

Supplemental Material

Targeted Serum Metabolomics Reveals Alterations in Amino Acid and Neurotransmitter Pathways in Parkinson's Disease

Jiaxin Yang^{1#}, Dong Wu^{2#}, Zhiwei Wang^{1#}, Zhijing Zhang¹, Xiaowen Zheng¹,
Sheng Wang³, Fen Huang⁴, Shuai Song^{1,*}, Qunlin Zhang^{1,*}

¹ *School of Pharmacy, Anhui Medical University, Hefei 230032, China*

² *Department of Pharmacy, Fuyang People's Hospital, Fuyang 236000, China*

³ *The Center for Scientific Research of Anhui Medical University, Hefei 230032, China*

⁴ *Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei 230032, China*

[#] *These authors contributed equally to this work.*

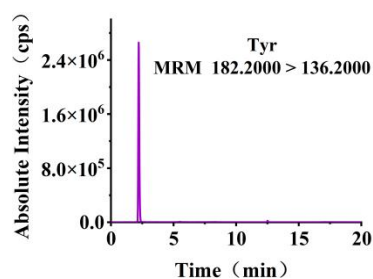
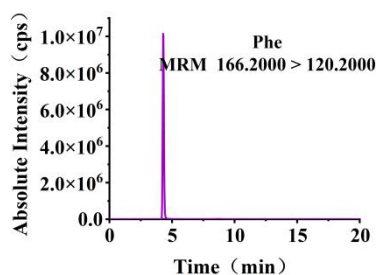
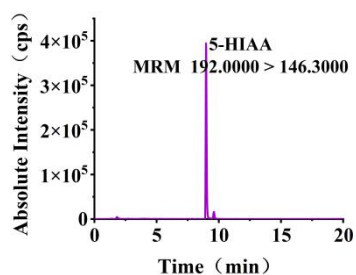
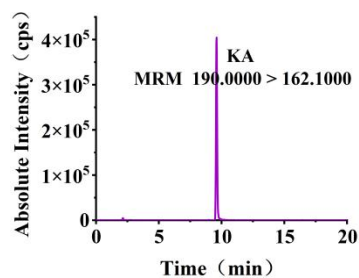
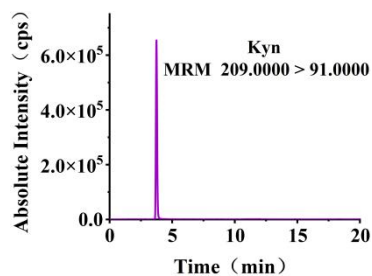
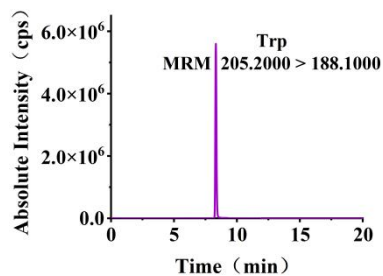
^{*}Corresponding authors. School of Pharmacy, Anhui Medical University, Hefei 230032, China.

E-mail addresses: songshuai@ahmu.edu.cn; qlzhang@ahmu.edu.cn

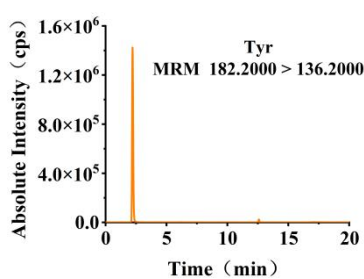
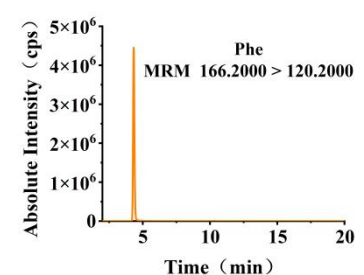
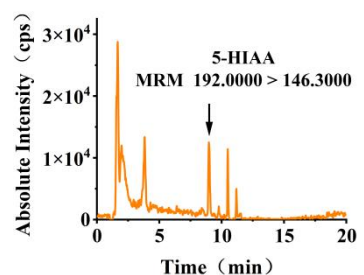
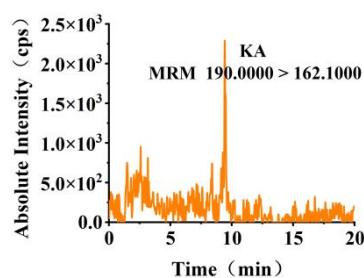
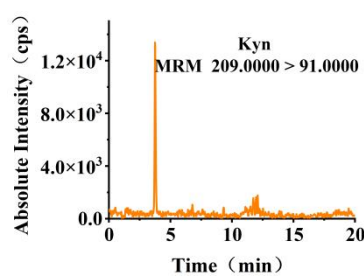
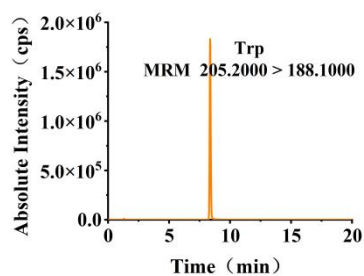
Supplemental Note S1 Correlation analysis

The Correlation analysis results show that the decrease of Trp (OR = 0.946, 95%CI = 0.917–0.976, $P < 0.001$), Kyn (OR = 0.961, 95%CI = 0.946–0.975, $P < 0.01$), Phe (OR = 0.979, 95%CI = 0.970–0.989, $P < 0.001$), E (OR = 0.979, 95%CI = 0.968–0.989, $P < 0.001$), Leu (OR = 0.986, 95%CI = 0.981–0.992, $P < 0.001$), Met (OR = 0.669, 95%CI = 0.5566–0.805, $P < 0.001$), Val (OR = 0.697, 95%CI = 0.614–0.791, $P < 0.001$), Ala (OR = 0.997, 95%CI = 0.995–0.999, $P = 0.005$), Arg (OR = 0.946, 95%CI = 0.926–0.966, $P < 0.001$), Asp (OR = 0.929, 95%CI = 0.898–0.962, $P < 0.001$), Cys–Cys (OR = 0.699, 95%CI = 0.5777–0.846, $P < 0.001$), Gln (OR = 0.811, 95%CI = 0.759–0.867, $P < 0.001$), Gly (OR = 0.687, 95%CI = 0.607–0.776, $P < 0.001$), Glu (OR = 0.313, 95%CI = 0.208–0.471, $P < 0.001$), Lys (OR = 0.133, 95%CI = 0.071–0.249, $P < 0.001$), Pro (OR = 0.057, 95%CI = 0.018–0.183, $P < 0.001$), Ser (OR = 0.975, 95%CI = 0.967–0.982, $P < 0.001$), Tau (OR = 0.838, 95%CI = 0.730–0.961, $P = 0.012$), Thr (OR = 0.692, 95%CI = 0.604–0.794, $P < 0.001$), KA (OR = 0.833, 95%CI = 0.7855–0.883, $P < 0.001$), 5-HIAA (OR = 0.785, 95%CI = 0.726–0.847, $P < 0.001$) and Cit (OR = 0.53, 95%CI = 0.426–0.659, $P < 0.001$) levels increased the risk of PD. ACh (OR = 2.131, 95%CI = 1.647–2.758, $P < 0.001$) levels were found to be associated with an increased risk of PD.

