Supporting Information for

Simultaneous analysis of coumarin derivatives in extracts of Radix angelicae pubescentis (Duhuo) by HPLC-DAD-ESI-MS^n technique

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Materials and instruments: Psoralen(**3**), osthole(**21**) and isoimperatorin(**23**) (purity>98%) were purchased from the National Institute for the China Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Umbelliferone(**1**), xanthotoxin(**5**), bergapten(**12**) and columbianadin(**22**) (purity>98%) obtained from Tianjin Shilan Technology Co., Ltd (Tianjin, China).

HPLC grade methanol was procured from Merck (Darmstadt, Germany). Formic acid (analytical grade) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Water for HPLC analysis was purified by a Milli Q water purification system (Millipore Corporation, MA, USA). The analytical grade methanol, acetonitrile, petroleum ether and chloroform used for extraction was from Tianjin Reagent Company (Tianjin, China). PTFE membrane filter (0.45 μm) was purchased from Waters Co. (Milford, MA). The samples was weighed in electronic balance (BP211D, Sartorius, Germany).

Duhuo (Dried roots of Angelica pubescens Maxim. f. biserrata Shan et Yuan) was purchased from Hubei province of China and was authenticated by Professor Mei Meng. The voucher specimen (DH-13-1002) was deposited at Key Laboratory of Chinese Medicine Research and Development in Anhui Province.

Standard solutions and sample preparation: Duhuo was ground into powder, passed through a 60 mesh sieve and dried at 50 ℃ for 6 h. An aliquot of 0.5 g of the dried sample was ultrasonically extracted using 10 ml of chloroform-methanol (1:1, v/v) for 30 min. The extractive of Duhuo was filtered and hence the sample solution was obtained. A stock solution containing seven standards (umbelliferone (**1**), psoralen (**3**), xanthotoxin (**5**), bergapten (**12**), osthole (**21**), columbianadin (**22**) and isoimperatorin (**23**)) was prepared in methanol and diluted with methanol to an appropriate concentration. All solutions were stored in the refrigerator at 4 °C and were filtered through a 0.45 μm syringe filter before use.

HPLC-DAD conditions: The HPLC system (Finnigan-Thermo Fisher Electron Corporation, San Jose, CA, USA) was equipped with a quaternary pump (Model Finnigan Surveyor Plus), an autosampler (Model Finnigan Surveyor Plus with 200-vial capacity sample) including a column oven controller, which was connected in a photo diode array detector (DAD) (Finnigan Surveyor Plus) quantitative analyzing and UV spectra acquisition. The chromatographic separation was performed on a C_{18}ultimate XB-C_{18} column (5 μm, ø 4.6 mm × 250 mm, Welch, USA). The mobile phase was methanol (A) and water with 0.1% formic acid (B). A gradient program was as follows: 0-5 min, 35% A; 6-20 min, 49% A; 25-35 min, 65% A; 40-50 min, 90% A. The beginning gradient was held for 10 min. The flow rate was 1.0 mL/min. The injection volume was 10 μL and the column temperature was maintained at 25 °C. The UV spectra was recorded from 200 nm to 400 nm. The detection wavelength was set at 246 nm, 274 nm and 320 nm. The chromatographic data were recorded and processed with an Xcalibur 2.0 chemstation workstation (Finnigan, San Jose, USA).

HPLC-ESI-MS^n Conditions: Identification of Duhuo extracts was confirmed by HPLC online coupled with the electrospray ionization (ESI) source of an ion trap mass spectrometer (MS)(Finnigan LCQ Advantage, San Jose, CA, USA). The chromatographic conditions were the same as HPLC-DAD analysis described above, by solvent splitting, 0.3 mL/min portion of the column effluent was delivered into the ion source of mass spectrometry. A ion-trap mass spectrometer equipped with a electrospray ionization source and Xcalibur software version 2.0 was used for data acquisition and processing. Optimal operating parameters of the ESI interface and quadropole/ion trap were found by infusing seven standard solutions above, the mobile phase at 300 μL/min using a Finnigan syringe pump. Ultrahigh pure helium (He) was used as the collision gas and high purity nitrogen (N_2) as the nebulizing gas. The optimum conditions of the interface were applied as follows: capillary temperature of 300 °C; sheath gas pressure and auxiliary gas pressure of 35 arb and 10 arb; spray, capillary, and tube lens voltages of 4.5 kV, 14V and 45V. For full scan

MS analysis, the total analysis time was 50 min. The mass spectrometer was operated with a scan range of m/z 150 to m/z 800 in the positive ion mode. MS^2 and MS^n (n=3-4) analyses were performed by the collision of the precursor ions with helium gas. The energy values of collision-induce dissociation (CID) were automatically selected.

Table S1. The extraction result of Duhuo samples with different solvents for 30 min

Extraction solvent	Ratio of Duhuo extract to sample ($mg \cdot g^{-1}$)	Peak area of osthole (%)
Petroleum ether	53.112	5.865
Chloroform	103.564	11.356
Methanol	129.689	12.587
Acetonitrile	117.856	9.467
Chloroform-methanol (1:1, v/v)	152.367	16.356
Chloroform-acetonitrile (1:1, v/v)	138.679	12.956

