Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

Sensitive and accurate determination of neurotransmitters from in vivo rat brain microdialysate of Parkinson's disease using in situ ultrasound-assisted derivatization dispersive liquid-liquid microextraction by UHPLC-MS/MS

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S3.1 The measurements of ionization efficiency (IE)

IE measurements were carried out as the works [34,35] for CCR-4-ethylphenol, LRSC-4-ethylphenol, and DNS-4-ethylphenol and benzoyl chloride--4-ethylphenol. The solvent composition was acetonitrile/ 0.1% aqueous formic acid in volume ratio 80:20. For every measurement, separate solutions of the analytes in the solvent were prepared and infused using two syringe pumps. Flows of the syringe pumps were combined prior to ESI source by means of tee-piece. The IE values were found according to Equation (1) and expressed as log IE values.

ESI source parameters were: drying gas temperature 300 °C; drying gas flow rate 9 L min^{-1}; nebulizer gas pressure 40 psi; sheath gas temp 280 °C; sheath gas flow 8 L min^{-1} and capillary voltage 3.5 kV. Only the MS parameter "target mass" (TM) was modified in order to tune ion transport. All IE measurements were carried out using M+ TM values (*m/z*).

S3.5 Method application: Content changes of NTs and DHBA of PD and the anti-Parkinsonian effect of baicalein

6-Hydroxydopamine (6-OHDA) is a dopamine analog, which specifically damages dopaminergiceither via uncoupling mitochondrial oxidative phosphorylation resulting in energy deprivation or alternatively, is associated with its ability to produce hydrogen peroxide, hydroxyl and superoxide radicals under physiological pH conditions. Therefore, 6-OHDA is used to investigate the cellular and molecular mechanisms underlying selective degeneration of dopaminergic neurons in PD.[S1] Therefore, 6-OHDA is used to investigate the cellular and molecular mechanisms underlying selective degeneration of dopaminergic neurons in PD. The results of spontaneous motor activity and rotation test[33] of all the drug-treated groups showed different degrees of improvement compared with the PD model group. Compared with the behavioral test, the method proposed in this study was applied for the determination of NTs and DHBA from all the groups to provide a more holistic and objective advantage for efficacy evaluation and drug screening. Compared with the control group, we found that

the concentrations of *L*-DOPA, DA, NE, 3-MT, NME, DOPAC, HVA, VMA, 5-HT and 5-HIAA of the PD rats were significantly lower ($P < 0.01$), while the levels of Glu and Asp were significantly higher ($P < 0.01$). E, ME, DOMA, DHPG, VMA, 5-HIAA, 2, 3-DHBA, 2, 5-DHBA, GABA and Gly was also decreased ($P < 0.05$). Nevertheless, treatment with of madopar and baicalein, showed a significantly tendency of bringing the levels of most NTs to normal. The returned levels of NTs after baicalein treatment compared to madopar group suggested that these two groups had the similar anti-Parkinsonian effect, which could be inferred that baicalein had obvious anti-Parkinsonian effect. The neuroprotective effect of baicalein on dopaminergic neurons may partly be due to its antioxidant, scavenge free-radical properties.[32] For example, it can effectively scavenge hydroxyl.

References

[S1] X. Mu, G.R. He, X. Yuan, X.X. Li, G.H. Du, *Pharmacol. Biochem. Be.*, 2011,**98**, 286-291.