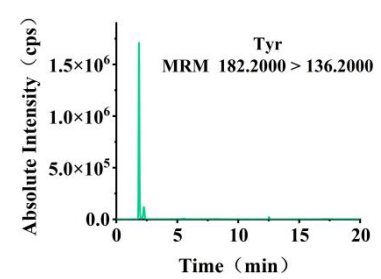
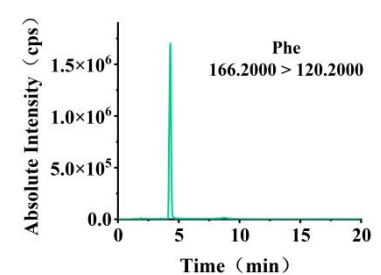
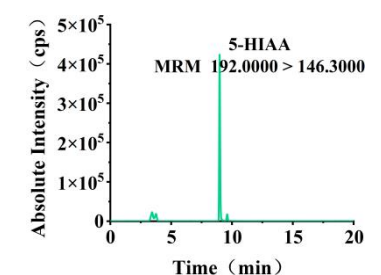
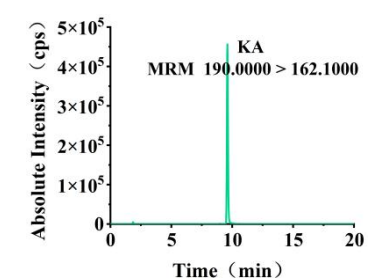
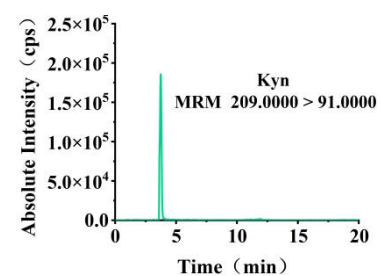
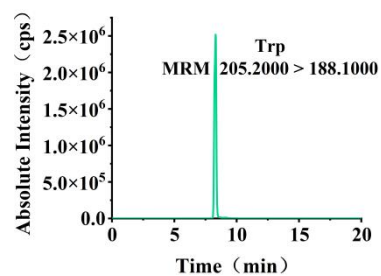
Mixed standard solution