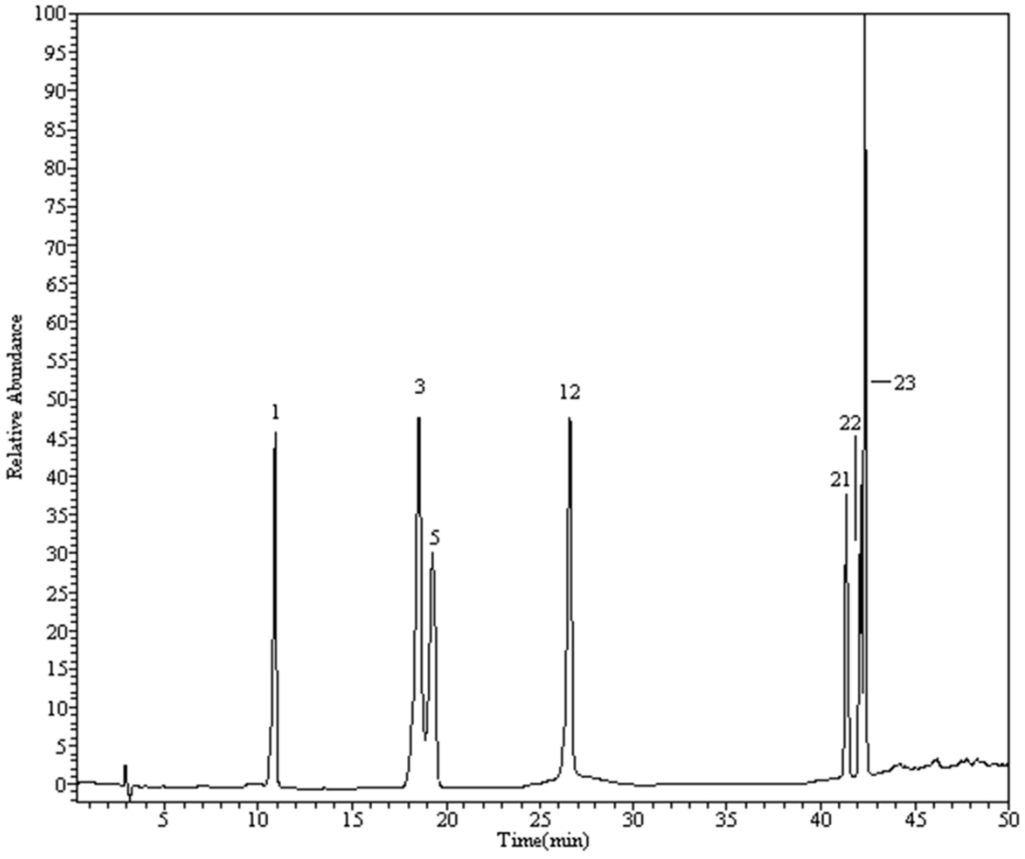


Figure S1. The HPLC chromatogram of seven standards

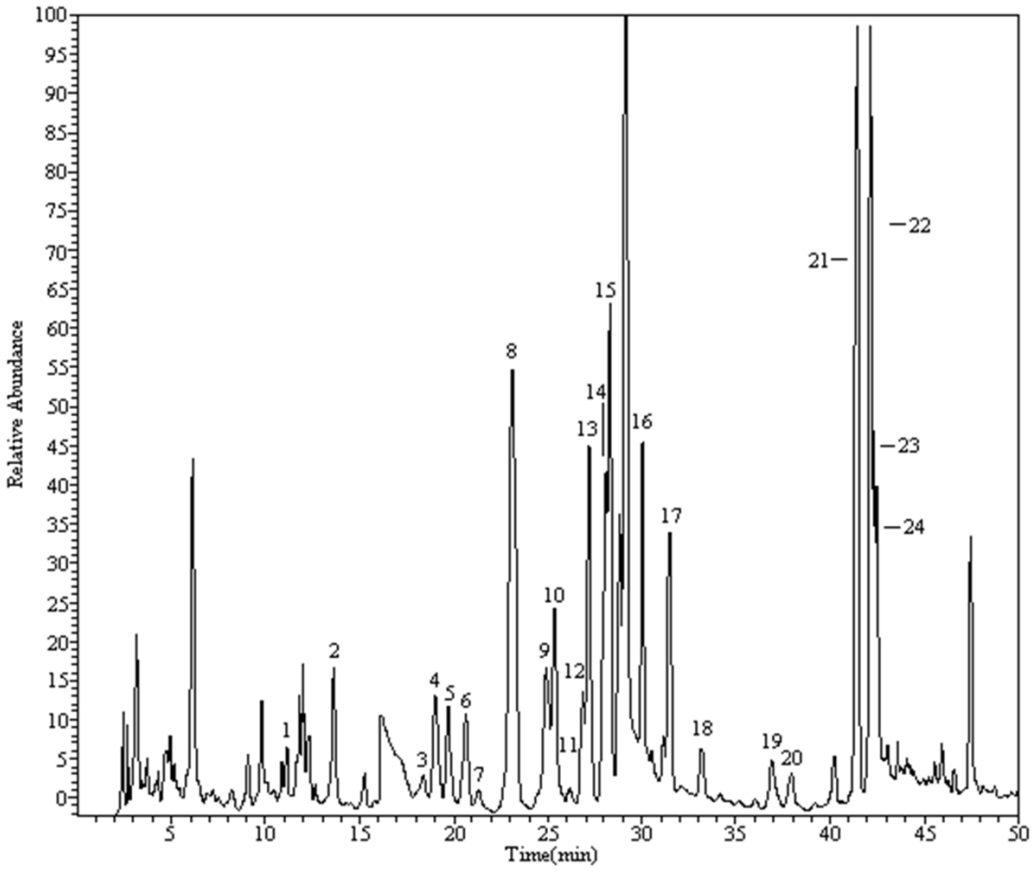


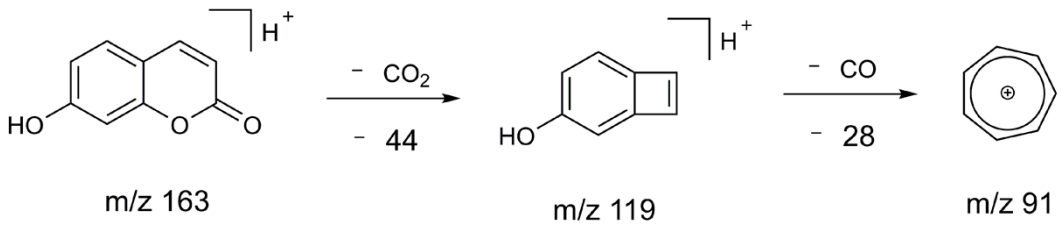
Figure S2.HPLC-DAD chromatogram of simultaneous separation of Duhuo extracts

Table S2.HPLC-DAD-ESI-MS^n data and identification of 24 coumarin constituents in

Duhuo extracts

Peak	t_R(min)	(+)ESI-MS (m/z)	HPLC-ESI(+)-MS^n experiment m/z(% base peak)	HPLC-DAD λ_{max}(nm)	Identification
1	11.21	163 $[M+H]^+$ 180 $[M+NH_4]^+$	MS^2[163]:119(100),91(85) MS^3[119]:91(100)	204,218, 327	Umbelliferone
2	13.67	409$[M+H]^+$	MS^2[409]:247 (100) MS^3[247]:229(93),201(30),187(38),175(100) MS^4[175]:147(100),119(36)	253,262, 331	β-D-glucosyl-columbianetin
3	19.01	187$[M+H]^+$	MS^2[187]:143(100) MS^3[143]:115(100)	210,247, 301,330	Psoralen
4	19.21	247$[M+H]^+$	MS^2[247]:229(100),175(95) MS^3[175]:147(100) MS^4[147]:119(100),103(86) MS^3[229]:187(100),175(40) MS^4[201]:173(100),159(10)	202,253, 261,331	Columbianetin
5	19.78	217$[M+H]^+$	MS^2[217]:202(100),189(23),185 (40) MS^3[202]:174(100) MS^4[174]:146 (100),117(38)	203,220, 250,304	Xanthotoxin
6	20.72	377$[M+H]^+$ 359$[M+H-H_2O]^+$	MS^2[377]:345(21),301(58),277(17),205(100) MS^3[205]:175(100) MS^4[175]:147(100) MS^2[359]:301(100),219(40) MS^3[301]:219(100) MS^4[219]:219(100),191(35)	205,224, 332	Angelol A
7	21.38	305$[M+H]^+$	MS^2[305]:203(100)MS^3[203]:175(51), 159(100),131(60) MS^4[159]:131(100)	202,249, 319	Oxypeucedanin hydrate
8	23.21	377$[M+H]^+$ 359$[M+H-H_2O]^+$	MS^2[377]:345(7),301(22),277(28),205(100) MS^3[205]:175(100) MS^4[175]:147(100) MS^2[359]:301(100),219(43) MS^3[301]:219(100) MS^4[219]:219(100),191(50)	207,223, 332	Isoangelol
9	25.02	379$[M+H]^+$ 361$[M+H-H_2O]^+$	MS^2[379]:347(5),303(15),277(36),205(67) MS^3[205]:175(100) MS^4[175]:147(100) MS^2[361]:303(100),219(18) MS^3[303]:219(100) MS^4[219]:219(100),191(55)	204,223, 332	Anpubesol
10	25.44	379$[M+H]^+$ 361$[M+H-H_2O]^+$	MS^2[379]:347(8),303(23),277(13),205(70) MS^3[205]:175(100) MS^4[175]:147(100) MS^2[361]:303(100),219(20) MS^3[303]:219(100) MS^4[219]:219(100),191(45)	203,223, 331	Angelol C
11	25.81	247$[M+H]^+$	MS^2[247]:232(100) MS^3[232]:217(100) MS^4[217]:189(100),161(15)	201,288, 327	Isopimpinellin
12	26.95	217$[M+H]^+$	MS^2[217]:202(100) MS^3[202]:174(100)	201,223,	Bergapten

