

Comparative Analysis of Hydrophilic Ingredients in Toad Skin and Toad Venom Using the UHPLC-HR-MS/MS and UPLC-QqQ-MS/MS Methods Together with the Anti-Inflammatory Evaluation of Indolealkylamines

Yu Zhang ¹, Bo Yuan ², Norio Takagi ², Hongjie Wang ¹, Yanyan Zhou ¹, Nan Si ¹, Jian Yang ¹, Xiaolu Wei ¹, Haiyu Zhao ^{1,*} and Baolin Bian ^{1,*}

¹ Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China; zhangyuddnh@163.com (Y.Z.); hjwang@icmm.ac.cn (H.W.); yyzhou@icmm.ac.cn (Y.Z.); nsi@icmm.ac.cn (N.S.); jyang@icmm.ac.cn (J.Y.); xlwei@icmm.ac.cn (X.W.)

² Department of Applied Biochemistry, Tokyo University of Pharmacy & Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan; yuanbo@toyaku.ac.jp (B.Y.); takagino@toyaku.ac.jp (N.T.)

* Correspondence: hyzhao@icmm.ac.cn (H.Z.); blbian@icmm.ac.cn (B.B.); Tel.: +136-610-784-32 (H.Z.); +139-012-278-22 (B.B.)

Table S1. Parameters of UPLC-QqQ-MS/MS analysis for eleven compounds

Compounds	Rt /min	MRM				
		Q1/Q3 ion ^a (<i>m/z</i>)	DP/V	CE/eV	CXP/V	EP/V
Valine	1.08	118.0/72.1	30	11	11	10
Adenine	1.39	136.1/119.2	34	31	16	10
Nicotinic acid	1.68	124.0/80.0	102	31	13	10
Hypoxanthine	2.10	137.2/120.1	100	32	7	10
Xanthine	2.68	153.0/136.2	149	25	15	10
Serotonin	4.44	177.1/160.2	92	14	10	10
N-methyl serotonin	4.95	191.0/160.1	45	15	11	10
Bufotenidine	5.35	219.2/160.1	42	23	25	10
Bufotenine	5.72	205.1/160.1	24	24	14	10
Dehydrobufotenine	7.30	203.0/188.1	102	31	17	10
Bufothionine	7.72	283.0/203.2	130	21	16	10
Diazepam	8.72	284.9/193.1	167	43	12	10
Ion spray voltage (IS) /V			5500			
Ion source (GS1) setting/Psi			55			
Ion source (GS2) setting/Psi			55			

^aQ1: precursor ion selected in Q1; Q3: product ion selected in Q3;

Table S2. The regression equation, LOD and LOQ of the 11 standards in toad skin samples

Analyte	Regression equations	<i>R</i>	Linear range ($\mu\text{g/mL}$)	LOD (ng/mL)	LOQ (ng/mL)	Repeatability ($n=6$)	Stability ($\text{RSD}\%$)	Intra-day precision ($\text{RSD}\%$, $n=6$)	Inter-day precision ($\text{RSD}\%$, $n=3$)
Valine	$Y=3.8355\times 10^6X+2.4571\times 10^6$	0.9997	0.21-43.48	0.0095	0.047	2.69	2.05	2.15	2.33
Adenine	$Y=1.0274\times 10^7X+1080200$	0.9997	0.15-6.05	0.33	3.30	0.71	2.95	1.15	1.95
Nicotinic acid	$Y=881906X+2016.4$	0.9995	0.11-4.60	2.30	11.50	2.37	1.24	1.50	1.84
Hypoxanthine	$Y=2.200\times 10^6X+3688.9$	0.9995	0.23-93.75	2.34	11.72	2.93	2.69	2.09	2.39
Xanthine	$Y=4400200X+2335.1$	0.9994	0.42-42.25	0.99	4.95	3.21	3.35	2.64	3.05
Serotonin	$Y=1.1181\times 10^6X+1.5718\times 10^6$	0.9988	2.38-38.20	24.76	49.52	1.37	2.24	0.25	3.38
N-methyl serotonin	$Y=1.97615\times 10^7X+713317$	0.9999	3.00-48.00	7.74	38.71	2.44	3.31	1.59	3.01
Bufotenidine	$Y=6.5895\times 10^7X+387000$	0.9995	23.37-374.00	1.30	6.50	1.36	2.34	2.26	2.76
Bufotenine	$Y=4.1411\times 10^6X+1395.8$	0.9989	12.93-414.00	25.88	103.52	2.90	3.46	1.30	3.19