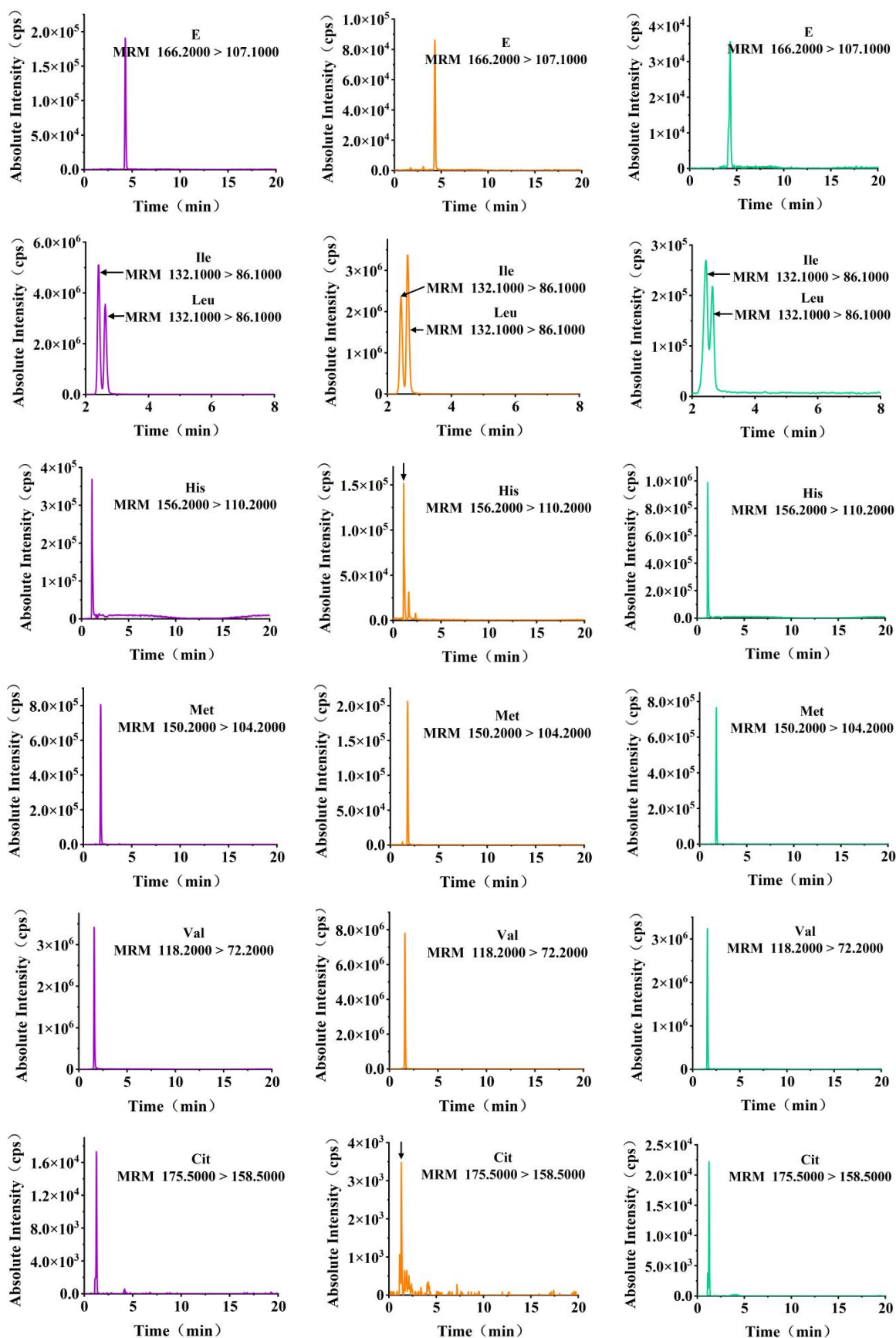


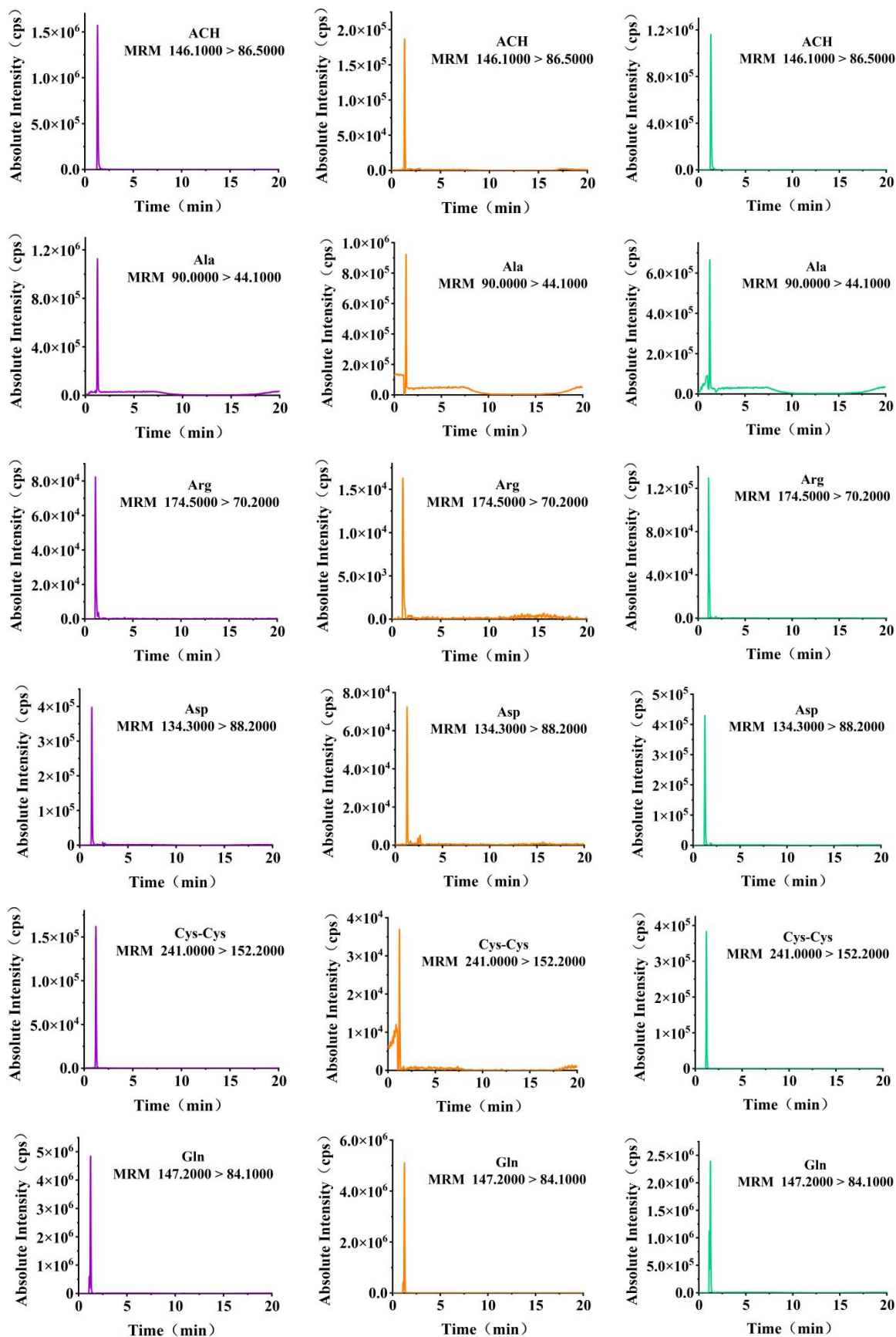
Serum samples

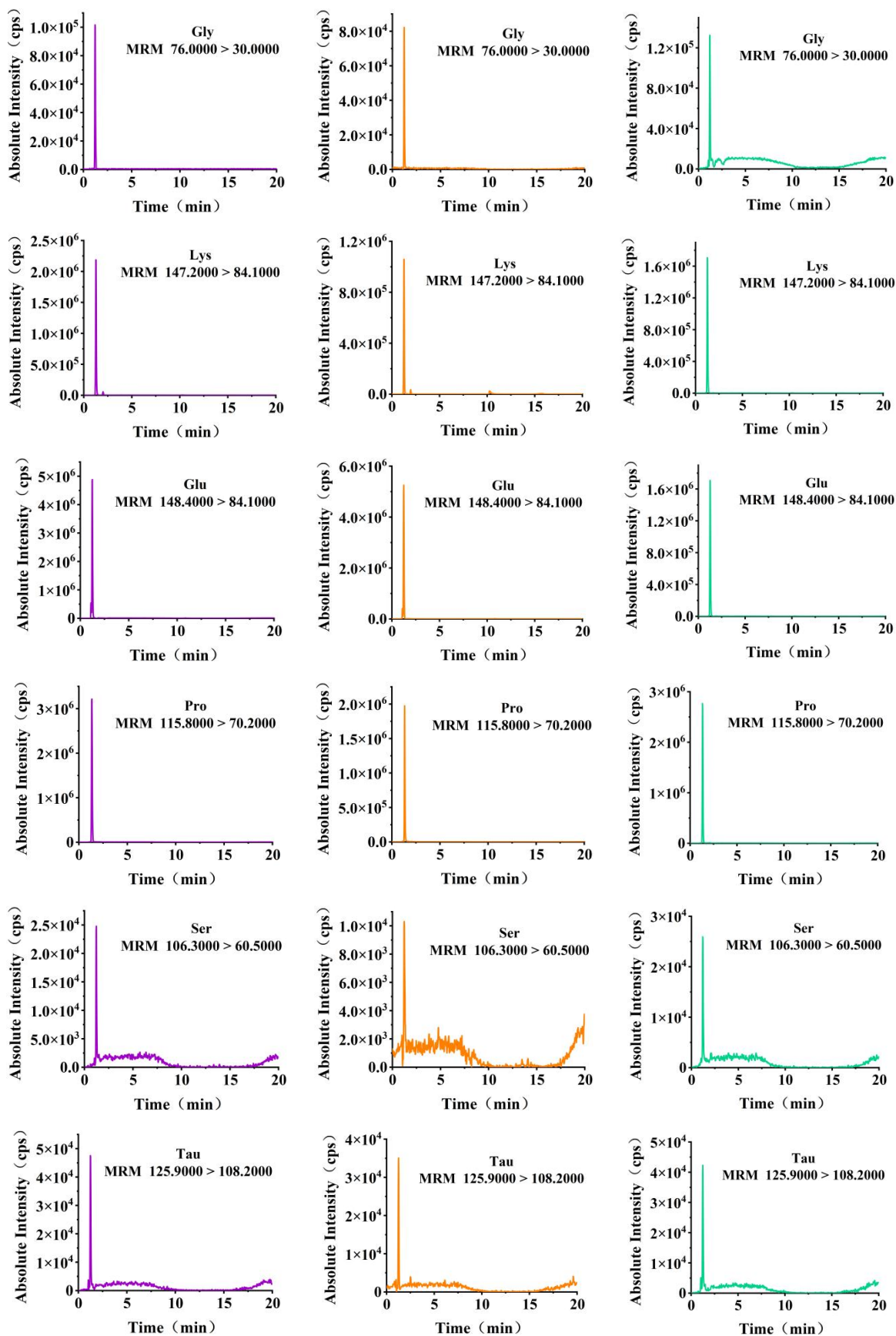


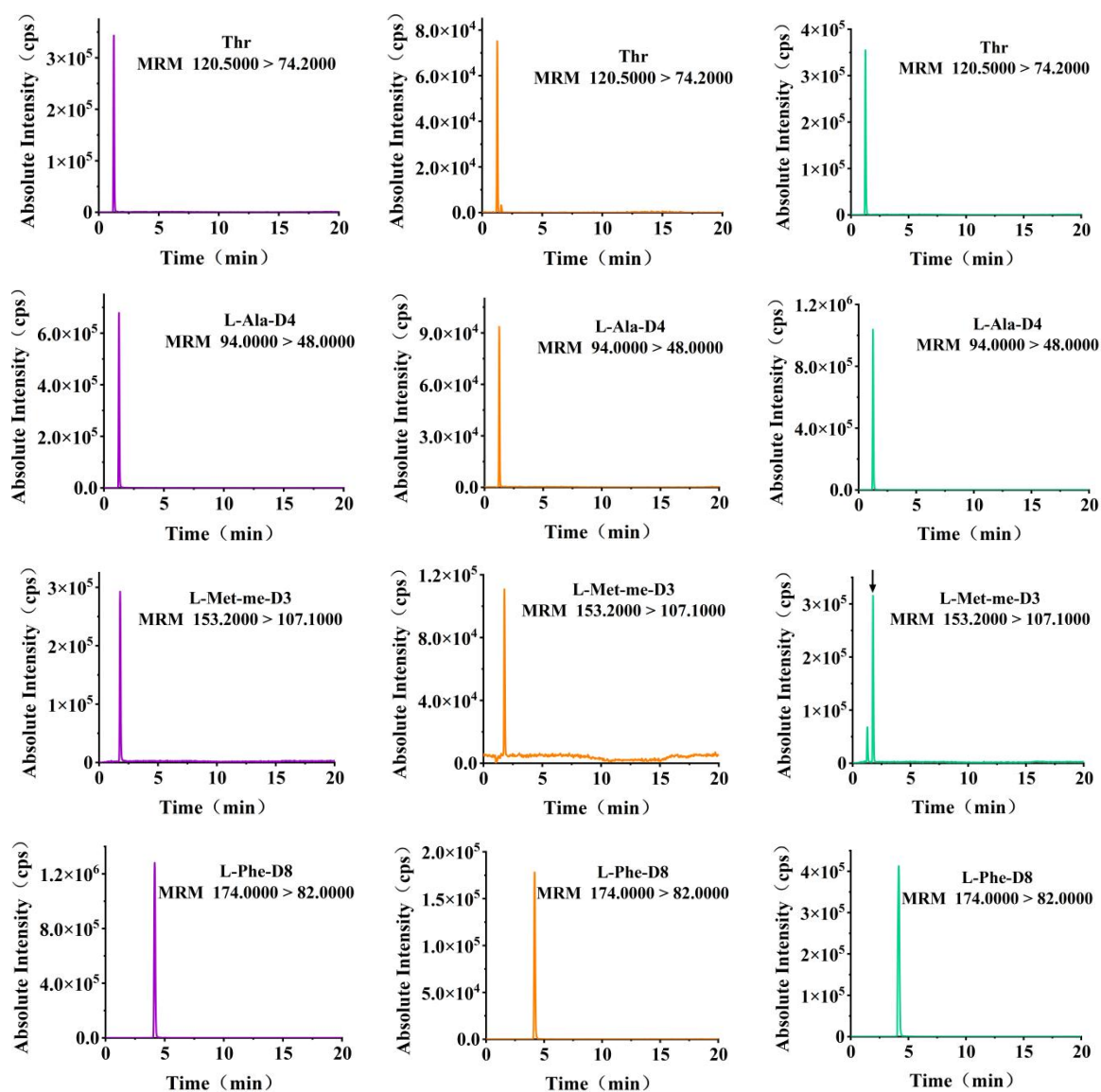
Serum samples spiked with standard solution











Supplementary Fig. 1. Representative MRM chromatograms of mixed standard solution, serum samples, and serum samples spiked with mixed standard solution are indicated in purple, orange and green, respectively.

Supplementary Table 1. Information of 26 analytes and 3 internal standards

English name of control substance	Abbreviation	Molecular weight	Production company	Batch number	Purity
L-Lysine	Lys	146.19	Chengdu Alfa Biotechnology	AF20080261	≥98%
L-Methionine	Met	149.21	Chengdu Alfa Biotechnology	AF21032151	≥98%
L-Glycine	Gly	75.07	Shanghai yuanye Bio-Technology	S29A10I96410	≥99%
L-Alanine	Ala	89.09	Chengdu Alfa Biotechnology	AF20071851	≥98%
L-Valine	Val	117.15	Chengdu Alfa Biotechnology	AF20050253	≥98%
L-Leucine	Leu	131.17	Chengdu Alfa Biotechnology	AF20080253	≥98%
L-Isoleucine	Ile	131.7	Chengdu Alfa Biotechnology	AF20080256	≥98%
L-Proline	Pro	155.13	Chengdu Alfa Biotechnology	AF20080255	≥98%
L-Serine	Ser	105.09	Chengdu Alfa Biotechnology	AF20080260	≥98%
L-Threonine	Thr	119.12	Chengdu Alfa Biotechnology	AF20080258	≥98%
L-Arginine	Arg	174.20	Chengdu Alfa Biotechnology	AF20080251	≥98%
L-Citrulline	Cit	175.19	Chengdu Alfa Biotechnology	AF21021555	≥98%