			MS^4[174]:146(100)	270,320	
13	27.29	359[M+H]$^+$	MS^2[359]:301(100),219(42) MS^3[301]:273(30),219(100) MS^4[219]:219(100),191(30)	225,330	Angelol Adehydration
14	28.14	361[M+H]$^+$	MS^2[361]:303(100),219(21) MS^3[303]:219(100) MS^4[219]:219(100),191(52)	210,224, 257,329	Anpubesol dehydration
15	28.35	361[M+H]$^+$	MS^2[361]:303(100),219(23) MS^3[303]:219(100) MS^4[219]:219(100),191(50)	209,225, 329	Angelol Cdehydration
16	30.09	289[M+H]$^+$ 311[M+Na]$^+$	MS^2[289]:229(100)MS^3[229]:187(100), 175(18) MS^4[187]:159(100),143(27),131(40)	253,262, 330	Columbianetin acetate
17	31.56	231[M+H]$^+$	MS^2[231]:175 (88),149(80),135(100) MS^3[175]:147(100) MS^3[149]:131(100),121(63) MS^3[135]:107(100) MS^4[147]:119(84),103(100)	201,249	Osthenol
18	33.25	229[M+H]$^+$	MS^2[229]:187(100),175(55) MS^3[187]:159(100),143(55) MS^4[159]:131(100)	202,331	Angenomalin
19	36.98	303[M+H]$^+$ 325[M+Na]$^+$	MS^2[303]:229(100) MS^3[229]:187(100),175(28) MS^4[187]:159(100),143(57)	202,331	Columbianetin propionate
20	37.89	359[M+H]$^+$	MS^2[359]:301(100),259(18),219(45) MS^3[301]:273,(20),219(100) MS^4[219]:219(100),191(45)	225,330	Isoangelol dehydration
21	41.45	245[M+H]$^+$	MS^2[245]:189(100) MS^3[189]:161(100),159(76) MS^4[161]:133(100)	207,252, 259,326	Osthole
22	42.20	329[M+H]$^+$ 351[M+Na]$^+$	MS^2[329]:229(100) MS^3[229]:201(43),187(100),175(34) MS^4[187]:159(100),143(42),131(35)	206,263, 330	Columbianadin
23	42.48	271[M+H]$^+$	MS^2[271]:203(100) MS^3[203]:175(42),159(100) MS^4[159]:131(100)	205,252, 261,313	Isoimperatorin
24	42.61	331[M+H]$^+$ 353[M+Na]$^+$	MS^2[331]:229(100) MS^3[229]:201(35),187(100),175(10) MS^4[187]:159(100),143(35),131(32)	204,252, 262,330	Dihydrocolumbianadin