Dehydrobufotenine	$Y=3.2817 \times 10^8 X + 154440$	0.9991	3.00-48.00	0.0069	0.069	2.56	3.38	1.26	1.68
Bufothionine	$Y=4.3126 \times 10^7 X + 1.1772 \times 10^5$	0.9995	3.07-153.75	0.77	3.84	2.08	2.29	0.66	1.49

Table S3. The regression equation, LOD and LOQ of the eleven standards in toad venom samples

Analyte	Regression equations	<i>R</i>	Linear range ($\mu\text{g/mL}$)	LOD (ng/mL)	LOQ (ng/mL)	Repeatability ($n=6$)	Stability ($\text{RSD}\%$)	Intra-day precision ($\text{RSD}\%$, $n=6$)	Inter-day precision ($\text{RSD}\%$, $n=3$)
Valine	$Y=5.3156 \times 10^6 X + 1.1318 \times 10^6$	0.9997	0.043-1.08	0.0095	0.047	1.04	2.85	2.15	2.33
Adenine	$Y=1.0274 \times 10^7 X + 1080200$	0.9997	0.15-6.05	0.33	3.30	1.87	3.31	1.15	1.95
Nicotinic acid	$Y=5.1613 \times 10^6 X + 8.2313 \times 10^4$	0.9995	0.023-1.15	2.30	11.50	2.69	2.72	1.50	1.84
Hypoxanthine	$Y=818537X + 167954$	0.9985	0.023-3.75	2.34	11.72	2.20	2.49	2.09	2.39
Xanthine	$Y=4129000X + 3264.3$	0.9999	0.016-8.45	0.99	4.95	2.34	1.62	2.64	3.05
Serotonin	$Y=1.3172 \times 10^6 X + 2.6083 \times 10^5$	0.9999	0.11-4.77	24.76	49.52	1.32	2.72	0.25	3.38
N -methyl serotonin	$Y=1.9476 \times 10^7 X + 13650$	0.9999	30.00-1920.00	7.74	38.71	1.98	2.00	1.59	3.01
Bufotenidine	$Y=6.5895 \times 10^7 X + 387000$	0.9995	233.70-3740.00	1.30	6.50	2.10	1.25	2.26	2.76
Bufotenine	$Y=4.1411 \times 10^6 X + 1395.8$	0.9989	129.37-1035.00	25.88	103.52	2.93	2.45	1.30	3.19

Dehydrobufotenine	$Y=4.7837 \times 10^6 X - 12281$	0.9995	60.00-480.00	0.0069	0.069	2.34	2.87	1.26	1.68
Bufothionine	$Y=3.3481 \times 10^7 X + 1.0016 \times 10^6$	0.9998	0.03-3.07	0.77	3.84	0.36	2.77	0.66	1.49

Table S4. Recovery of eleven compounds in toad skin ($n=3$)

Analyte	Initial amount (μg)	Added amount (μg)	Total recovered amount(μg)	Recovery (%)	RSD (%)
Valine	19.9426	15.9859	36.1297	101.2583 \pm 3.0115	2.97
	19.9028	19.9824	40.2451	101.8012 \pm 0.5798	0.56
	19.9824	23.9789	43.5476	98.2748 \pm 2.1667	2.20
Adenine	1.2409	0.9969	2.2281	99.0232 \pm 1.2490	1.26
	1.2384	1.2461	2.4996	101.2111 \pm 2.7558	2.72
	1.2434	1.4953	2.7646	101.7285 \pm 2.0047	1.97
Nicotinic acid	3.1768	2.5219	5.7724	102.9229 \pm 1.6638	1.61
	3.1704	3.1524	6.3636	101.2923 \pm 1.9745	1.94
	3.1831	3.7829	6.8594	97.1825 \pm 1.0219	1.05
Hypoxanthine	17.3134	13.9372	30.9724	99.3258 \pm 1.7687	1.78
	17.2788	17.4215	34.8688	96.6490 \pm 0.6166	0.63
	17.3479	20.9058	38.2977	102.3880 \pm 2.0592	2.01
Xanthine	12.2107	9.7004	21.8125	103.0415 \pm 0.3191	0.31
	12.1863	12.1255	24.3112	103.2720 \pm 1.2903	1.24
	12.2350	14.5506	26.7197	101.7960 \pm 1.1414	1.12
Serotonin	12.4092	9.8867	22.3880	100.9306 \pm 2.6421	2.61
	12.3845	12.3584	25.0499	102.4840 \pm 1.3794	1.34
	12.4340	14.8301	27.4797	101.4540 \pm 3.1334	3.08
N -methyl serotonin	10.2749	8.3142	18.3882	97.5843 \pm 1.4335	1.46
	10.2544	10.3927	20.9582	102.9942 \pm 0.3299	0.29
	10.2953	12.4712	23.0443	102.2271 \pm 0.4390	0.42
Bufotenidine	102.2819	81.0846	186.4367	103.7865 \pm 0.8486	0.82