L-Histidine	His	155.15	Chengdu Alfa Biotechnology	AF20080527	≥98%
L-Aspartic acid	Asp	133.1	Shanghai yuanye Bio-Technology	S24A8I34463	≥99%
L-Glutamic acid	Glu	147.13	Shanghai yuanye Bio-Technology	S12A10I85582	≥99%
L-Cystine	Cys-Cys	240.30	Chengdu Alfa Biotechnology	AF20080262	≥98%
L-Phenylalanine	Phe	165.19	Chengdu Alfa Biotechnology	AF20080254	≥98%
L-Tyrosine	Tyr	181.19	Chengdu Alfa Biotechnology	AF20080252	≥98%
Epinephrine	E	183.21	National Institutes for Food and Drug Control	100154-202106	≥99%
L-Tryptophan	Trp	204.23	Shanghai Standard Technology	6135	≥98%
L-Kynurenine	Kyn	208.22	Shanghai McLean Biochemical Technology	C13900275	≥98%
4-Hydroxyquinoline-2-carboxylic acid	KA	189.17	Shanghai Standard Technology	10558	≥98%
5-Hydroxyindole-3-acetic acid	5-HIAA	191.18	Sigma-Aldrich	BCBZ1118	≥98%
Acetylcholine chloride	ACh	181.66	Shanghai yuanye Bio-Technology	Z08N8H847718	≥98%
Taurine	Tau	125.15	Shanghai yuanye Bio-Technology	108K1305V	≥98%
L-Glutamine	Gln	146.14	Shanghai yuanye Bio-Technology	011M01991V	≥98%
L-phenylalanine-D8	L-phe-D8	173.24	Shanghai ZZBio	21B143-B1	≥99%
L-Alanine-D4	L-Ala-D4	93.12	Shanghai ZZBio	21B184-C2	≥99%
L-Methionine-methyl-D3	L-met-me-D3	152.23	Shanghai yuanye Bio-Technology	O29GB165934	≥99%

Supplementary Table 2. Multiple reaction monitoring parameters for 26 tested and 3 internal standards (positive ion mode)