Scheme S1. Proposed MS fragmentation pathway for the $[M+H]^+$ ion of Peak **1**

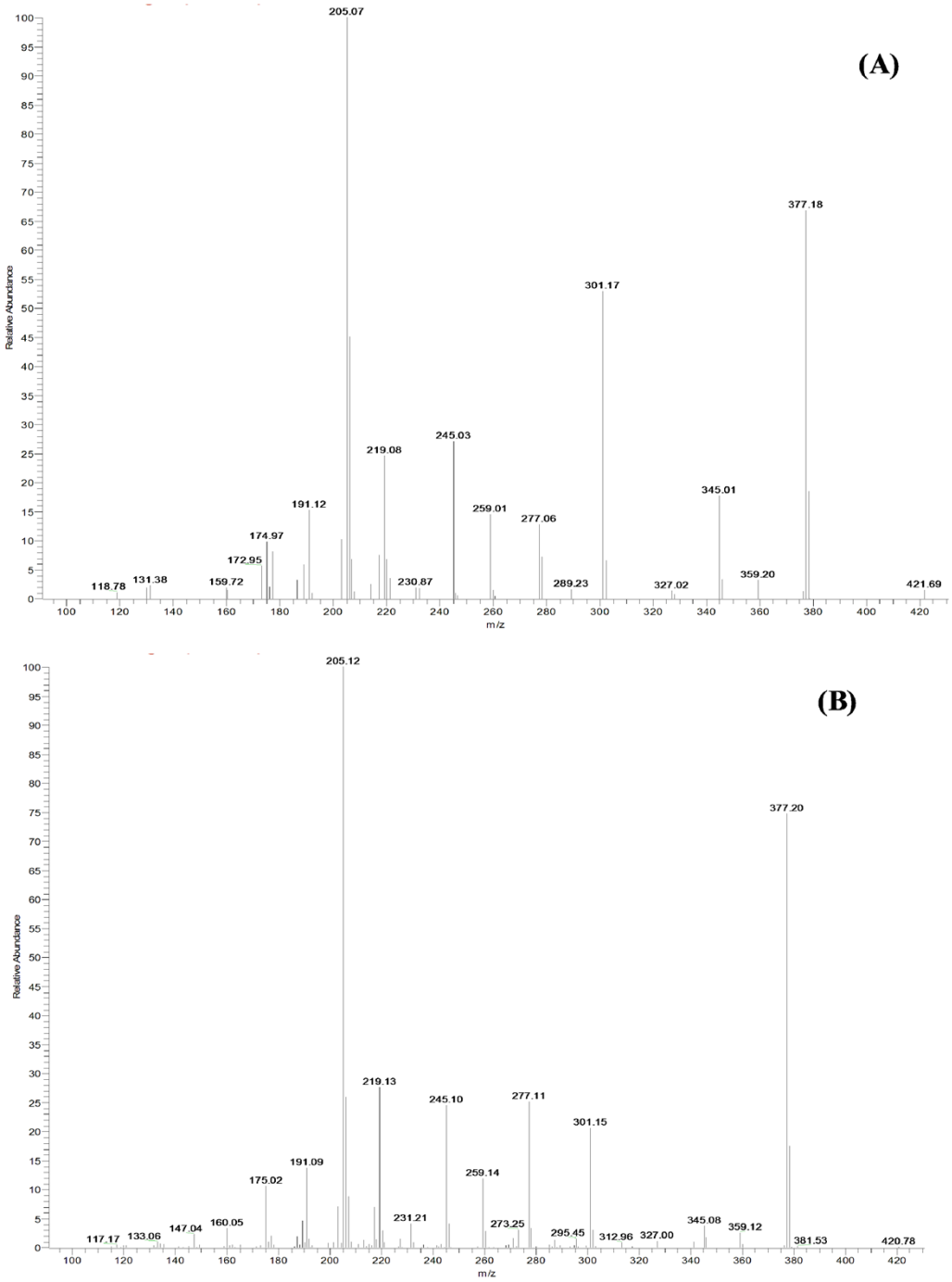
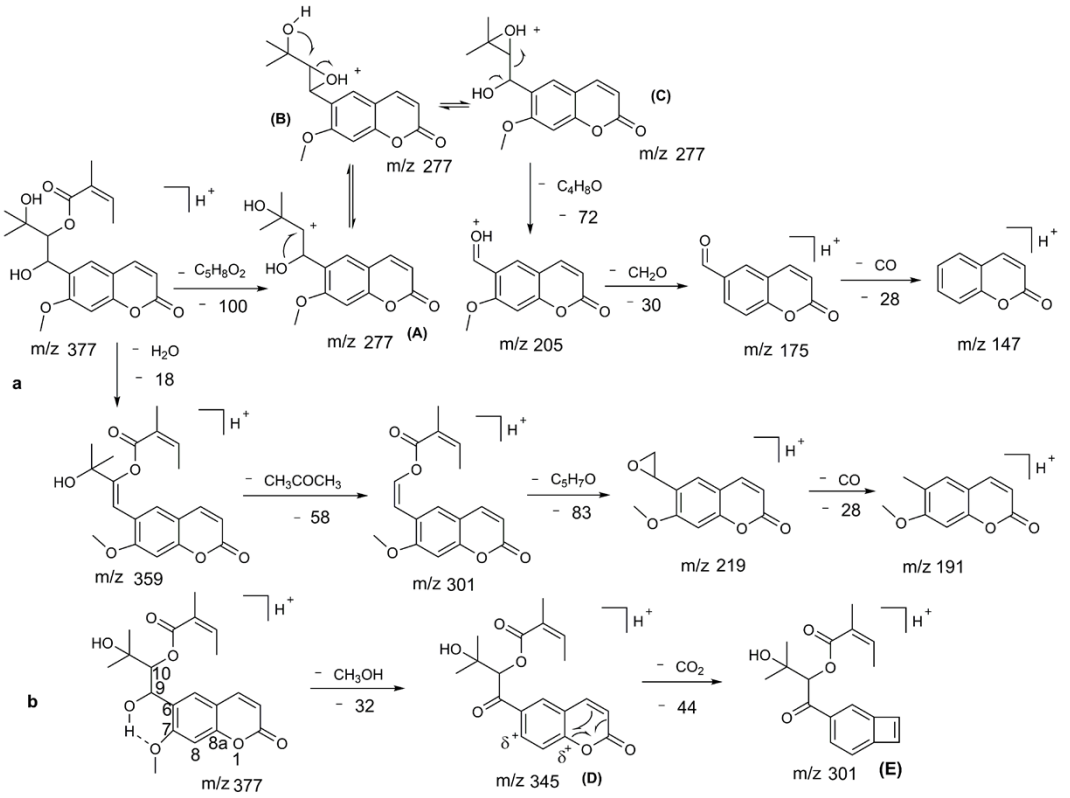
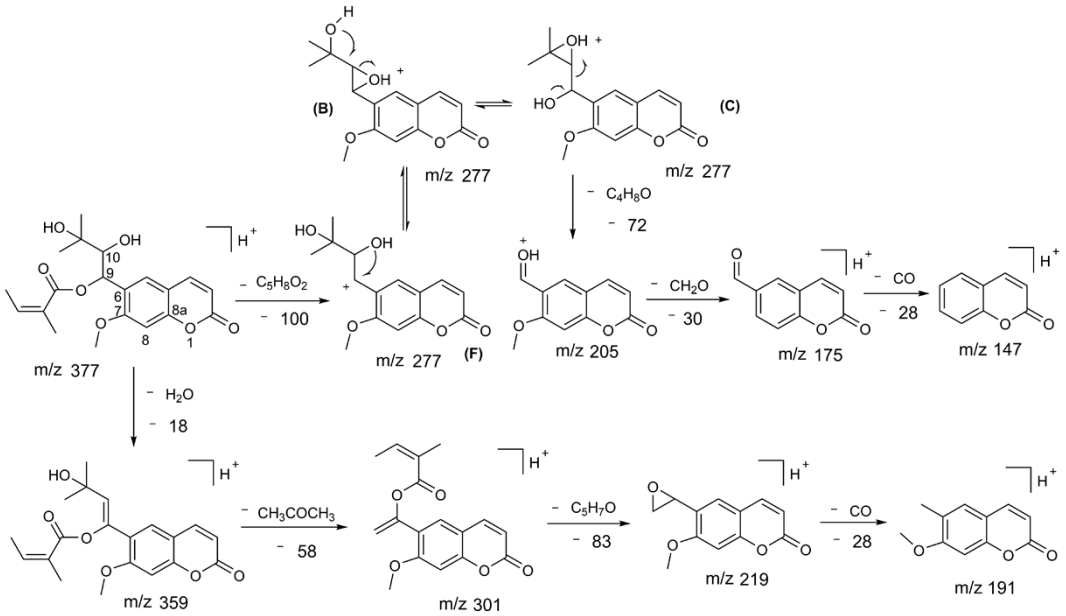


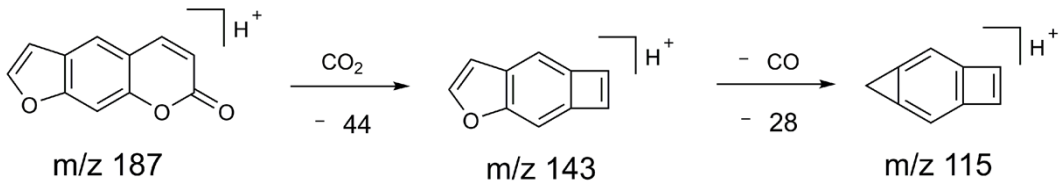
Figure S3. MS^2 spectrum of the ion at m/z 377 for Peak **6** (A) and Peak **8** (B)



