Study of forced degradation behaviour of cobicistat and atazanavir using LC/ESI/QTOF/MS; Combination of in-source and collision induced dissociation for evaluate the fragmentation patterns of degradation products

Supplementary Data

Table S5 Robustness data of UPLC method for COB and ATZ

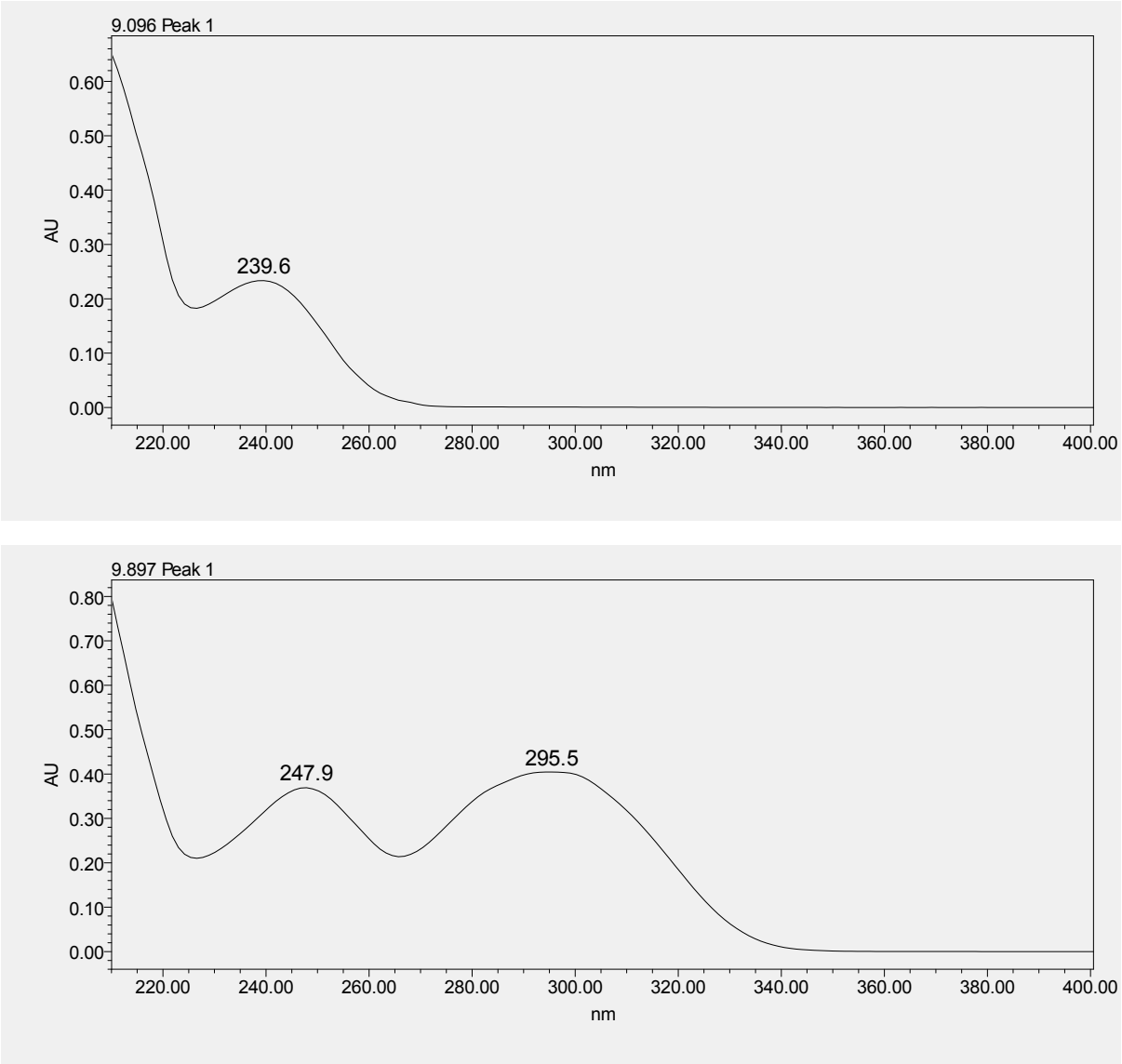


Fig. S1 UV spectra of cobicistat and atazanavir

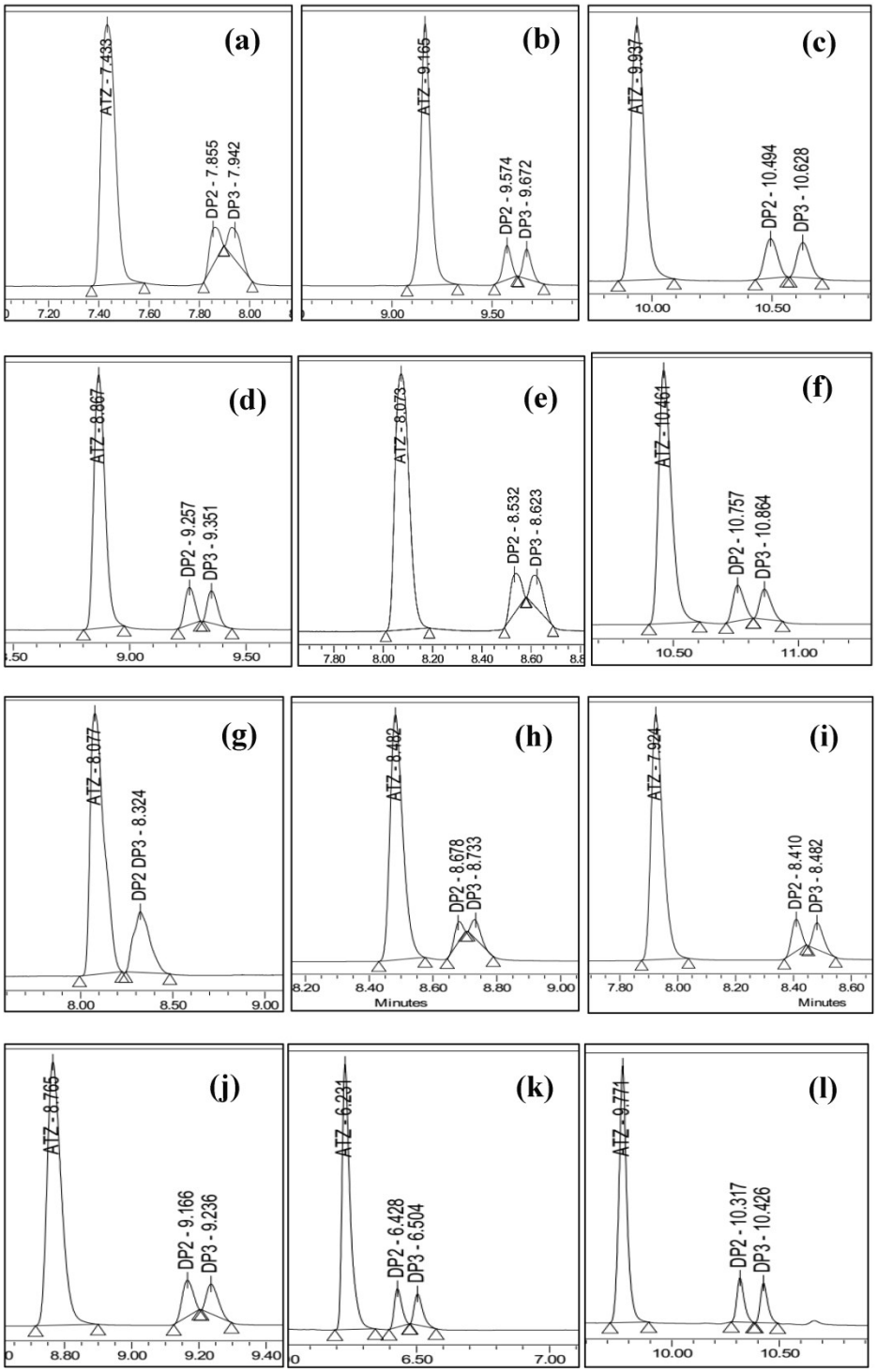


Fig. S2. UPLC/PDA chromatograms of trials were performed to separate the critical pair ATZ