	102.0779	101.3557	202.7663	99.3417±1.2894	1.29
	102.4859	121.6268	226.1888	101.7068±0.8010	0.78
Bufotenine	47.5919	38.4235	87.7697	104.5655±0.3481	0.33
	47.4970	48.0294	95.6302	100.2161±0.7315	0.73
	47.6869	57.6353	104.3142	98.2512±1.3342	1.35
Dehydrobufotenine	22.6642	16.5515	38.9057	98.1267±2.0924	2.13
	22.6190	20.6894	43.3865	100.3772±1.7402	1.73
	22.7094	24.8273	48.4912	103.8443±1.5376	1.48
Bufothionine	54.3128	44.0750	99.4469	102.4029±2.6966	2.63
	54.2044	55.0938	107.1919	96.1876±0.0893	0.09
	54.4211	66.1126	118.4191	96.8016±0.6902	0.71

Table S5. Recovery of eleven compounds in toad venom (*n*=3)

Analyte	Initial amount (μg)	Added amount (μg)	Total recovered amount(μg)	Recovery (%)	RSD (%)
Valine	0.2507	0.2010	0.4457	97.0180 \pm 1.6119	1.66
	0.2467	0.2513	0.5000	100.7959 \pm 2.8695	2.84
	0.2508	0.3016	0.5499	99.1732 \pm 3.0881	3.11
Adenine	4.0673	3.3012	7.3935	100.7571 \pm 2.5815	2.56
	4.0131	4.1265	8.2363	102.3448 \pm 1.7186	1.67
	4.0809	4.9518	9.1164	101.6906 \pm 2.2498	2.21
Nicotinic acid	0.2212	0.1751	0.3905	96.7029 \pm 1.2627	1.30
	0.2182	0.2189	0.4314	97.3785 \pm 1.0159	1.04
	0.2219	0.2627	0.4735	95.8006 \pm 1.2395	1.29
Hypoxanthine	0.2212	0.1751	0.3905	99.9552 \pm 3.3.2176	3.21
	0.2182	0.2189	0.4314	101.7055 \pm 0.9623	0.94
	0.2219	0.2627	0.4735	102.5305 \pm 2.9664	2.89
Xanthine	1.7115	1.3219	3.0640	98.5530 \pm 2.8588	2.90
	1.6887	1.6524	3.3693	99.4378 \pm 2.2129	2.22
	1.7172	1.9829	3.7503	97.1840 \pm 2.0844	2.14
Serotonin	0.8173	0.6283	1.4461	100.0699 \pm 1.7300	1.72
	0.8064	0.7854	1.5732	97.6347 \pm 2.1333	2.18
	0.8200	0.9425	1.7311	96.6671 \pm 2.3877	2.47
N -methyl serotonin	141.5192	111.3772	252.0478	99.2381 \pm 0.8129	0.82
	139.6323	139.2215	282.2270	102.4229 \pm 1.0194	0.99
	141.9910	167.0658	308.9295	99.9238 \pm 2.2751	2.27
Bufotenidine	1292.2692	1030.5726	2330.4032	100.7337 \pm 3.0255	3.00