Table S1 Analytical performance of the *in situ* UA-DDLLME by UHPLC-MS/MS

Analytes	Linear range (nM)	LODs (pM)	LOQs (nM)	R	Added concentrations in QCs (nM)	Intra-and Inter-day Precision (RSD, %) (*n* = 6)	Intra-and Inter-day accuracy (%) (*n* = 6)	Matrix effect (%) (*n* = 3)	Recovery (RSD, %) (*n* = 3)	Stability (Peak area RSDs, %)
L-DOPA	0.05-200	0.2	0.01	0.995	0.1	6.3-9.9	101.3	88.2	80.2	4.2-8.1
					5.0	3.9-9.4	102.7	98.5	96.3	
					100.0	532-8.4	94.3	103.1	105.4	
DA	0.05-200	0.1	0.01	0.998	0.1	5.3-11.5	96.1	98.5	82.2	4.4-8.3
					5.0	4.9-11.5	104.5	101.8	96.5	
					100.0	4.21.0	95.2	81.2	104.6	
NE	0.05-200	0.5	0.01	0.994	0.1	5.3-11.4	96.1	97.3	87.2	4.2-7.9
					5.0	5.5-10.9	105.3	101.1	97.2	
					100.0	4.6-11.3	95.2	79.2	103.5	
E	0.05-200	0.5	0.01	0.993	0.1	5.0-9.2	95.5	93.4	85.4	6.9-8.4
					5.0	5.9-11.5	105.5	97.8	96.2	
					100.0	5.3-10.3	94.2	109.1	104.3	
3-MT	0.05-200	0.5	0.01	0.995	0.1	3.5-11.2	106.6	94.2	96.2	4.3-7.8
					5.0	4.5-9.4	102.7	80.6	104.1	
					100.0	5.1-10.9	94.3	102.6	78.7	
NME	0.05-200	0.5	0.01	0.996	0.1	5.3-10.7	108.9	98.2	97.2	4.0-8.2
					5.0	7.1-11.3	104.7	106.7	102.3	
					100.0	5.9-105	92.3	94.0	80.0	
ME	0.05-200	0.5	0.01	0.995	0.1	5.1-10.6	102.8	95.0	83.0	4.3-8.4
					5.0	5.3-11.7	109.2	108.6	95.7	
					100.0	4.9-11.2	92.2	104.3	102.9	
DOPAC	0.05-200	0.5	0.01	0.996	0.1	4.1-8.9	103.7	85.8	87.6	3.9-8.1
					5.0	4.5-11.7	106.8	97.8	95.2	
					100.0	6.1-10.8	92.2	109.7	107.1	
DOMA	0.05-200	0.5	0.01	0.993	0.1	4.6-11.6	94.2	96.7	95.3	4.3-8.3
					5.0	6.1-11.5	102.4	107.5	104.3	
					100.0	5.2-10.6	96.7	80.4	88.6	
DHPG	0.05-200	0.5	0.01	0.994	0.1	6.1-10.5	87.5	87.0	84.2	4.5-8.2