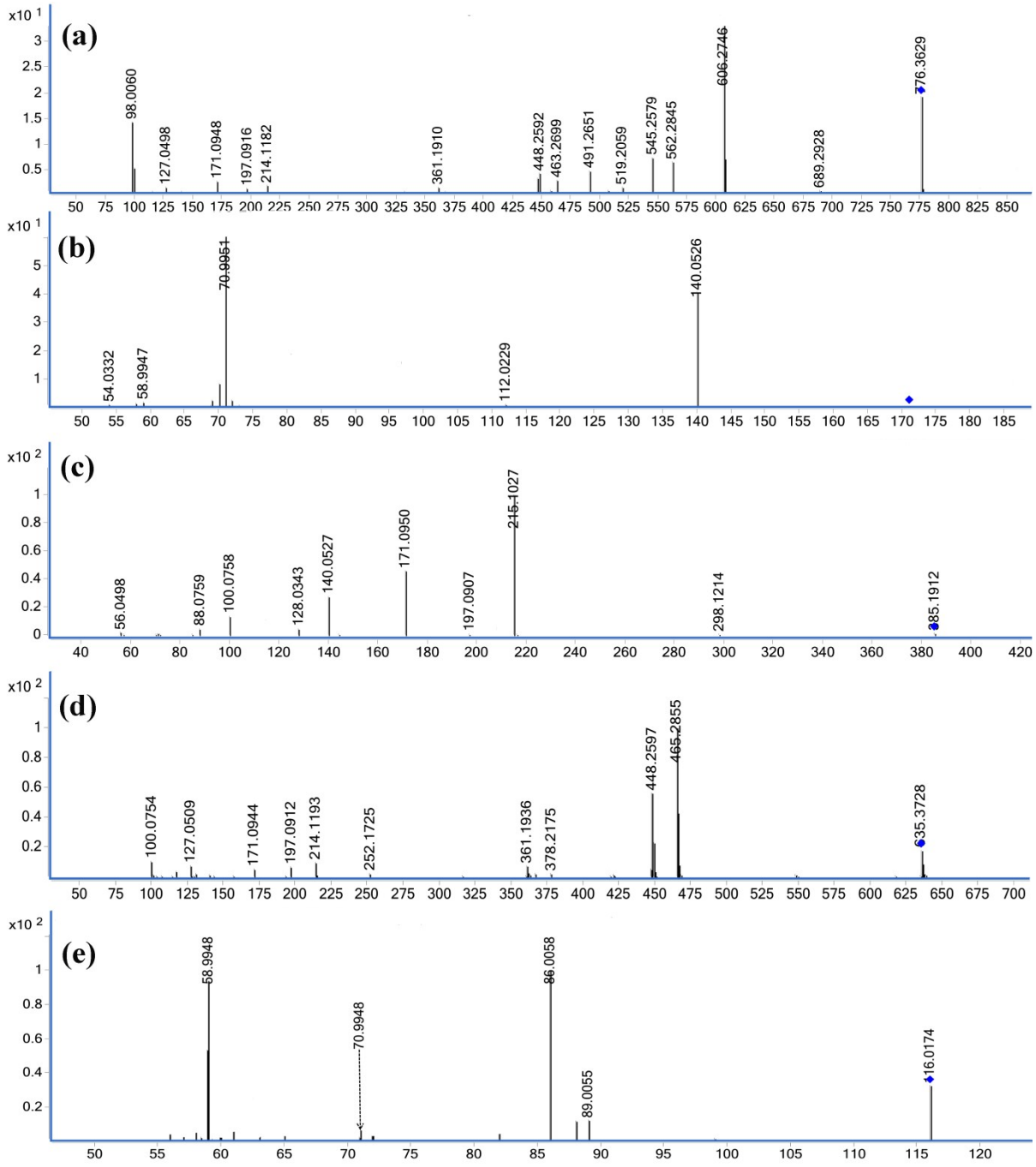


Fig. S3. LC/MS/MS spectra (Count (%) vs. mass-to-charge (*m/z*)) of the $[M+H]^+$ ions of (a) COB, (b) C1, (c) C2, (d) C4, and (e) C7

