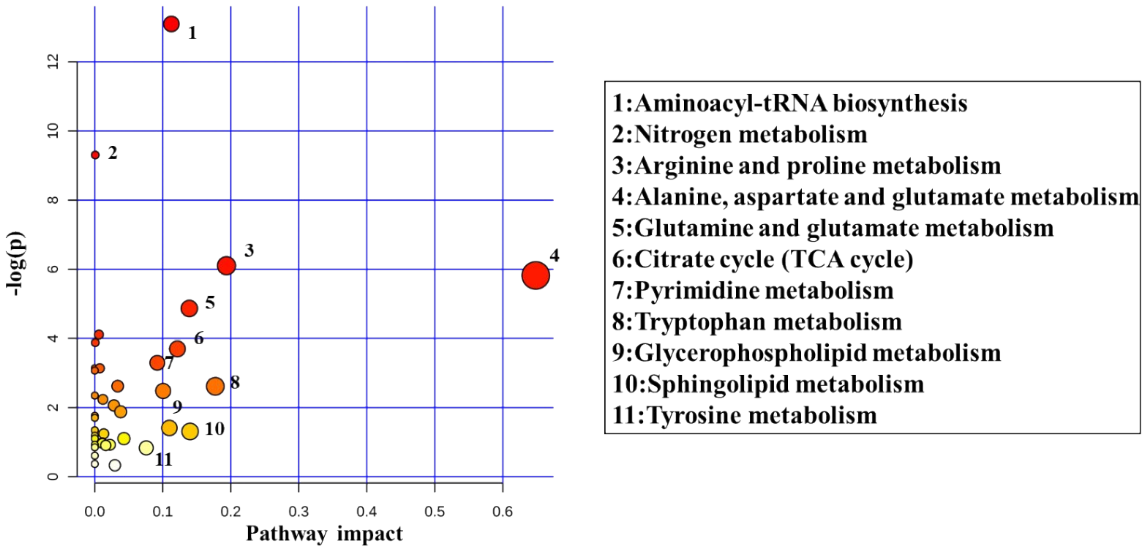

Fig. S6 Metabolic pathways enrichment analysis of potential biomarkers.

Table S1. The detailed elution gradient and flow rate

Time (min)	First dimension		Second dimension		Valve position
	Flow rate (mL/min)	Gradient ACN (v%)	Flow rate (mL/min)	Gradient ACN (v%)	
0-2.5	0.18	2%	0	/	A
2.5-8. 5	0	/	0.2	2%	B
		8.5-15 min, 2-70%		8.5-18 min, 2%-100% (washing)	
8.5-20	0.25	15-20 min, 70-100%	0.25	18-27 min, 2% (pre-equilibration)	C□
20-28	0.18	2%			

Table S2. Regression equation, linear range, correlation coefficient of the detection of 13 metabolites

NO	Compound	Formula	*m/z*	IS	Linear equation of calibration curves	R	liner range(ng mL^{-1})
1	Trp	$C_{11}H_{12}N_2O_2$	205.0972	Trp-d_5	Y = -0.00345422+0.000125887*X	0.9966	100-20000
2	5-HTP	$C_{11}H_{12}N_2O_3$	221.0921	Trp-d_5	Y = -7.67882e-006+3.20041e-005*X	0.9915	1-200
3	5-HT	$C_{10}H_{12}N_2O$	177.1022	5-HT-d_4	Y = -0.00215224+0.000583969*X	0.9927	20-4000
4	5-HIAA	$C_{10}H_9NO_3$	192.0655	IAA-d_2	Y = 0.000188532+0.00271059*X	0.998	2-400
5	Kynurenine	$C_{10}H_{12}N_2O_3$	209.0921	IAA-d_2	Y = -2.52919+0.310782*X	0.9928	10-2000
6	Tyr	$C_9H_{11}NO_3$	182.0812	Tyr-d_2	Y = 0.000828546+0.000537593*X	0.9987	200-40000
7	L-DOPA	$C_9H_{11}NO_4$	198.0761	Tyr-d_2	Y = -1.56258e-005+3.84442e-006*X	0.9982	20-4000
8	Glu	$C_5H_9NO_4$	148.0604	Glu-d_5	Y = 0.656039+0.00452585*X	0.9967	500-100000
9	Gln	$C_5H_{10}N_2O_3$	147.0764	Gln-^{15}N	Y = 0.447101+0.00354935*X	0.9925	200-40000
10	GABA	$C_4H_9NO_2$	104.0706	GABA-d_2	Y = 0.102702+0.00399208*X	0.9961	100-20000
11	Ach	$C_7H_{16}NO_2$	146.1176	Choline-d_9	Y = 0.0639203+0.0107657*X	0.9961	20-4000
12	Asn	$C_4H_8N_2O_3$	133.0608	Asn-d_1	Y = 0.00420051+0.00213585*X	0.9985	40-8000
13	Taurine	$C_2H_7NO_3S$	126.0219	Taurine-^{15}N	Y = 0.227581+0.00972572*X	0.9981	100-20000