Scheme S2. Proposed MS fragmentation pathway for the $[M+H]^+$ ion of Peak **6**



Scheme S3. Proposed MS fragmentation pathway for the $[M+H]^+$ ion of Peak **8**



Scheme S4. Proposed MS fragmentation pathway for the $[M+H]^+$ ion of Peak **3**

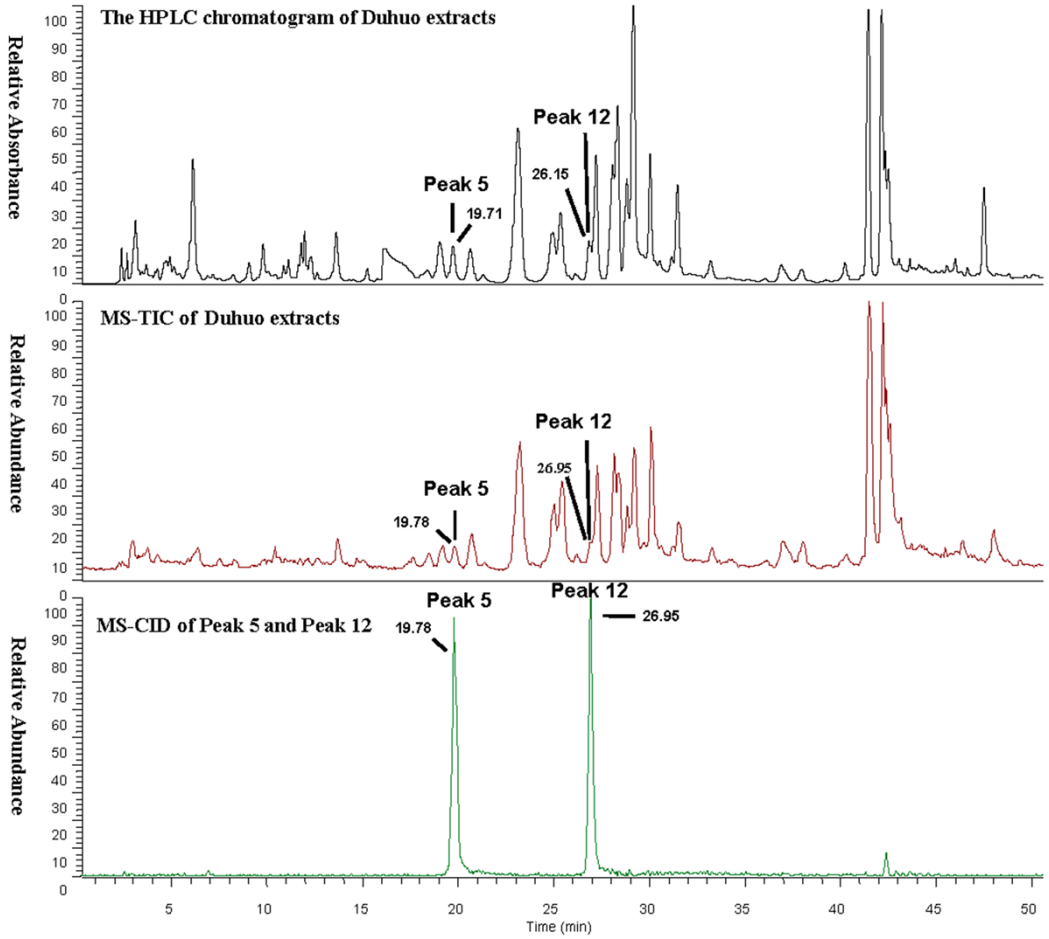


Figure S4. The HPLC chromatogram and Mass spectra of xanthotoxin (Peak **5**) and bergapten (Peak **12**) in sample

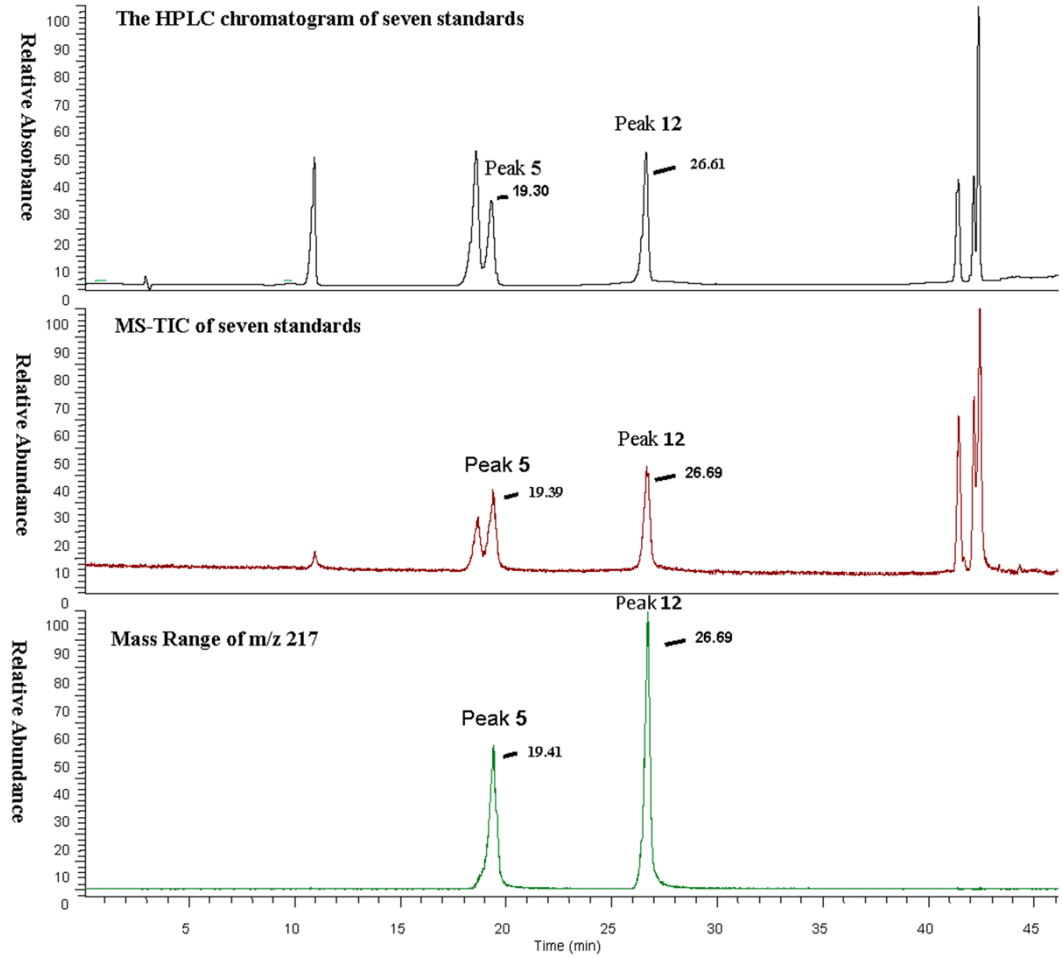


Figure S5. The HPLC chromatogram and Mass spectra of xanthotoxin (Peak **5**) and bergapten (Peak **12**) in standards