Amine acid	Precursorion	Production	Q1 (V)	CE (V)	Q3 (V)
Lys	147.2	84.1	-11.0	-18.0	-15.0
Met	150.2	104.2	-11.0	-12.0	-20.0
Gly	76.0	30.2	-29.0	-23.0	-28.0
Ala	90.0	44.0	-10.0	-13.0	-15.0
Val	118.2	72.2	-11.0	-13.0	-14.0
Leu	132.2	86.1	-13.0	-24.0	-16.0
Ile	132.2	86.1	-13.0	-24.0	-16.0
Pro	115.8	70.2	-10.0	-13.0	-14.0
Ser	106.0	60.5	-11.0	-14.0	-22.0
Thr	120.5	74.5	-14.0	-14.0	-14.0
Arg	174.5	70.2	-24.0	-25.0	-16.0
Cit	175.5	158.5	-11.0	-11.0	-10.0
His	156.2	110.2	-11.0	-16.0	-22.0
Asp	134.3	88.2	-10.0	-13.0	-26.0
Glu	147.2	84.1	-11.0	-16.0	-16.0
Cys-Cys	241.0	152.2	-12.0	-16.0	-25.0
Phe	166.2	120.2	-13.0	-15.0	-22.0
Tyr	182.2	136.2	-14.0	-15.0	-26.0
E	166.2	107.1	-12.0	-21.0	-19.0
Trp	205.2	188.1	-15.0	-11.0	-19.0
Kyn	209.0	91.0	-24.0	-41.0	-17.0
KA	190.0	162.0	-23.0	-20.0	-30.0
5-HIAA	192.0	146.0	-10.0	-17.0	-29.0
ACh	146.1	86.5	-10.0	-17.0	-16.0
Tau	125.9	108.2	-15.0	-5.0	-19.0
Gln	147.2	84.1	-11.0	-19.0	-16.0
L-Phe-D8	174.4	82.1	-14.0	-45.0	-14.0
L-Ala-D4	94.0	48.0	-14.0	-13.0	-18.0
L-Met-me-D3	153.2	107.1	-14.0	-15.0	-16.0

Supplementary Table 3. Limit of detection, limit of quantitation, linear equation and correlation coefficient results of compounds

Compounds	Retention time	linear equations	R^2	LOD		LOQ		IS
				ng/mL	ng/mL	RSD	RE	
His	1.115	$y = 707154x + 0.0031$	0.9993	0.1	1	1.42	102.03	L-phe-D8
Arg	1.12	$y = 192766x - 0.0037$	0.9988	5	10	0.95	98.63	L-Met-me-D3
Cys-Cys	1.199	$y = 797963x - 0.01$	0.9998	0.1	1	1.1	112.71	L-Met-me-D3
Gly	1.215	$y = 220732x + 0.0013$	0.9993	0.1	1	0.21	112.48	L-Met-me-D3
Ser	1.23	$y = 33161x + 0.0026$	0.9992	1	10	5.1	108.48	L-Met-me-D3
Gln	1.242	$y = 4863821x - 0.0503$	0.9999	0.1	1	7.25	109.2	L-Met-me-D3
Lys	1.244	$y = 4955863x - 0.0527$	0.9999	0.1	1	9.61	114.01	L-Met-me-D3
Ala	1.249	$y = 800921x + 0.0168$	0.9995	0.1	1	0.2	92.66	L-Ala-D4
Tau	1.255	$y = 49087x + 0.002$	0.9999	1	10	17.04	97.92	L-Ala-D4
Cit	1.264	$y = 24952x - 0.0005$	0.9990	1	10	1.12	109.59	L-Met-me-D3
Asp	1.267	$y = 385834x + 0.001$	1.0000	1	10	1.83	113.73	L-Ala-D4
Thr	1.267	$y = 271571x - 0.002$	0.9993	2	10	1.74	101.06	L-Met-me-D3
Glu	1.282	$y = 3179641x - 0.0171$	0.9995	0.1	2	16.82	118.49	L-Met-me-D3
ACh	1.324	$y = 1386394x + 0.0206$	0.9999	0.1	1	0.98	88.18	L-Ala-D4
Pro	1.351	$y = 547766x + 0.0689$	0.9951	0.1	1	3.05	107.72	L-phe-D8
Val	1.599	$y = 5657394x + 0.01783$	0.9998	0.1	2	0.51	114.95	L-Met-me-D3
Met	1.792	$y = 1542353x + 0.0078$	0.9999	0.1	1	2.03	101.14	L-Met-me-D3
Tyr	2.225	$y = 790761x + 0.0058$	0.9998	0.1	10	7.71	79.98	L-phe-D8
Ile	2.417	$y = 3864065x - 0.0258$	0.9997	0.1	1	3.09	94.89	L-Ala-D4
Leu	2.622	$y = 3218680x - 0.0221$	0.9995	0.1	1	4.19	120.05	L-Ala-D4
Kyn	3.736	$y = 376376x + 4.5999$	0.9997	2	10	2.81	98.4	L-phe-D8
Phe	4.287	$y = 3099506x + 0.0254$	0.9999	0.1	1	0.57	107.21	L-phe-D8
E	4.291	$y = 61769x + 0.0007$	0.9999	1	10	3.36	111.88	L-phe-D8
Trp	8.317	$y = 2111951x + 0.0016$	1.0000	0.1	1	2.29	104.45	L-phe-D8
5-HIAA	8.999	$y = 374956x + 0.0004$	1.0000	1	1.5	8.16	112.69	L-phe-D8
Ka	9.618	$y = 318245x + 0.0009$	0.9999	0.1	2	4.18	112.93	L-phe-D8

Supplementary Table 4. Extraction recovery and matrix effect results of 26**compounds**