	1275.0390	1288.2158	2553.2371	99.2224±1.3035	1.31
	1296.5768	1545.859	2848.4041	100.3861±1.0842	1.08
Bufotenine	495.9808	384.7958	881.5789	100.2085±1.0827	1.08
	489.3677	480.9947	968.6293	99.6397±2.9791	2.98
	497.6340	577.1936	1064.7046	98.2462±1.1273	1.14
Dehydrobufotenine	237.5481	184.3548	425.5484	101.9774±1.2692	1.24
	234.3808	230.4435	466.2928	100.6373±1.5951	1.58
	238.3399	276.5322	513.0391	99.3372±0.7294	0.73
Bufothionine	1.9904	1.5723	3.5007	96.0586±2.4862	2.58
	1.9638	1.9654	3.9211	99.5872±2.7544	2.76
	1.9970	2.3585	4.3017	97.7189±1.2760	1.30

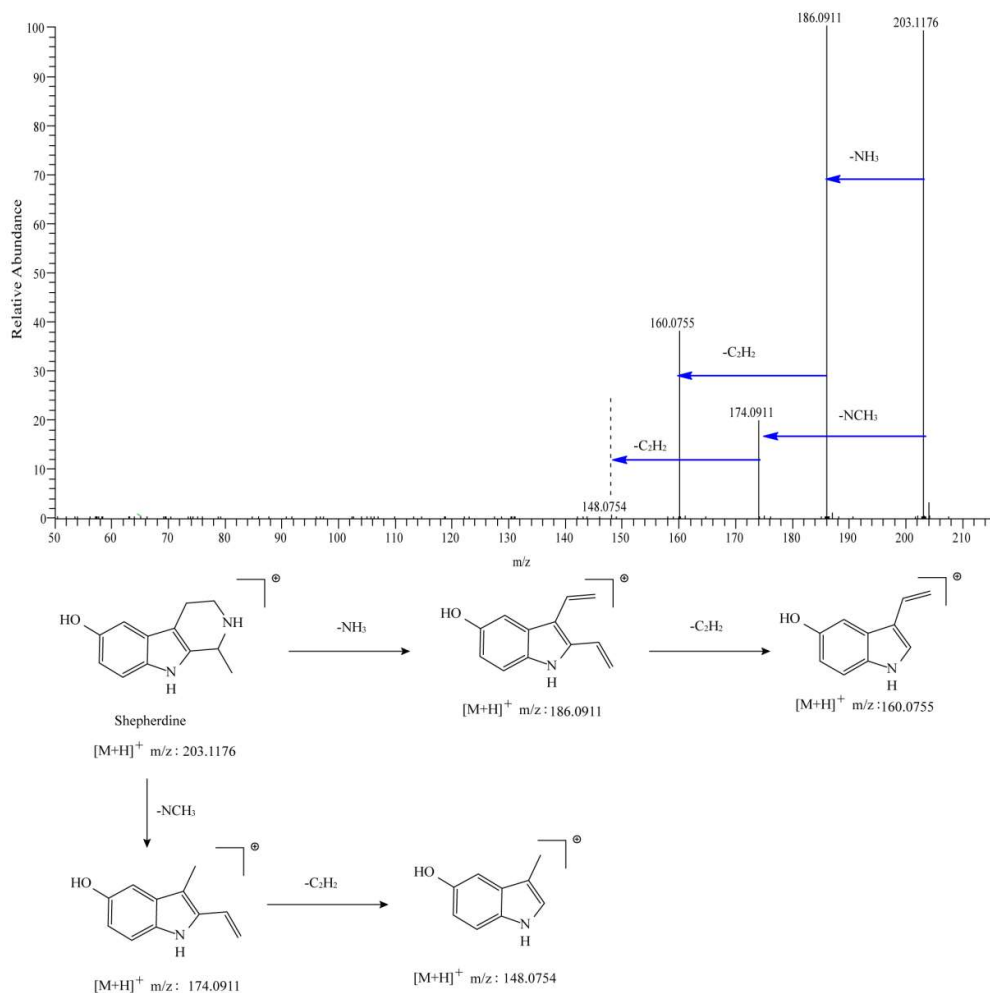


Figure S1. The MS/MS spectrum and fragmentation patterns of shepherdine

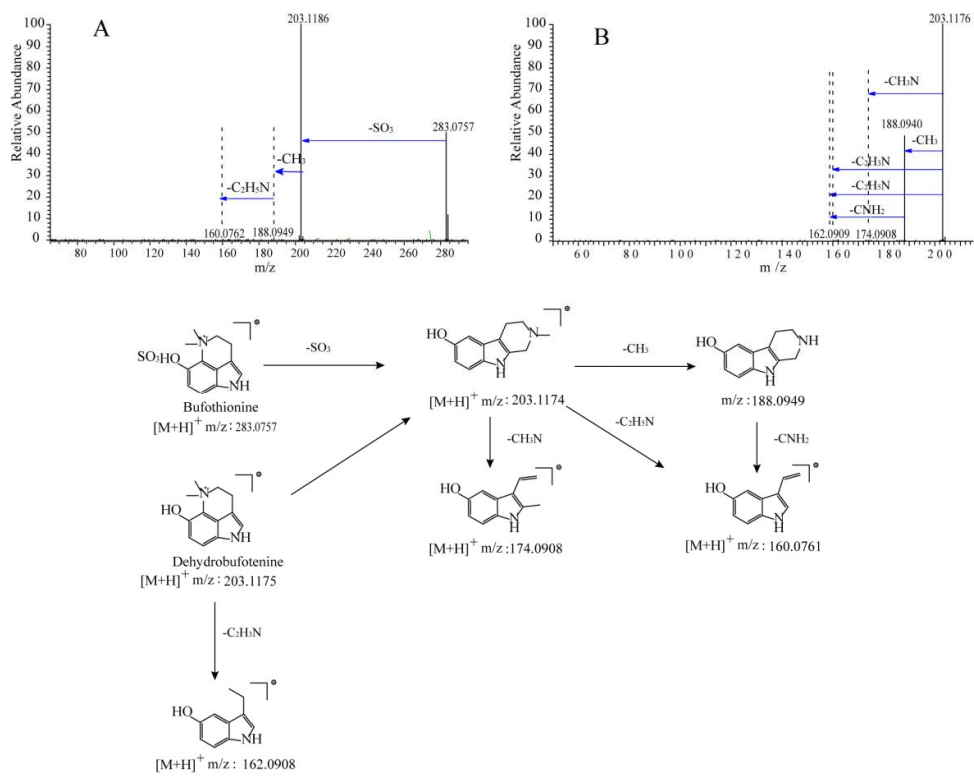


Figure S2. The MS/MS spectrum and fragmentation patterns of A: bufothionine; B: dehydrobufotenin

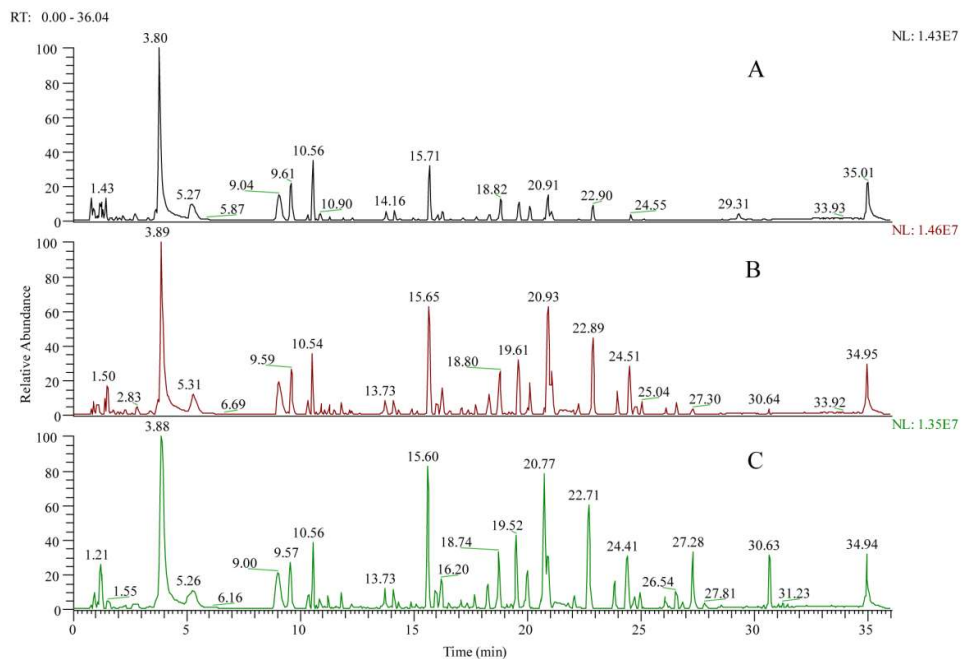


Figure S3. Optimized extraction conditions selected from three extraction solvents. A: water; B: 50% methanol; C: 50% ethanol.