					5.0	4.2-8.9	107.1	97.2	106.3	
					100.0	3.8-102	93.7	104.3	103.5	

					0.1	5.3-11.4	106.6	95.5	104.3	
HVA	0.05-200	0.5	0.01	0.995	5.0	5.9-10.4	105.4	104.9	106.2	4.3-8.3
					100.0	4.5-11.4	93.4	85.9	81.7	
					0.1	4.4-10.2	92.1	86.6	80.4	
VMA	0.05-200	0.5	0.01	0.993	5.0	6.3-9.9	108.1	105.3	102.6	4.0-8.4
					100.0	3.9-9.4	95.4	10.3.6	101.9	
					0.1	4.8-10.6	96.2	88.9	90.0	
MHPG	0.05-200	0.5	0.01	0.992	5.0	5.2-10.4	90.6	98.7	102.6	4.7-8.0
					100.0	5.3-11.4	89.3	112.3	106.3	
					0.1	5.3-10.9	97.7	98.4	84.2	
5-HT	0.05-200	0.4	0.01	0.995	5.0	4.7-11.3	92.6	102.9 6	96.4	4.9-8.2
					100.0	5.8-11.4	103.7	105.2	104.4	
					0.1	4.1-9.5	89.7	78.8	80.8	
5-HIAA	0.05-200	0.5	0.01	0.997	5.0	5.0-9.4	90.6	97.6	98.6	4.8-8.4
					100.0	5.8-11.9	106.5	106.3	104.3	
					1.0	3.1-8.9	89.4	86.0	86.2	
Glu	3-5000	1	0.5	0.995	10.0	4.1-9.3	92.3	98.2	97.5	4.0-8.0
					500.0	6.1-11.5	105.5	105.3	105.5	
					1.0	5.7-10.4	88.9	88.0	80.2	
GABA	3-5000	2	0.5	0.996	10.0	4.3-11.4	105.5	97.2	98.3	3.8-7.5
					500.0	6.2-11.7	106.5	107.3	102.1	
					1.0	4.1-9.2	99.9	85.0	83.2	
Gly	3-15000	3	0.5	0.992	100	5.3-11.6	104.5	98.8	96.2	4.1-8.3
					500.0	5.9-11.4	94.8	106.3	102.3	
					1.0	5.2-10.7	97.9	78.9	89.4	
Asp	3-15000	3	0.5	0.994	10.0	4.6-11.5	95.4	96.4	97.8	4.4-7.7
					500.0	6.1-11.3	110.4	104.3	104.3	
					1.0	4.2-8.2	88.6	78.7	81.4	
2,3-DHBA	1-5000	3	0.05	0.994	10.0	4.3-10.4	91.4	97.2	104.4	4.6-8.2
					500.0	5.9-10.9	95.8	103.3	101.2	
					1.0	4.3-9.9	97.4	88.4	85.4	
2,5-DHBA	1-5000	3	0.05	0.993	10.0	3.8-9.6	92.4	96.7	97.4	5.0-7.9
					500.0	6.5-12.8	107.9	104.3	103.2	