compounds	Extraction recovery (n = 5)			Matrix effect (n = 5)		
	mean $\pm$ SD (%)			mean $\pm$ SD (%)		
	10 ng/mL	100 ng/mL	1000 ng/mL	10 ng/mL	100 ng/mL	1000 ng/mL
Trp	80.93 $\pm$ 4.35	93.79 $\pm$ 10.21	108.67 $\pm$ 4.09	101.11 $\pm$ 5.63	104.26 $\pm$ 6.71	112.18 $\pm$ 16.64
Kyn	83.35 $\pm$ 10.02	101.1 $\pm$ 2.97	107.33 $\pm$ 3.14	103.45 $\pm$ 6.22	103.93 $\pm$ 3.37	90.85 $\pm$ 8.06
Ka	98.38 $\pm$ 14.12	105.42 $\pm$ 6.48	106.21 $\pm$ 3.2	103.98 $\pm$ 4.45	105.06 $\pm$ 2.7	105.45 $\pm$ 11.26
5-HIAA	102.39 $\pm$ 17.94	98.97 $\pm$ 4.77	106.33 $\pm$ 3.44	108.44 $\pm$ 3.88	109.75 $\pm$ 6.34	89.04 $\pm$ 16.89
Phe	90.16 $\pm$ 14.66	82.79 $\pm$ 14	105.63 $\pm$ 4.35	102.05 $\pm$ 10.8	109.34 $\pm$ 9.07	102.38 $\pm$ 3.62
Tyr	83.95 $\pm$ 8.94	103.27 $\pm$ 13.76	86.77 $\pm$ 3.2	98.24 $\pm$ 7.96	108.98 $\pm$ 8.14	112.08 $\pm$ 7.71
E	95.64 $\pm$ 7.92	104.13 $\pm$ 14.9	107.2 $\pm$ 2.46	82.18 $\pm$ 4.53	114.63 $\pm$ 17.27	116.93 $\pm$ 8.4
Lle	89.25 $\pm$ 1.5	81.11 $\pm$ 7.87	82.04 $\pm$ 9.61	88.63 $\pm$ 4.61	104.44 $\pm$ 4.44	117.27 $\pm$ 9.66
Leu	95.42 $\pm$ 10.85	101.73 $\pm$ 17.08	118.31 $\pm$ 7.58	94.24 $\pm$ 8.76	110.15 $\pm$ 14.96	89.28 $\pm$ 14.51
His	82.01 $\pm$ 12.04	84.55 $\pm$ 13.91	90.3 $\pm$ 2.61	81.5 $\pm$ 7.99	102.82 $\pm$ 9.45	120.09 $\pm$ 19.41
Met	83.41 $\pm$ 12.97	92.86 $\pm$ 2.62	95.01 $\pm$ 9.35	105 $\pm$ 10.93	88.92 $\pm$ 7.86	82.64 $\pm$ 11.36
Val	86.17 $\pm$ 4.24	83.48 $\pm$ 16.76	97.88 $\pm$ 16.38	82.75 $\pm$ 9.54	80.24 $\pm$ 5.48	111.88 $\pm$ 21.23
Cit	115.91 $\pm$ 17.86	90.24 $\pm$ 12.33	106.58 $\pm$ 6.66	81.66 $\pm$ 7.78	91.63 $\pm$ 16.31	92.48 $\pm$ 0.89
Ach	90.67 $\pm$ 11.65	104.27 $\pm$ 3.87	102.46 $\pm$ 5.69	107.04 $\pm$ 7.58	103.13 $\pm$ 1.59	99.96 $\pm$ 4.5
Ala	104.7 $\pm$ 12.31	88.37 $\pm$ 15.86	80.92 $\pm$ 13.31	100.57 $\pm$ 8.62	97.09 $\pm$ 14.76	117.54 $\pm$ 3.03
Arg	81.5 $\pm$ 17.6	80.93 $\pm$ 4.55	89.45 $\pm$ 4.07	83.04 $\pm$ 14.56	83.16 $\pm$ 9.64	101.65 $\pm$ 8.41
Asp	85.69 $\pm$ 10.46	99.01 $\pm$ 8.49	102.6 $\pm$ 2.29	94.94 $\pm$ 5.23	92.22 $\pm$ 6.86	89.49 $\pm$ 8.23
Cys-Cys	81 $\pm$ 9.86	88.87 $\pm$ 9.77	93.67 $\pm$ 3.48	81.85 $\pm$ 1.34	110.72 $\pm$ 1.33	91.04 $\pm$ 3.35
Gln	104.02 $\pm$ 19.29	84.11 $\pm$ 13.13	95.3 $\pm$ 11.54	85.36 $\pm$ 5.57	113.5 $\pm$ 20.81	115.34 $\pm$ 8.18
Gly	111.02 $\pm$ 1.33	82.39 $\pm$ 7.28	83.03 $\pm$ 10.84	88.5 $\pm$ 3.48	95.12 $\pm$ 5.26	89.79 $\pm$ 10.89
Glu	103.4 $\pm$ 6.56	82.17 $\pm$ 6.43	102.05 $\pm$ 5.46	92.64 $\pm$ 8.81	97.86 $\pm$ 4.54	109.62 $\pm$ 20.98
Lys	88.93 $\pm$ 4.73	94.89 $\pm$ 12.13	93.96 $\pm$ 9.73	83.34 $\pm$ 4.4	104.6 $\pm$ 19.5	117.09 $\pm$ 9.13
Pro	81.6 $\pm$ 1.06	84.59 $\pm$ 11.29	99.96 $\pm$ 3.63	91.55 $\pm$ 4.72	95.88 $\pm$ 12.48	119.98 $\pm$ 3.87
Ser	85.38 $\pm$ 4.17	101.07 $\pm$ 2.43	81.89 $\pm$ 13.04	98.48 $\pm$ 10.19	95.44 $\pm$ 8.54	82.17 $\pm$ 10.24
Tau	105.72 $\pm$ 7.33	80.18 $\pm$ 14.34	82.14 $\pm$ 10.8	97.84 $\pm$ 12.15	82.97 $\pm$ 10.11	114.94 $\pm$ 9.66
Thr	99.26 $\pm$ 7.17	85.18 $\pm$ 0.14	100.63 $\pm$ 7.2	95.38 $\pm$ 13.88	100.95 $\pm$ 19.42	115.62 $\pm$ 11.83

Supplementary Table 5. Intra-day and inter-day accuracy results of 26 compounds
(n = 5)

compounds	Accuracy (RE%)					
	10 ng/mL		100 ng/mL		1000 ng/mL	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Gly	100.97	98.71	104.97	114.52	104.93	100.53
Gln	105.89	91.25	96.44	95.92	88.1	110.9
Ile	110.49	109.28	103.95	112.14	105.38	92.51
Leu	88.54	88.37	97.54	106.71	112.1	93.44
His	88.54	87.42	94.81	91.84	108.59	91.75
Phe	106.57	106.24	93.1	101.28	111.58	93.88
5-HIAA	90.39	86.35	105.52	110.81	90.81	101.5
ACh	108.21	99.16	98.31	90.43	107.04	89.97
Ala	95.94	93	103.35	111.62	98.3	112.69
Arg	95.65	96.56	93.4	108.23	99.22	93.98
Asp	95.95	92.88	112.53	108.07	110.78	106.23
Cit	113.16	87.83	105.86	108.99	114.69	90.23
Cys-Cys	109.53	108.51	105.96	114.24	101.02	112.24
E	93.33	91.84	100.71	110.51	86.17	94.39
Glu	106.09	90.21	112.87	105.87	108.88	97.09
Ka	101.09	93.87	95.82	97.46	93.4	96.91
Lys	96.05	87.25	104.67	108.31	105.18	94.94
Met	89.52	85.06	104.38	90.82	104.73	110.06
Pro	103.14	96.76	94.03	88.93	102.54	111.97
Ser	99.27	95.31	94.21	98.5	86.78	112.73
Tau	96.82	92.4	99.1	99.1	87.12	113.49
Thr	90.92	88.64	102.51	99.57	106.77	93.85
Trp	98.83	98.24	105.93	109.11	95.49	108.3
Tyr	96.42	94.73	101.07	105.13	111.6	99.51
Val	90.69	88.98	102.4	89.5	100.43	112.86
Kyn	96.2	94.03	99.36	103.1	106.37	89.2