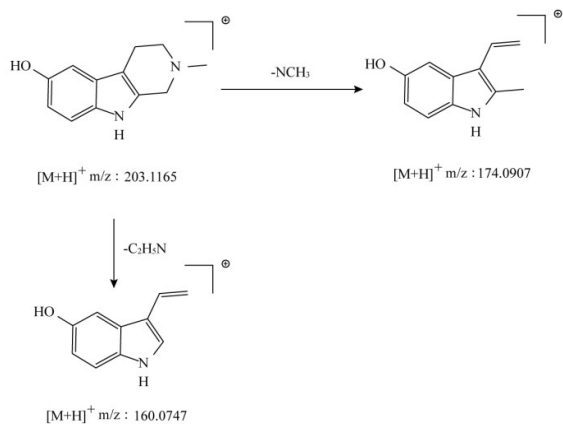
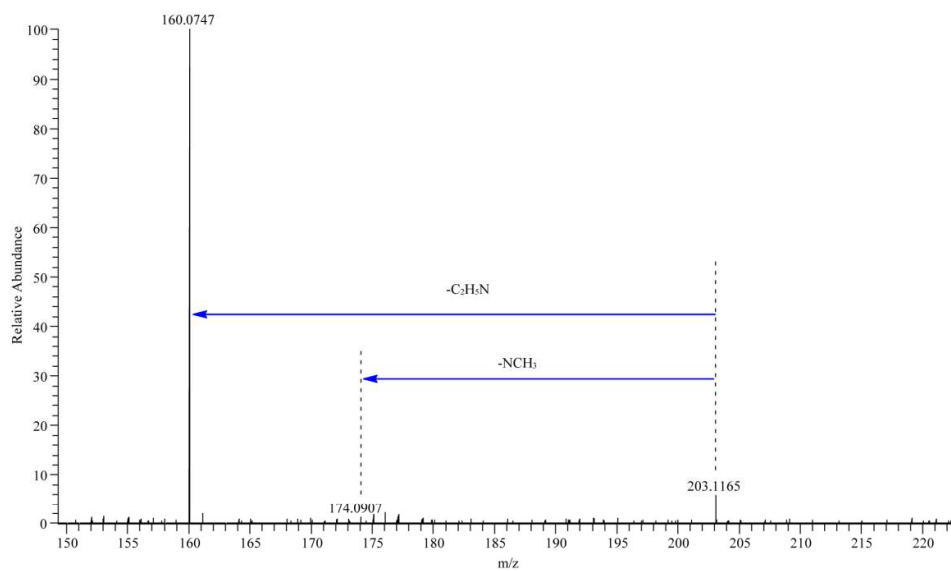