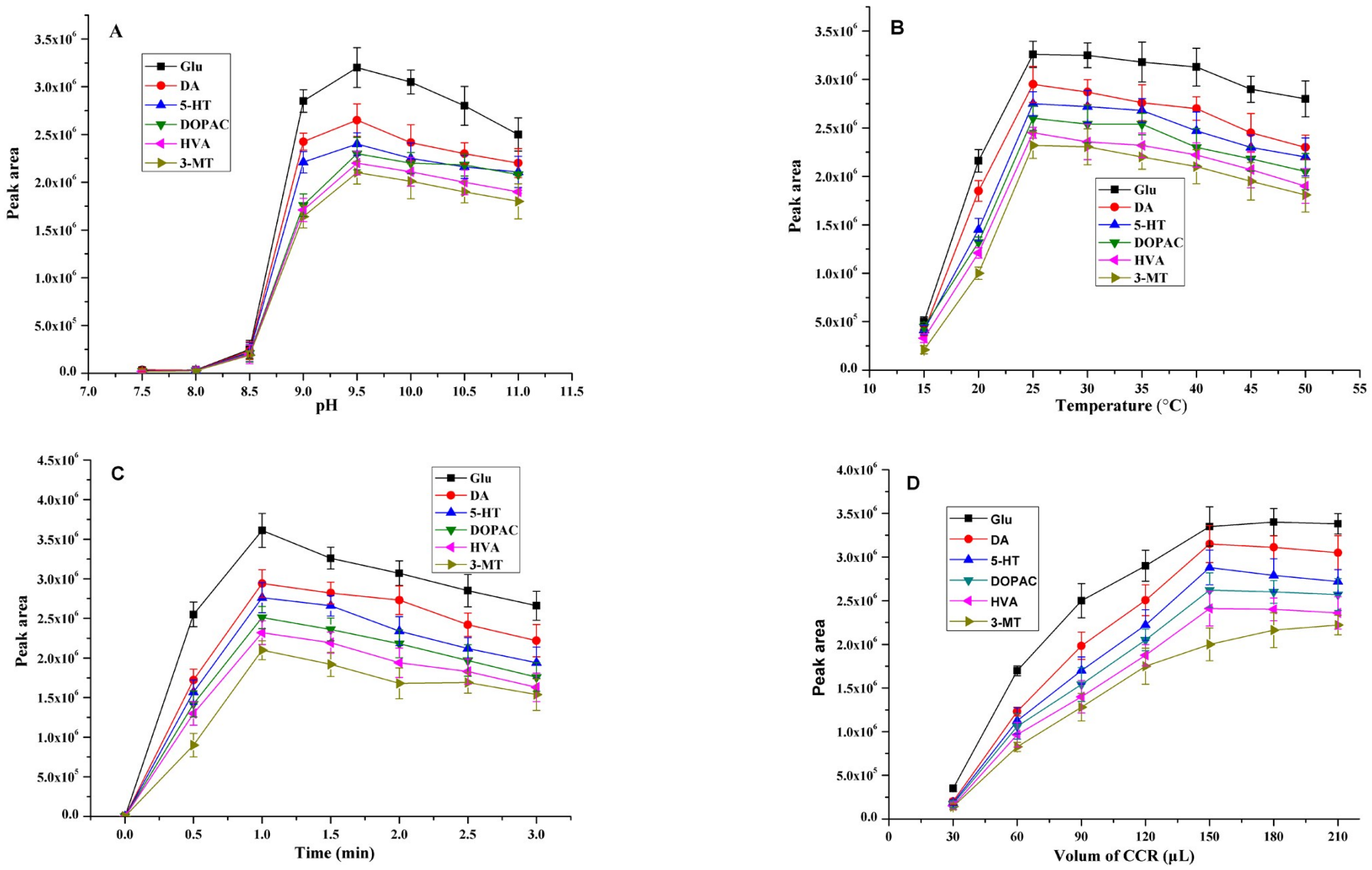


Fig. S1 Optimization of in situ UA-DDLLME conditions: (A) pH, (B) temperature, (C) ultrasound time, and (D) volume of CCR