Supplementary Table 6. Intra-day and inter-day precision results of 26 compounds
(n = 5)

compounds	Precision (RSD%)					
	10 ng/mL		100 ng/mL		1000 ng/mL	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Gly	3.12	1.69	1.43	2	3.59	1.9
Gln	12.6	11.67	1.62	1.78	5.31	11.66
Ile	1.1	0.93	1.56	0.72	3.98	3.15
Leu	1.17	0.69	0.63	0.72	4.99	4.29
His	7.08	2.66	1.44	3.83	6.22	9.9
Phe	0.89	0.59	0.73	0.52	4.64	3.86
5-HIAA	3.52	2.17	1.58	1.13	7.43	2.01
ACh	12.11	10.76	2.71	6.21	0.92	0.89
Ala	11.87	9.52	12.52	16.57	10.62	7.56
Arg	12.02	12.01	15.32	14.17	8.24	13.68
Asp	5.57	5.09	1.69	2.33	6.9	11.7
Cit	12.67	14.27	12.89	11.86	13.02	13.4
Cys-Cys	3.45	0.85	2.3	2.12	3.25	5.72
E	0.73	0.65	1.52	1.63	10.44	4.7
Glu	6.19	8.11	1.15	3.41	7.55	12.94
Ka	1.67	1.89	1.02	0.92	3.23	1.54
Lys	7.94	11.62	0.93	1.97	4.16	9.36
Met	3.01	2.57	1.23	1.22	7.76	1.58
Pro	6.71	5.89	3.27	2.79	2	2.86
Ser	7	3.87	1.63	2.79	3.73	7.76
Tau	1.75	2.51	3.42	3.03	8.8	10.69
Thr	11.36	12.85	4.12	4.42	13.9	8.35
Trp	0.99	1.12	1.05	0.67	3.93	1.13
Tyr	1.13	1.35	0.89	0.66	5.35	4.36
Val	4.66	2	0.9	0.33	3.49	4.97
Kyn	1.21	1.66	0.84	0.92	3.75	1.5

Supplementary Table 7. Precision and accuracy results of 26 compounds assessed under short-term (0, 6, and 24 h at room temperature) and freeze–thaw (three cycles) conditions (n = 5)

0 h	10 ng/mL		100 ng/mL		1000 ng/mL	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
	(RE%)	(RSD%)	(RE%)	(RSD%)	(RE%)	(RSD%)
Gly	95.44	1.46	93.52	4.65	96.44	3.74
Gln	95.33	1.02	92.49	1.01	98.05	0.86
Ile	89.3	1.83	88.5	1.48	97.33	1.96
Leu	102.24	0.94	96.21	1.6	103.75	0.34
His	89.28	4.78	92.1	15.66	94.52	9.31
Phe	93.13	0.31	91.5	2.05	92.09	1.79
5-HIAA	96.51	3.01	90.54	1.79	96.49	1.8
Ach	94.18	0.56	94.77	0.82	99.2	5.82
Ala	104.98	0.24	100.76	1.42	113.07	1.32
Arg	97.41	4.77	85.43	10.64	103.7	4.32
Asp	92.39	2.13	87.28	3.22	94.88	4.16
Cit	92.99	8.02	89.31	10.41	110.8	21.47
Cys-Cys	90.26	1.18	88.21	3.59	91.13	12.63
E	97.55	0.85	103.79	2.15	93.14	1.59
Glu	96.02	3.1	96.04	2.9	94.55	2.53
KA	107.71	1.63	103.93	0.88	104.89	9.37
Lys	95.85	1.87	85.91	0.58	100.4	1.39
Met	93.5	0.47	93.25	0.49	93.92	1.24
Pro	89.05	2.48	88.44	2.07	98.77	1.1
Ser	107.16	3.01	96.07	4.63	97.36	4.5
Tau	96.87	1.52	91.23	2.77	98.59	7.24
Thr	104.84	2.61	98.98	3.39	107.7	9.5
Trp	100.85	0.93	98.64	2.05	97.28	0.59
Tyr	100.32	0.79	97.2	1.53	97.29	1.24
Val	98.65	1.93	89.87	3.29	106.92	1.66
Kyn	91.12	0.71	94.25	3.84	97.5	3.19

6 h	10 ng/mL		100 ng/mL		1000 ng/mL	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
	(RE%)	(RSD%)	(RE%)	(RSD%)	(RE%)	(RSD%)
Gly	94.43	2.32	112.18	3.33	88.39	2.9
Gln	87.06	0.98	102.03	1.78	89.19	1.91
Ile	100.27	1.43	88.9	1.09	92.94	0.85
Leu	99.24	0.57	106.08	0.72	102.2	1.89
His	102.34	8.1	92.01	6.75	81.2	3.06
Phe	93.03	2.37	93.93	1.96	90.19	0.85
5-HIAA	90.47	3.27	92.1	0.86	93.58	11.96
Ach	93.46	3.66	100.83	2.1	91.9	0.94
Ala	96.71	1.41	112.47	2.29	104.29	1.19
Arg	114.37	2.6	107.74	10.97	89.58	4.33
Asp	89.09	2.06	94.3	1.91	88.12	2.59
Cit	96.1	0.46	108.09	11.88	92.34	1.61
Cys-Cys	87.8	2.65	103.89	3.05	88.4	0.83
E	103.99	1.91	92.56	1.76	103.94	1.48
Glu	97.4	1.34	112.13	1.08	98.98	0.82
KA	100.28	1.29	103.34	0.78	100.63	6.42
Lys	92.92	0.86	120.84	1.59	93.8	1.13
Met	89.81	2.37	105.12	2.79	89.04	5.98
Pro	87.79	3.58	97.15	1.13	87.41	2.8
Ser	102.27	2.1	121.51	5.98	105.46	5.03
Tau	93.36	3.65	100.68	5.56	87.43	8.02
Thr	89.05	1.09	105.35	0.34	88.98	3.28
Trp	93.92	0.93	97.66	0.4	95.26	1.96
Tyr	99.38	1.71	99.97	1.51	98.33	0.08
Val	100.53	2.89	104.59	2.04	88.61	3.28
Kyn	95.72	0.76	94.26	0.9	97.65	2.09

24 h	10 ng/mL		100 ng/mL		1000 ng/mL	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
	(RE%)	(RSD%)	(RE%)	(RSD%)	(RE%)	(RSD%)
Gly	95.6	3.56	113.28	7.53	108.64	4.65
Gln	89.17	6.31	107.99	2.18	108.29	2.09
Ile	88.98	5.35	89.6	0.94	90.01	1.1
Leu	92.72	4.72	93.11	1.71	92.88	0.71
His	105.39	7.43	94.35	5.8	105.45	1.25
Phe	87.59	7.88	89.43	4.99	96.2	2.15
5-HIAA	97.36	5.96	101.29	3.2	94.02	10.04
Ach	97.65	3.44	91.82	1.31	94.19	4.62
Ala	108.24	4.5	95.71	1.24	108.15	0.92
Arg	107.43	14.82	94.9	4.54	102.65	4.86
Asp	88.51	11.81	95.66	4.92	104.72	6.65
Cit	96.73	14.41	93.81	7.76	106.16	11.74
Cys-Cys	94.54	6.81	95.39	4.26	96.96	7.7
E	88	7.3	107.07	6.45	108.2	3.99
Glu	106.04	4.76	105.09	4.32	112.22	1.22
KA	111.06	0.98	103.34	3.45	102.17	8.63
Lys	94.08	7.3	97.53	3.21	107.08	3.07
Met	90.13	7.82	92.15	2.56	93.73	4.87
Pro	97.65	6.53	99.37	1.66	102.97	1.22
Ser	89.92	8.96	105.47	10.79	108.76	5.86
Tau	107.69	2.19	109.79	6.81	104.56	5.33
Thr	88.46	6.92	104.11	6.55	106.04	5.35
Trp	85.4	6.98	88.99	3.66	91.85	2.19
Tyr	93.54	7.63	93.6	2.64	100.82	1.7
Val	90.03	6.99	92.38	4.55	95.77	1.67
Kyn	112.92	5.79	108.83	2.83	111.27	11.98