Table S3. Intra -day assay accuracies and precisions of the detection 13 metabolites

NO	Compound	Low		Medium		High	
		Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)
1	GABA	101.4	0.9	101.3	0.3	98.1	4.1
2	Ach	108.9	5.2	108.1	1.8	99.9	7.1
3	Asn	88.9	3.4	100.7	4.7	103.5	5.2
4	Taurine	90.8	3.1	99.4	3.4	107.8	1.3
5	Gln	106.4	1.3	111.4	1.9	96.8	4
6	Glu	102.9	4.8	107.3	1.2	98.3	5.6
7	L-DOPA	108.9	3.5	110.7	2.3	112.7	0.6
8	Tyr	96.7	3.7	104.6	2.2	105.8	1.3
9	5-HT	90.3	3.1	89	1.7	98.8	8.8
10	5-HTP	93.6	2.4	98.2	4.8	109.1	2.5
11	kynurenine	106.6	2.8	107.8	2.1	107.3	3.4
12	Trp	92.6	2.9	89.8	2.6	100.9	6.2
13	5-HIAA	106.4	4.4	106.9	3.5	95.7	4.7

Table S4. Inter-day assay accuracies and precisions of 13metabolites

		Low		Medium		High	
NO	Compound	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)
1	GABA	103.2	5.4	104.4	2.8	96.1	6.6
2	Ach	108.3	4.5	104.8	5.8	100.6	10.2
3	Asn	96.1	9.5	105.8	6.1	105.9	4.9
4	Taurine	89.4	2.8	98.1	6.5	105.3	5.8
5	Gln	110.5	3	112.2	1.7	92.4	4.7
6	Glu	100.9	7.2	103.5	5.5	93.9	5.3
7	L-DOPA	99.8	10.8	106.2	8.9	108.9	4.8
8	Tyr	102.4	6.2	104.3	4.9	106.5	3.5
9	5-HT	95.9	9.2	102.6	10.1	101.3	8.2
10	5-HTP	91	4.1	94.8	7.5	107.9	5.9
11	kynurenine	99.1	9.4	104.93	8.6	107.8	4.9
12	Trp	98.5	6.6	99.8	8.6	106.7	5.9
13	5-HIAA	100.9	6.7	98.3	8.4	98.5	7.1

Table S5. Extraction recoveries for the detection of the 13 metabolites.

		Low		Medium		High	
NO	Compound	Extraction Recovery(%)	Precision(%)	Extraction Recovery(%)	Precision(%)	Extraction Recovery(%)	Precision(%)
1	GABA	72.9	1.2	77.9	6.6	75.1	7.8
2	Ach	94.7	5.4	92.9	5.6	86.7	5.2
3	Asn	71.9	6.4	89.4	2.3	79.3	2.7
4	Taurine	90.3	4.5	88.9	5.7	91.7	8.9
5	Gln	72.6	4.7	73.9	8.2	79.4	4.3
6	Glu	76	3	82.8	8.8	88.1	9.7
7	L-DOPA	97.1	7.8	107.8	3.6	114.7	3.1
8	Tyr	101.2	3.9	103.4	4.8	113.7	6.5
9	5-HT	114.9	4.6	114.5	11.1	100.3	5.4
10	5-HTP	77.9	2.1	74.1	2.6	88.5	7.1
11	kynurenine	72.4	5.8	74.9	0.6	72.7	6.7
12	Trp	74.2	4.2	79.2	5.3	84.1	9.2
13	5-HIAA	73.4	3.3	74.4	2.9	77.2	11.9