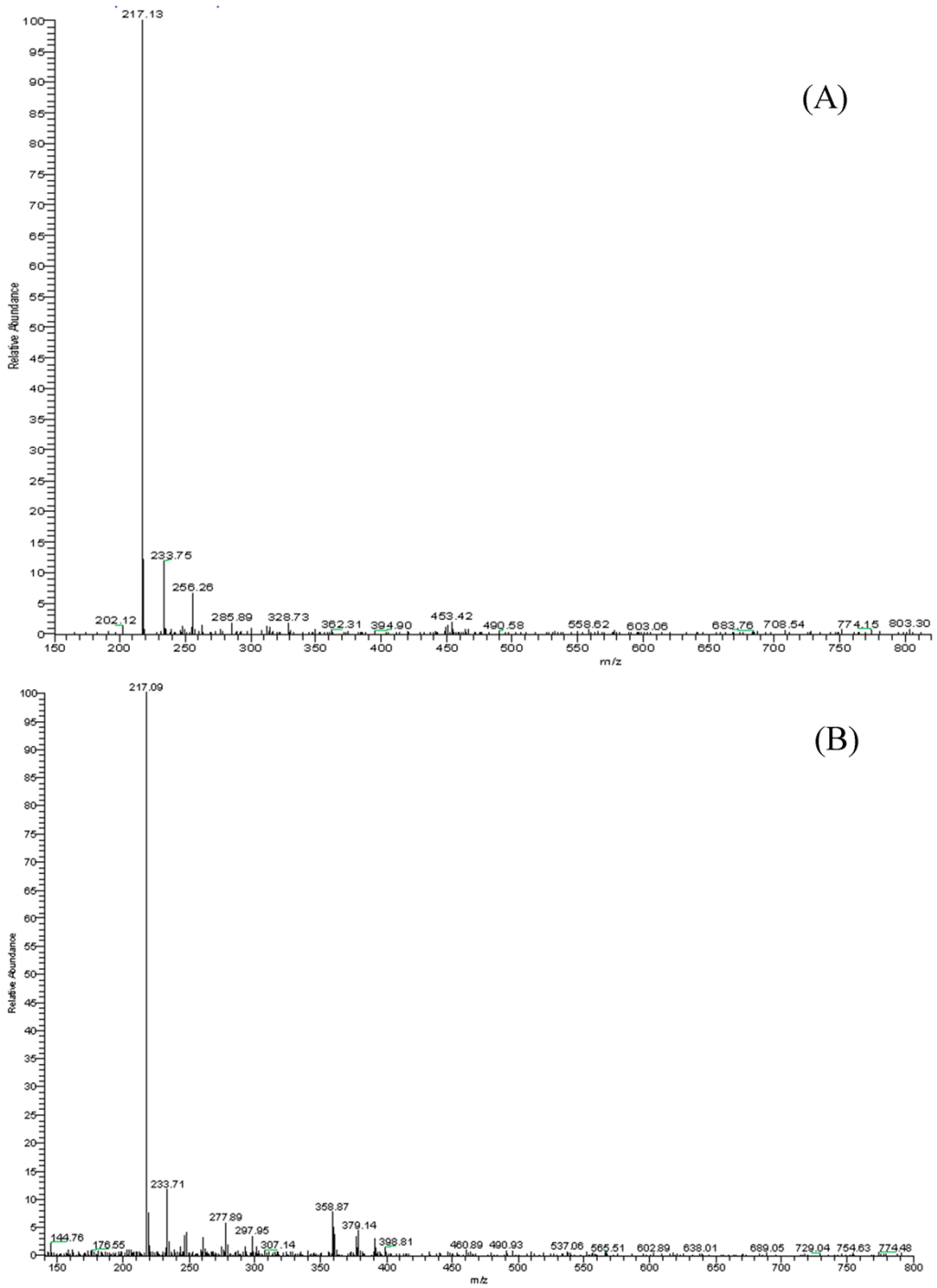


Figure S6.MS spectrum of the ion at m/z 217 for Peak **5**(A) and Peak **12**(B).

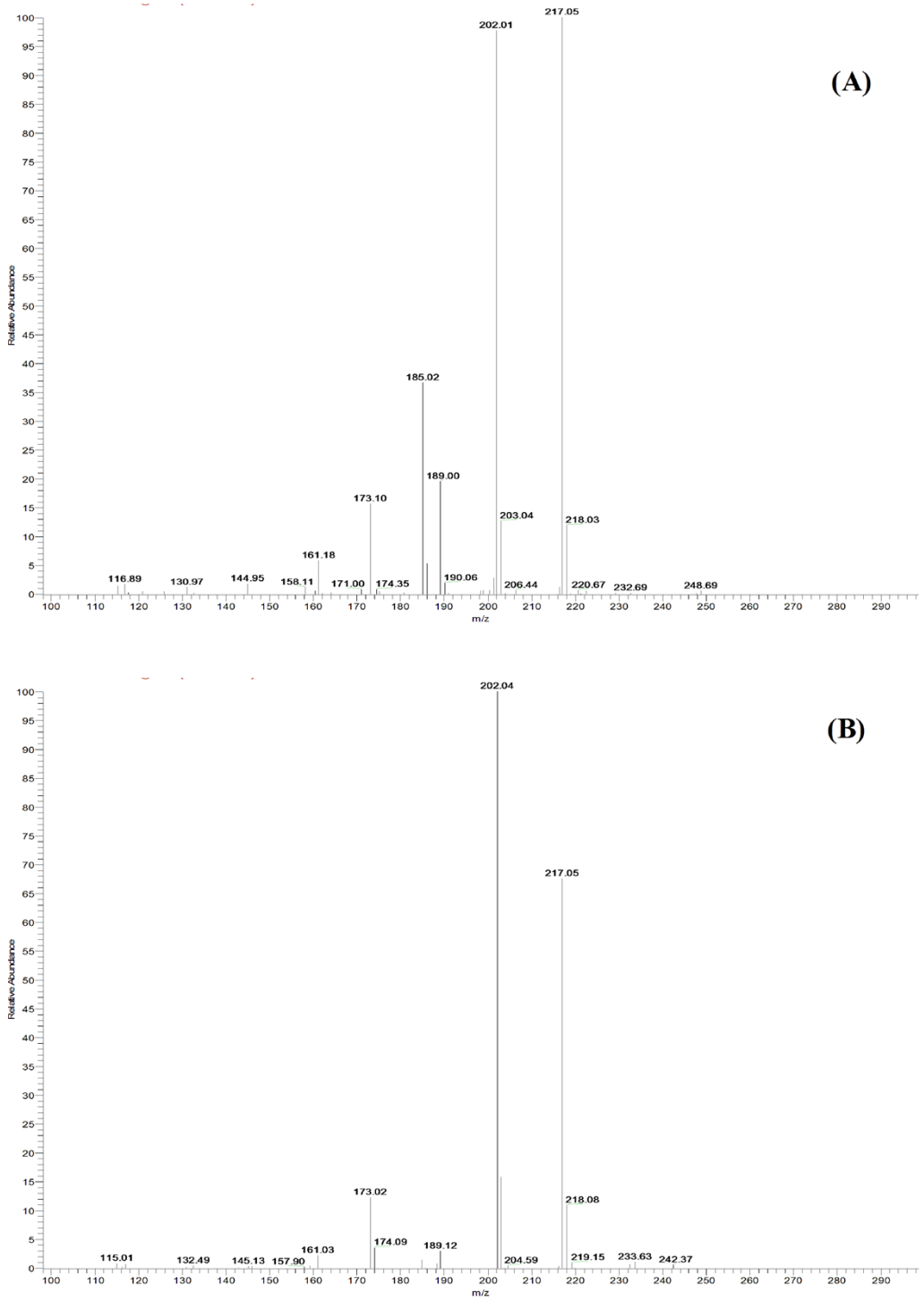


Figure S7. MS^2 spectrum of the ion at m/z 217 for Peak **5**(A) and Peak **12**(B).