3 Times	10 ng/mL		100 ng/mL		1000 ng/mL	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
	(RE%)	(RSD%)	(RE%)	(RSD%)	(RE%)	(RSD%)
Gly	100.3	1.6	106.38	6.98	98.74	0.55
Gln	109.12	0.92	98.96	1.14	101.94	2.05
Ile	85.08	1.27	89.1	1.69	89.17	0.39
Leu	100.03	1.1	96.05	1.39	96.74	2.37
His	97.84	1.21	97.5	2.77	92.12	8.92
Phe	93.92	2.48	91.5	0.71	92.36	2.19
5-HIAA	97.63	1.48	94.2	2.84	94.78	8.71
Ach	96.15	1.33	93.74	2.3	96.07	2.25
Ala	110.96	0.54	108.04	0.95	108.45	1.97
Arg	105.28	1.53	94.71	7.07	92	14.24
Asp	95.04	1.34	93.63	2.55	93.09	4.54
Cit	105.53	6.88	109.24	6.76	96.37	7.73
Cys-Cys	91.38	2.38	86.82	2.28	87.34	1.62
E	104.27	2.02	108.18	3.86	107.6	2.6
Glu	111.59	2.43	110.27	1.77	109.44	2.38
KA	110.51	1.35	109.41	4.22	108.62	9.77
Lys	93.66	1.67	95.52	0.25	91.56	1.71
Met	93.75	0.95	91.47	1.3	92.53	4.69
Pro	92.45	0.73	90.03	1.73	94.58	3.78
Ser	92.86	6.7	110.95	3.84	95.17	7.04
Tau	103.99	3.35	95.64	4.41	101.73	3.88
Thr	109.31	7.98	101.92	2.64	111.03	9.58
Trp	95.86	2.3	93.2	0.59	93.53	1.03
Tyr	100.74	1.51	99.99	1.59	100.53	3.95
Val	95.68	1.06	85.37	0.9	86.82	2.12
Kyn	112.83	2.73	110.25	1.38	110.81	2.66

Supplementary Table 8. Multiple comparison of serum metabolite levels among ePD, maPD and normal groups

compounds	e PD		ma PD		<i>P</i> values		
	Mean	SD	Mean	SD	Control VS e PD	Control VS ma PD	e PD VS ma PD
Trp ^(1/2) , μM	67.4	9.87	70.47	9.32	0.001	0.01	0.774
Kyn ^(1/2) *10 ² , μM	150.03	27.96	147.77	26.67	< 0.001	< 0.001	0.746
Phe, μM	117.43	27.5	127.12	30.96	< 0.001	0.003	0.792
Tyr, μM	104.37	34.87	129.32	53.13	0.039	1	0.112
E, μM	107.34	25.68	115.88	27.66	0.001	0.01	0.813
Ile, μM	202.64	79.28	194.01	76.77		0.544 ^a	
Leu, μM	226.23	58.17	235.11	61.27	< 0.001	< 0.001	0.631
His ^(1/2) *10, μM	49.28	9.94	49.13	8.75		0.772 ^a	
Met ^(1/4) *10, μM	23.04	1.36	23.64	1.96	< 0.001	< 0.001	0.697
Val ^(1/2) , μM	14.4	4.06	14.72	3.25	< 0.001	< 0.001	0.697
Ala, μM	492.35	130.75	552.66	141.21	0.001	0.084	0.104
Arg ^(1/2) *10, μM	64.75	22.47	56.84	17.72	0.009	< 0.001	0.258
Asp, μM	40.22	9.23	39.98	10.65	0.001	< 0.001	1
Cys-Cys ^(1/5) *10, μM	13.91	1.64	14.27	2.11	0.001	0.002	0.442
Gln ^(1/2) , μM	16.48	3.92	17.06	6.39	< 0.001	< 0.001	1
Gly ^(1/2) , μM	13.33	3.1	12.8	3.52	< 0.001	< 0.001	1
Glu ^(1/3) , μM	4.28	0.92	4.45	0.98	< 0.001	< 0.001	1
Lys ^(1/4) , μM	4.11	0.44	4.12	0.68	< 0.001	< 0.001	1
Pro ^(1/4) , μM	3.17	0.26	3.25	0.35	< 0.001	< 0.001	0.97
Ser, μM	103.23	45.6	103.35	62.66	< 0.001	< 0.001	1
Tau ^(1/2) , μM	11.97	2.4	12.56	2.02	0.007	0.05	0.335
Thr ^(1/5) *10, μM	24.64	3.01	25	3.02	< 0.001	< 0.001	1
KA ^(1/2) *10 ² , μM	33.8	8.62	30.8	7.44	< 0.001	< 0.001	0.134
5-HIAA ^(1/4) *10 ² , μM	69.82	9.09	68.59	7.82	< 0.001	< 0.001	0.558
Cit ^(1/4) *10, μM	13.04	1.77	12.4	1.94	< 0.001	< 0.001	0.724
ACh ^(1/4) *10, μM	18.16	1.51	18.03	2.47	< 0.001	< 0.001	0.991

^a No significant difference was detected in the overall test, and no multiple comparisons were made.

Supplementary Table 9. Pathway analysis of differential metabolites in serum

Pathway Name	Match Status	<i>P</i>	$-\log(P)$	Holm <i>P</i>	FDR	Impact
Arginine biosynthesis	3/14	4.5583E-5	4.3412	0.0036466	0.0036466	0.30457
Glyoxylate and dicarboxylate metabolism	3/32	5.897E-4	3.2294	0.046587	0.023588	0.14815
Glycine, serine and threonine metabolism	2/33	0.013984	1.8544	1.0	0.3729	0.4744

Supplementary Table 10. Enrichment analysis of differential metabolites in serum

Metabolite Set	Total	Hits	Expect	<i>P</i> value	Holm <i>P</i>	FDR
Urea Cycle	28	4	0.307	1.41E-4	0.0138	0.0105
Ammonia Recycling	31	4	0.34	2.13E-4	0.0207	0.0105
Aspartate Metabolism	35	4	0.384	3.47E-4	0.0333	0.0113
Arginine and Proline Metabolism	52	4	0.571	0.00163	0.155	0.0399
Glycine and Serine Metabolism	59	4	0.648	0.00263	0.247	0.0515