Table S6. Freeze-thaw stability of the detection 13metabolites

		Freeze-thaw		Freeze-thaw		Freeze-thaw	
		Low		Medium		High	
NO	Compound	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)
1	GABA	92.2	3.8	95.8	2.6	92.9	1.9
2	Ach	92.8	3.7	93.3	2.7	89.1	4.4
3	Asn	90.6	3.5	96	4.2	95.7	1.8
4	Taurine	98.7	6.2	95.6	3.6	90.9	4.98
5	Gln	101.9	1.37	103.6	2.4	94.6	3.88
6	Glu	95.7	2.97	91.2	2.9	98.9	3.28
7	L-DOPA	98.8	4.77	101.6	2.9	104.5	3.98
8	Tyr	91.3	4.97	89.9	2.2	101.6	3.7
9	5-HT	91.2	5.4	95.9	1.7	95.4	6.6
10	5-HTP	100.2	4.6	98.7	1.9	92.5	3.2
11	kynurenine	95.5	11.47	96.2	3.9	90.3	2
12	Trp	90.8	2.4	91.8	2.8	98.1	2.9
13	5-HIAA	101.3	3.8	103.5	1.8	102.1	1.2

Table S7. Results of stability test under different storage conditions: autosampler stability for 4 °C for 24 h

		4℃ for 24h		4℃ for 24h		4℃ for 24h	
		Low		Medium		High	
NO	Compound	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)
1	GABA	102.9	2.1	102.7	1.2	100.3	0.6
2	Ach	107.9	2.9	101.8	0.5	92.9	6.2
3	Asn	99.5	7.6	104.8	3.6	94.3	5.2
4	Taurine	102	1.9	101.2	2.8	100.7	5.6
5	Gln	101.6	1.5	102.9	1.5	93.5	2.5
6	Glu	103.7	4.1	104.6	1.4	102.9	2.9
7	L-DOPA	105.3	3.9	101.7	0.9	106.7	1.5
8	Tyr	99.7	7.9	107.3	3.2	101.2	5.7
9	5-HT	105.8	2.6	109.1	3.7	96.9	8.4
10	5-HTP	90	3.3	96.6	8.2	102.2	2.8
11	kynurenine	102.4	5.6	107.5	6.5	107.7	2.4
12	Trp	99.9	6.9	95	3.2	96.4	6.4
13	5-HIAA	107.2	4.1	104.9	7.4	101.2	2.2

Table S8. Results of stability test under different storage conditions: autosampler stability for 4 °C for 48 h

		4℃ for 48h		4℃ for 48h		4℃ for 48h	
		Low		Medium		High	
NO	Compound	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)	Accuracy(%)	Precision(%)
1	GABA	101.9	5.1	103.2	1.6	93.8	2.7
2	Ach	99.3	7.3	98.3	3.7	91.2	3.9
3	Asn	98	5.5	99.4	3.4	92.9	2.6
4	Taurine	104.2	1.6	95	2.5	94.2	3.4
5	Gln	102.7	1.8	102.5	1.8	107.6	3.8
6	Glu	101.3	7.5	101.9	5	95.5	4.6
7	L-DOPA	99.9	7.7	101.4	1.8	104.6	5.3
8	Tyr	99.8	5.2	96.2	3.8	99.6	3.7
9	5-HT	101	3.1	102.9	3.9	90.8	11.3
10	5-HTP	89.7	2.2	95.9	8.5	95.6	8.2
11	kynurenine	89.8	4.4	94.9	7	89.4	5.1
12	Trp	95.8	6.8	99.2	3.8	101.9	7.9
13	5-HIAA	104.2	3.2	104.3	3.7	102.6	2.3

Table S9. Information list of differential metabolites in plasma between before taking drug group and after taking drug group identified by LC-MS/MS