Figure S4. The MS/MS spectrum and fragmentation patterns of peak 19

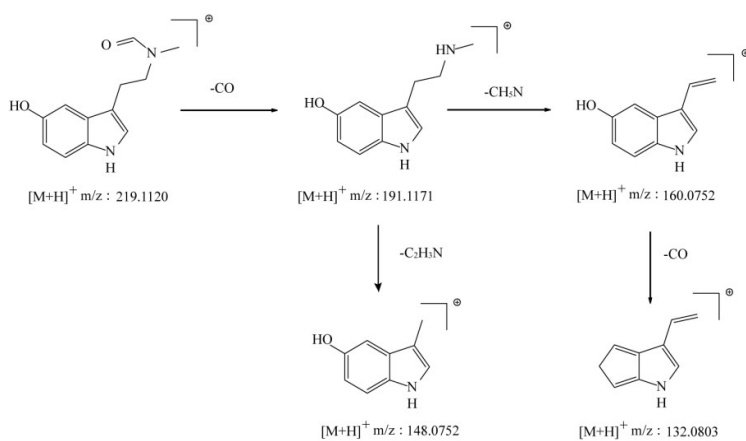
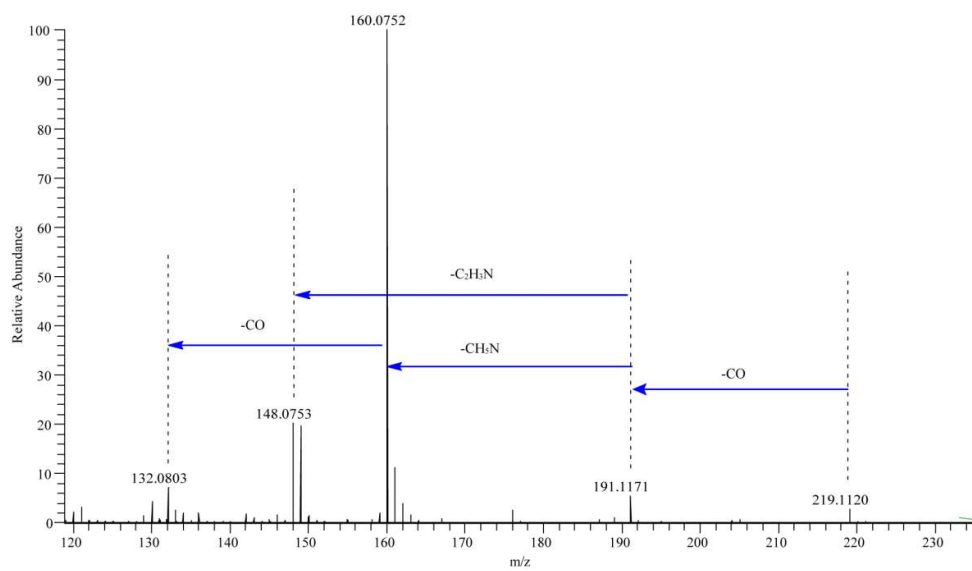


Figure S5. The MS/MS spectrum and fragmentation patterns of peak 20

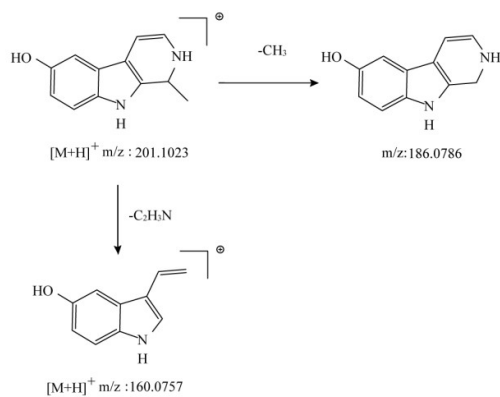
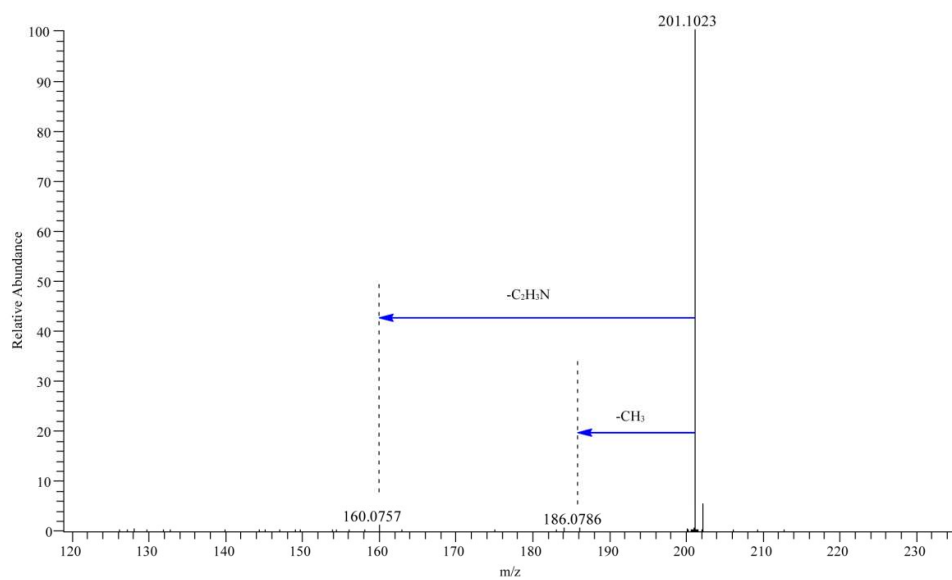


Figure S6 The MS/MS spectrum and fragmentation patterns of peak **31**