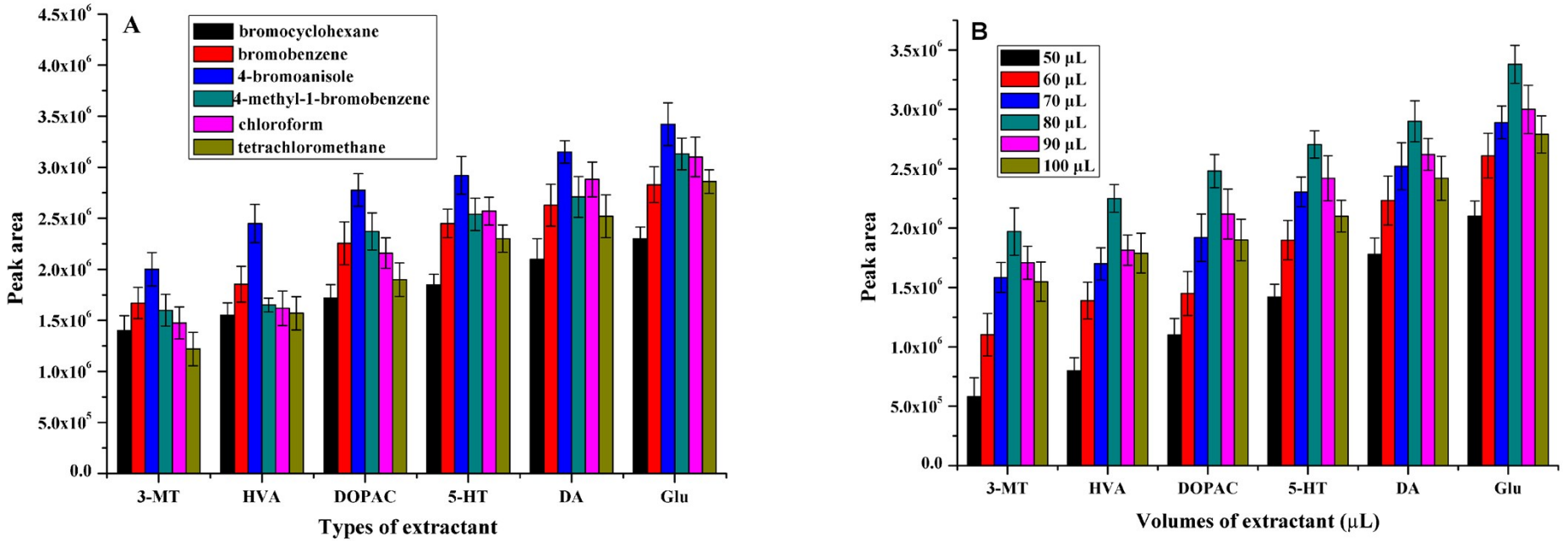
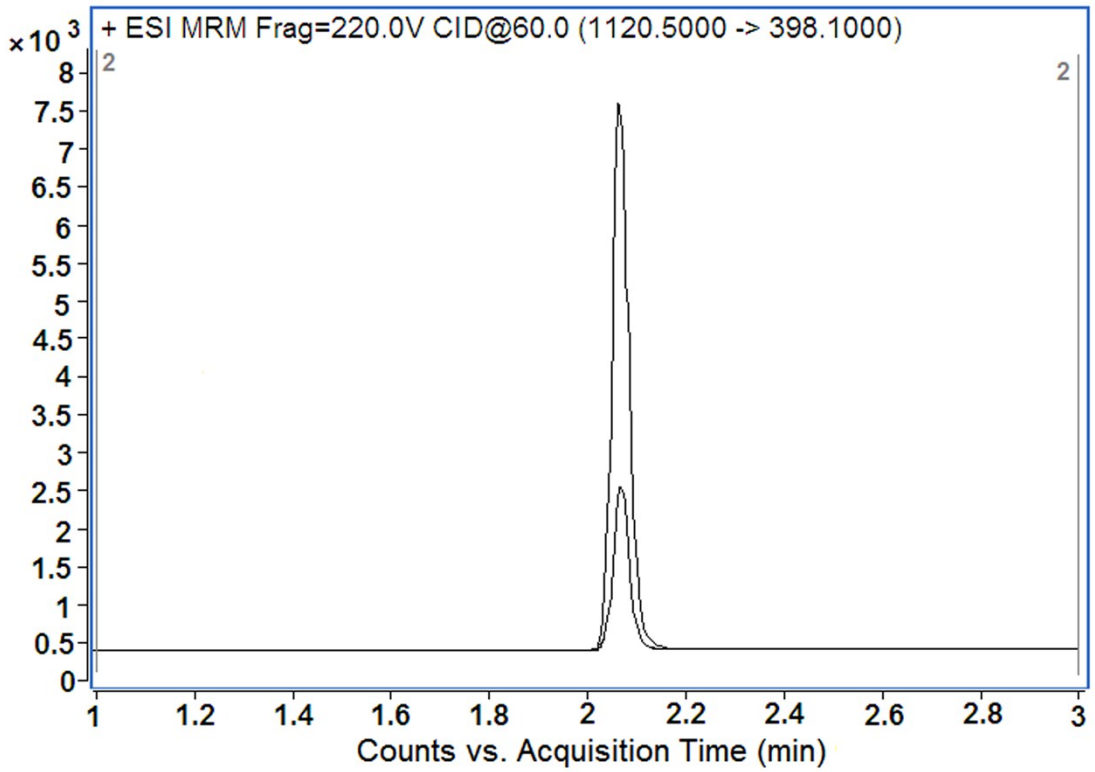


Fig. S2 Optimization of parameters of *in situ* UA-DDLLME: (A) type of extractant, (B) volume of extractant

(A)


+ ESI MRM Frag=220.0V CID@60.0 (1120.5000 -> 398.1000)
×10 3
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
Counts vs. Acquisition Time (min)

(B)