Name	Formula	Precursor Ion (*m/z*)	MS error (ppm)	Product Ion (*m/z*)	RT (min)	FC
1-Methylhistidine	$C_7H_{11}N_3O_2$	170.0919	-2.94	68.0501;97.0766;107.0795;109.0726;110.0795;124.0869;126.1025	4.27	2.69
LysoPE(0:0/18:2)	$C_{23}H_{44}NO_7P$	478.2911	-3.55	216.0626;263.2363;337.2727;460.2823	17.54	2.58
PC(14:0/20:2)	$C_{42}H_{80}NO_8P$	758.5665	-3.82	63.2733;184.0733	18.11	1.69
L-Tyrosine	$C_9H_{11}NO_3$	182.0809	-1.65	107.0495;118.0651;119.0491;136.0755;147.0438;165.0544	9.12	1.62
L-Arginine	$C_6H_{14}N_4O_2$	175.1186	-2.28	60.0564;70.0657;116.0706	4.24	1.61
L-Leucine	$C_6H_{13}NO_2$	132.1018	-0.76	55.0542;56.0502;69.0706;72.0808	9.45	1.57
4-Hydroxycinnamic Acid	$C_9H_8O_3$	165.0542	-2.42	53.0395;91.0545;95.0494;103.0543;119.049;123.0439;147.04737	9.12	1.55
Creatine	$C_4H_9N_3O_2$	132.0765	-2.27	87.0556;90.0552;114.0661	6.11	1.49
L-Methionine	$C_5H_{11}NO_2S$	150.058	-2	56.0502;61.0113;74.0236;102.055;104.0529	5.75	1.31
L-Acetylcarnitine	$C_9H_{17}NO_4$	204.1223	-3.43	60.0815;85.0287;145.0491	5.5	0.68
Linoleyl carnitine	$C_{25}H_{45}NO_4$	424.3404	-6.83	85.0287;144.0106;245.2251	17.12	0.67
Sphinganine	$C_{18}H_{39}NO_2$	302.3042	-3.97	266.2843;284.2936	16.56	0.59
Uracil	$C_4H_4N_2O_2$	113.1345	-0.88	70.0293;98.0081	9.05	0.52
Cortisol	$C_{21}H_{30}O_5$	363.2151	-4.13	121.0647;267.1734;285.1337;309.184;327.1955;345.2026	14.97	0.24
Tryptophan	$C_{11}H_{12}N_2O_2$	205.0964	-3.9	74.0244;132.081;146.0602;159.0918;188.0708	12.53	1.08
5-hydroxytryptophan	$C_{11}H_{12}N_2O_3$	221.0917	-2.71	74.0245;94.0657;146.0603;158.0603;175.0869	12.53	1.02
5-hydroxytryptamine	$C_{10}H_{12}N_2O$	177.1016	3.95	73.0292;115.0542;132.0807;148.0763;160.0756	11.18	0.84
5-Hydroxyindoleacetic acid	$C_{10}H_9NO_3$	192.0682	-2.08	94.0608;104.0500;132.0446;146.0601;162.0511;174.0552	11.39	0.95
L-Dopa	$C_9H_{11}NO_4$	198.0751	2.02	74.0972;100.0762;157.0835;170.0807	9	1.91