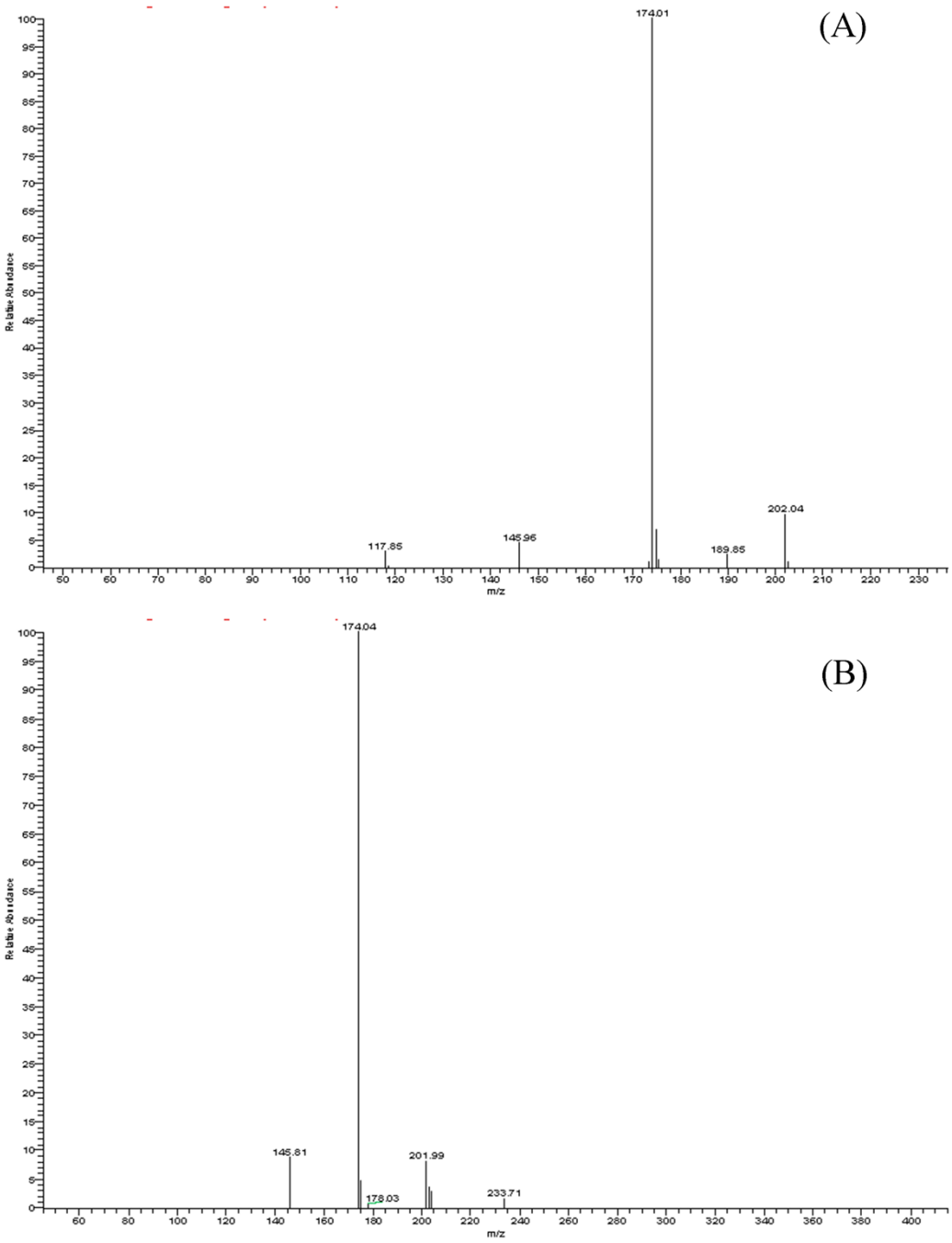


Figure S8. MS^3 spectrum of the ion at m/z 217 for Peak **5**(A) and Peak **12**(B).

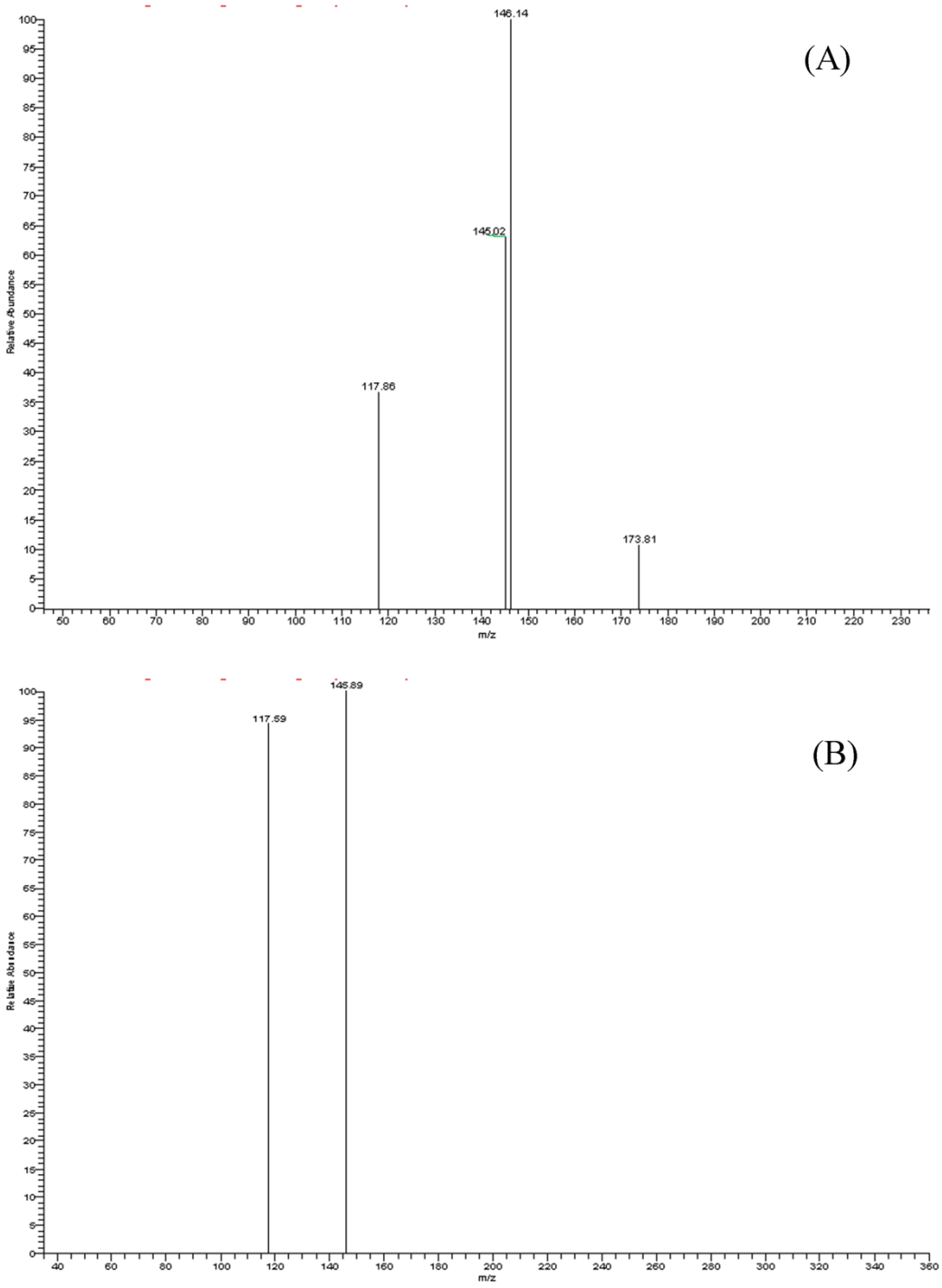


Figure S9. MS^4 spectrum of the ion at m/z 217 for Peak **5**(A) and Peak **12**(B).