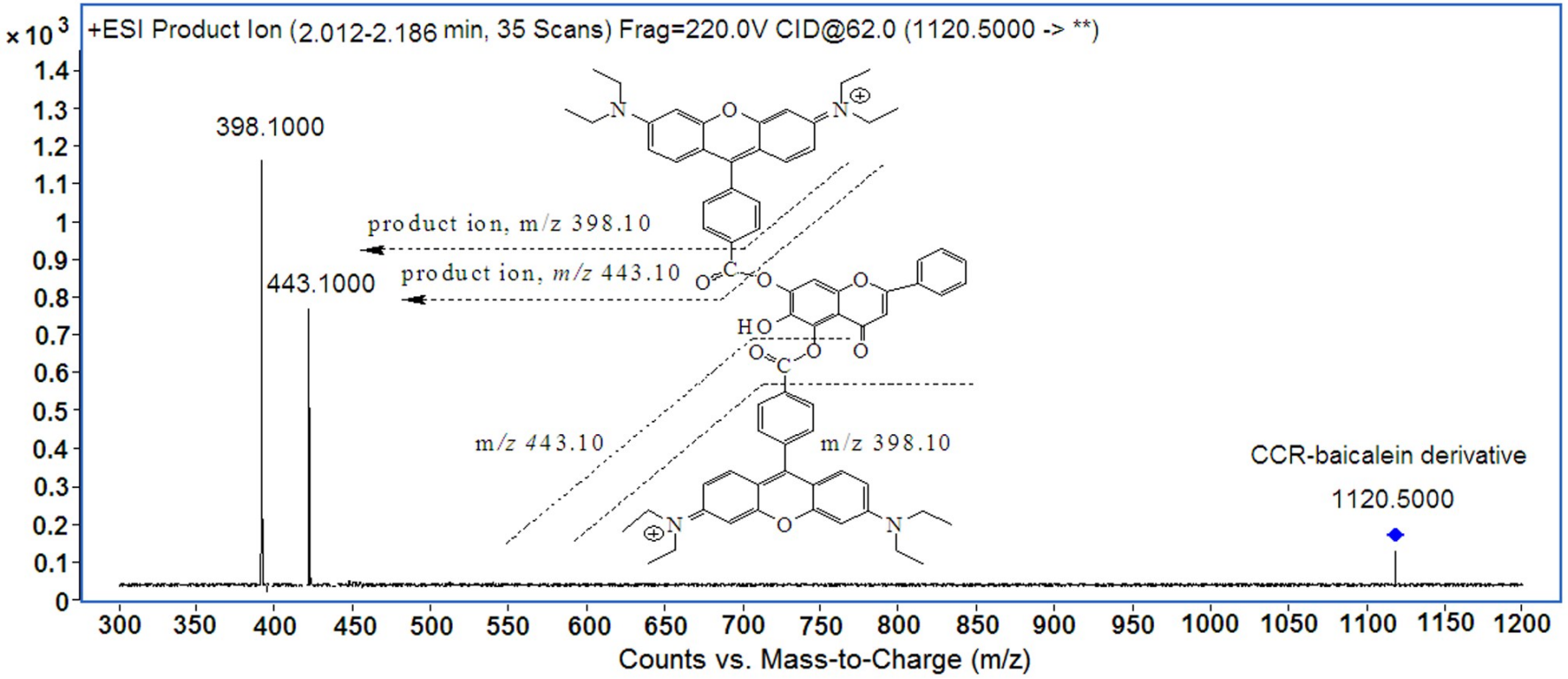


Fig. S3 (A) Representative MRM chromatogram of baicalein and (B) product ion spectrum and proposed fragmentation schematics of CCR-baicalein derivative

Table S2 The dynamic concentration changes of NTs and DHBA in rat brain microdialysates of two rat groups (mean ± SD, *n* = 8)