Glutamate	$C_5H_9NO_4$	148.0691	-2.03	56.0503;74.0971;84.045;88.0398;102.0553;130.0500	3.98	1.29
Glutamine	$C_5H_{10}N_2O_3$	147.0759	-3.4	56.0502;74.0971;84.0449;86.0492;101.0713;130.0499	3.8	1.49
Aspartic acid	$C_4H_7NO_4$	134.0444	-2.98	60.0452;86.079;88.0398;105.0701	3.81	0.17
Acetylcholine	$C_7H_{16}NO_2$	146.1171	-3.42	60.0817;84.0451;87.0447;102.0554;130.0510	3.86	1.04
LysoPE(18:1/0:0)	$C_{23}H_{46}NO_7P$	478.2936	1.67	78.9574;140.0106;196.0373;214.0481	17.98	3.65
LysoPE(14:0/0:0)	$C_{19}H_{40}NO_7P$	424.2462	0.71	196.0375;227.2011	17.16	2.95
Galactosylglycerol	$C_9H_{18}O_8$	253.0932	5.53	61.029;71.0122;89.0227;103.0395;161.0447	7.74	2.99
LysoPE(0:0/18:3)	$C_{23}H_{42}NO_7P$	474.2626	2.32	78.9575;140.0109;196.0375;214.0486;277.2175	17.25	2.79
Octadecanedioic acid	$C_{18}H_{34}O_4$	313.2386	4.15	99.0800;113.0957;183.1381;277.2174;295.2276	17.27	2.73
Ferulic acid 4-sulfate	$C_{10}H_{10}O_7S$	273.0072	3.3	149.0597;193.0499	13.03	2.4
Histidinyl-Tryptophan	$C_{17}H_{19}N_5O_3$	340.1418	4.12	74.0248;108.0552;153.0772;203.0818	11.98	2.03
Glutamyltryptophan	$C_{16}H_{19}N_3O_5$	332.1255	4.22	128.0341;159.0765;185.0561;203.0821;244.1445;288.1361	12.5	2.09
Citric acid	$C_6H_8O_7$	191.0191	2.62	85..0280;87.0073;111.0074;129.0181;173.0083	8.63	1.91
LysoPE(0:0/22:5)	$C_{27}H_{46}NO_7P$	526.2947	3.61	78.9571;196.0376;285.2586;329.2491	17.83	1.93
LysoPE(0:0/16:1)	$C_{21}H_{42}NO_7P$	450.2633	4.00	78.9573;152.9949;196.0376;253.2173;405.2939	17.36	1.92
Isocitric acid	$C_6H_8O_7$	191.0191	2.62	85.0281;103.0385;111.0073;129.0181;147.029;154.9976	11.2	1.91
LysoPE(0:0/20:3)	$C_{25}H_{46}NO_7P$	502.2959	6.17	78.9575;140.0107;196.0374;214.0489;305.2491	17.78	1.78
Calendic acid	$C_{18}H_{30}O_2$	277.2174	4.33	59.0123;83.0486;205.19858;259.2068	18.74	1.71
LysoPE(18:0/0:0)	$C_{23}H_{48}NO_7P$	480.3091	1.25	78.9574;140.0105;196.0373;214.0479;283.2642	18.6	1.56
LysoPE(0:0/16:0)	$C_{21}H_{44}NO_7P$	452.2784	2.65	78.9574;140.0106;196.0373;214.0481;255.2327	17.81	1.57
Glucaric acid	$C_6H_{10}O_8$	209.0298	2.87	85.0279;111.0073;191.0194	7.93	1.3
Uridine	$C_9H_{12}N_2O_6$	243.0623	4.53	66.0334;82.0283;110.0247;122.0234;152.0343;200.0559	9.2	0.65

Table S10. Pathway with PI greater than 0.1 analysis of the potential differential metabolite between before and after administration group

Pathway Name	Total	Hits	P	-log(p)	FDR	Expected	Impact
Aminoacyl-tRNA biosynthesis	75	8	2.06E-06	13.095	0.00016447	0.93477	0.11268
Arginine and proline metabolism	77	5	0.0022383	6.1021	0.059302	0.9597	0.19377
Alanine, aspartate and glutamate metabolism	24	3	0.0029651	5.8208	0.059302	0.29913	0.64863
D-Glutamine and D-glutamate metabolism	11	2	0.0077044	4.866	0.12327	0.1371	0.13904
Citrate cycle (TCA cycle)	20	2	0.024828	3.6958	0.24828	0.24927	0.12153
Tryptophan metabolism	79	3	0.073214	2.6144	0.41836	0.98463	0.1773
Glycerophospholipid metabolism	39	2	0.083742	2.48	0.44662	0.48608	0.10053
Tyrosine metabolism	76	2	0.24429	1.4094	0.88833	0.94724	0.10975
Sphingolipid metabolism	25	1	0.27031	1.3082	0.90103	0.31159	0.1402