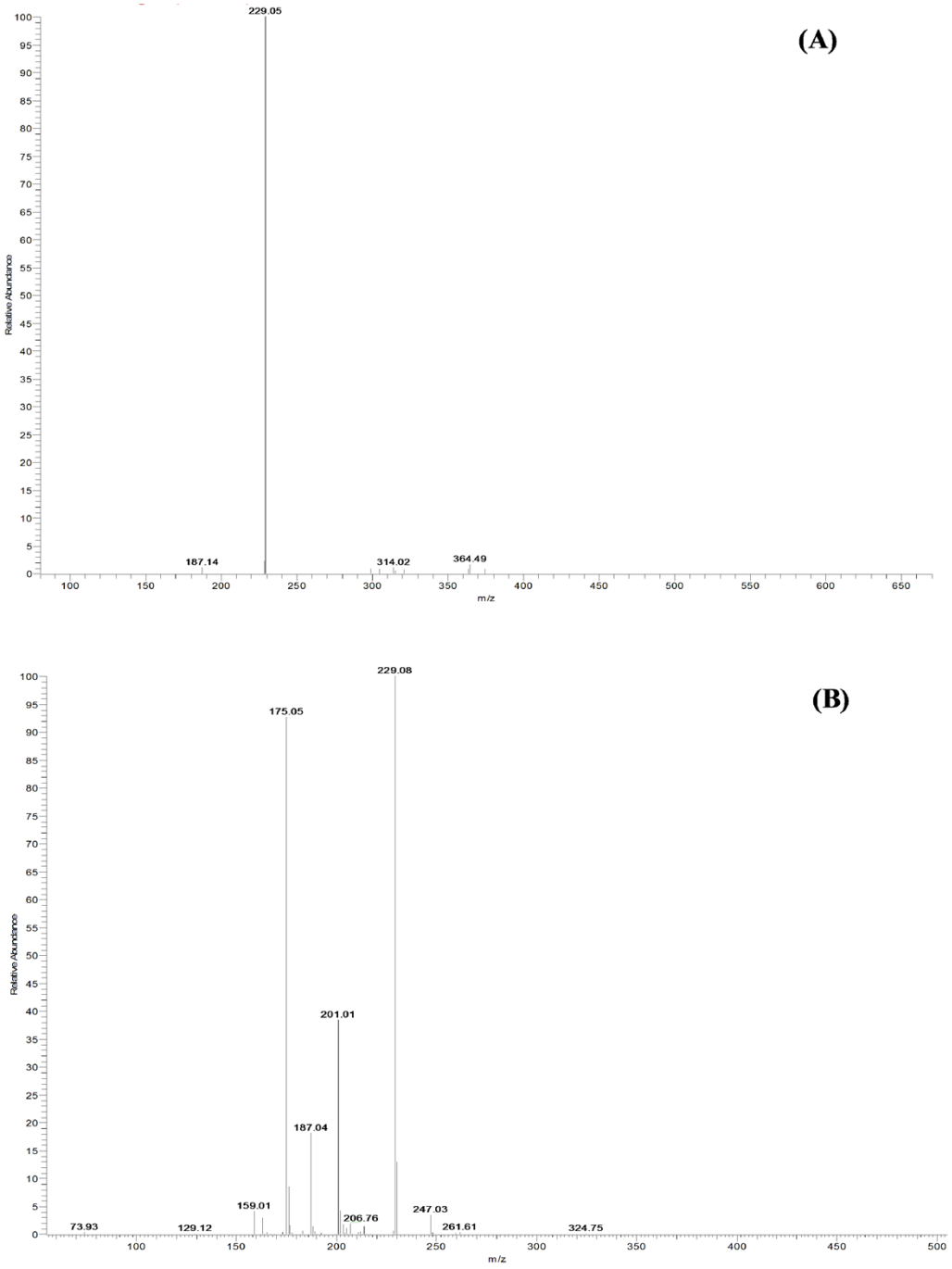
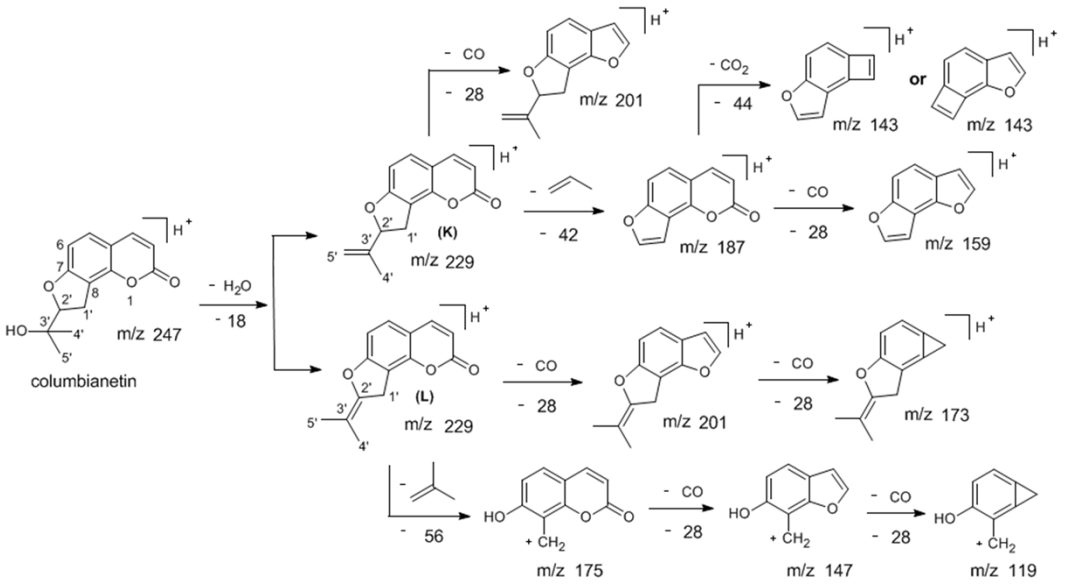


Figure S10. MS^2 spectrum of the ion at m/z 329 for Peak **23** (A) and the ion at m/z 247 for Peak **4** (B)



Scheme S5. Proposed MS fragmentation pathway for the $[M+H]^+$ ion of Peak **4**