Analytes	Normal group (μM)	PD Stage A (μM)	PD Stage B (μM)	PD Stage C (μM)
L-DOPA	0.130 ± 0.102	$0.065 \pm 0.007^{\Delta\Delta\Delta}$	$0.109 \pm 0.07^{\#\#\#}$	$0.117 \pm 0.009^{\#\#\#}$
DA	0.030 ± 0.007	$0.012 \pm 0.004^{\Delta\Delta\Delta}$	$0.026 \pm 0.003^{\#\#\#}$	$0.029 \pm 0.004^{\#\#\#}$
NE	0.015 ± 0.005	$0.010 \pm 0.002^{\Delta\Delta\Delta}$	$0.013 \pm 0.005^{\#\#\#}$	$0.014 \pm 0.002^{\#\#\#}$
E	0.018 ± 0.002	$0.011 \pm 0.003^{\Delta}$	$0.015 \pm 0.006^{\#}$	$0.015 \pm 0.005^{\#\#\#}$
3-MT	0.015 ± 0.003	$0.008 \pm 0.001^{\Delta\Delta\Delta}$	$0.012 \pm 0.005^{\#\#\#}$	$0.013 \pm 0.004^{\#\#\#}$
NME	0.015 ± 0.005	$0.008 \pm 0.004^{\Delta\Delta\Delta}$	$0.013 \pm 0.002^{\#\#\#}$	$0.013 \pm 0.002^{\#\#\#}$
ME	0.015 ± 0.007	$0.012 \pm 0.005^{\Delta}$	$0.014 \pm 0.004^{\#}$	$0.014 \pm 0.002^{\#}$
DOPAC	0.105 ± 0.004	$0.039 \pm 0.028^{\Delta\Delta\Delta}$	$0.070 \pm 0.009^{\#}$	$0.070 \pm 0.011^{\#\#\#}$
HVA	0.035 ± 0.008	$0.017 \pm 0.009^{\Delta\Delta\Delta}$	$0.027 \pm 0.005^{\#\#\#}$	$0.028 \pm 0.009^{\#\#\#}$
DOMA	0.034 ± 0.005	$0.024 \pm 0.012^{\Delta}$	$0.031 \pm 0.008^{\#}$	$0.032 \pm 0.007^{\#}$
DHPG	0.009 ± 0.002	$0.005 \pm 0.001^{\Delta}$	$0.007 \pm 0.002^{\#}$	$0.008 \pm 0.001^{\#}$
VMA	0.042 ± 0.009	$0.022 \pm 0.007^{\Delta\Delta\Delta}$	$0.035 \pm 0.007^{\#}$	$0.036 \pm 0.003^{\#\#\#}$
MHPG	0.011 ± 0.003	$0.008 \pm 0.001^{\Delta}$	$0.010 \pm 0.003^{\#}$	$0.010 \pm 0.002^{\#}$
5-HT	0.031 ± 0.017	$0.015 \pm 0.008^{\Delta\Delta\Delta}$	$0.025 \pm 0.007^{\#\#\#}$	$0.028 \pm 0.009^{\#\#\#}$
5-HIAA	0.025 ± 0.009	$0.013 \pm 0.007^{\Delta\Delta\Delta}$	$0.020 \pm 0.002^{\#}$	$0.021 \pm 0.007^{\#\#\#}$
Glu	2.145 ± 0.87	$3.163 \pm 1.25^{\Delta\Delta\Delta}$	$2.411 \pm 1.01^{\#\#\#}$	$2.361 \pm 0.74^{\#\#\#}$
GABA	3.374 ± 1.58	$2.765 \pm 1.01^{\Delta}$	$3.062 \pm 1.03^{\#}$	$3.170 \pm 1.14^{\#}$
Gly	5.333 ± 1.46	7.524 ± 1.73	$5.762 \pm 1.54^{\#}$	$5.758 \pm 1.39^{\#}$
Asp	8.617 ± 2.11	$11.013 \pm 2.31^{\Delta\Delta\Delta}$	$9.363 \pm 2.14^{\#\#\#}$	$9.248 \pm 1.58^{\#\#\#}$
2,3-DHBA	1.245 ± 0.32	$2.305 \pm 1.01^{\Delta\Delta\Delta}$	$2.014 \pm 0.58^{\#\#\#}$	$2.475 \pm 0.69^{\#\#\#}$
2,5-DHBA	1.556 ± 0.21	$3.083 \pm 0.58^{\Delta\Delta\Delta}$	$1.628 \pm 0.45^{\#\#\#}$	$1.532 \pm 0.32^{\#\#\#}$

$^{\Delta}P < 0.05$ or $^{\Delta\Delta\Delta}P < 0.05$ compared with normal group, $^{\#}P < 0.05$ or $^{\#\#\#}P < 0.01$ compared with PD group with 6-OHDA treatment.