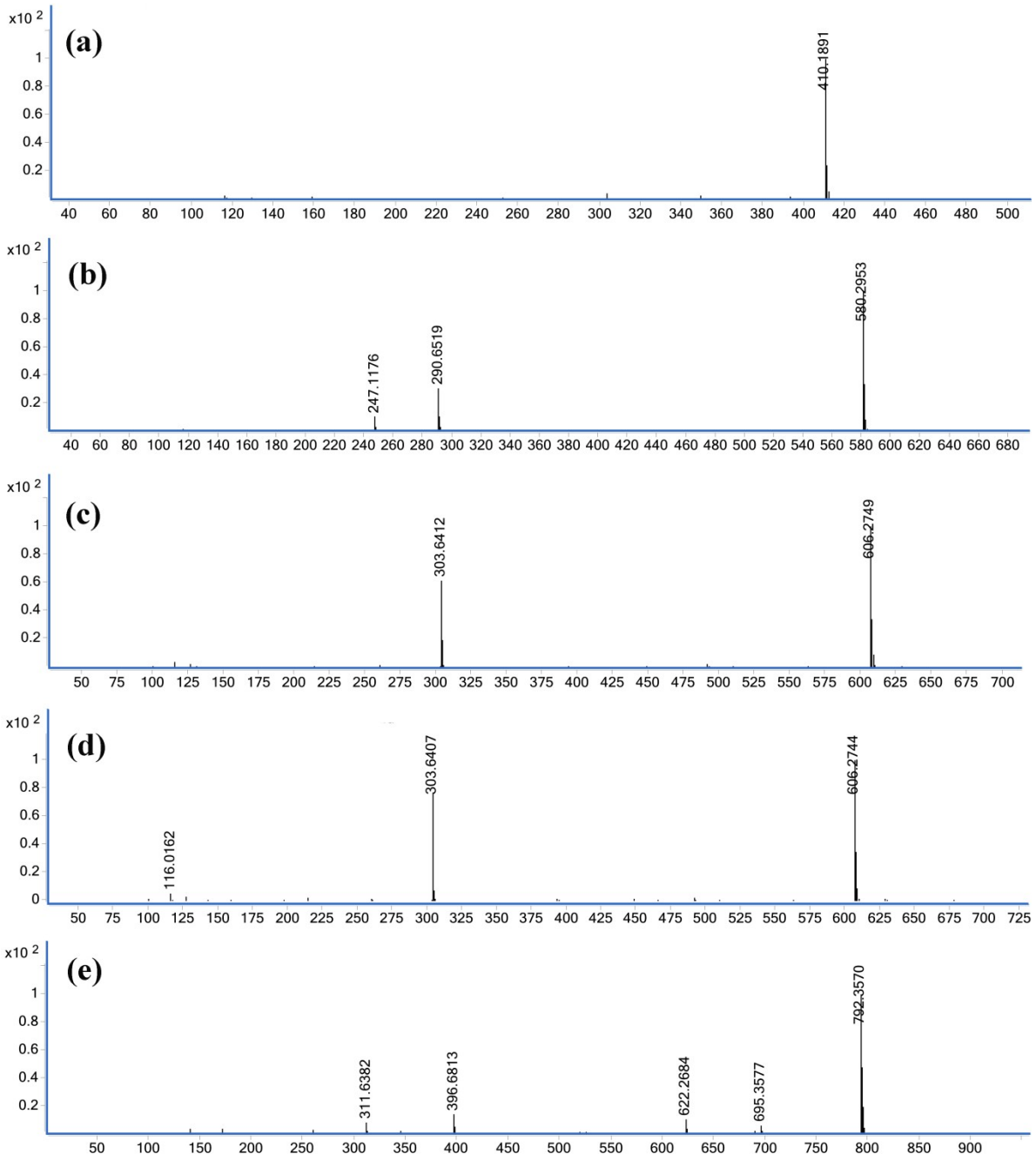


Fig. S4. LC/MS spectra (Count (%) vs. mass-to-charge (*m/z*)) of the $[M+H]^+$ ions of (a) C3, (b) C5, (c) C6, (d) C8, and (e) C9

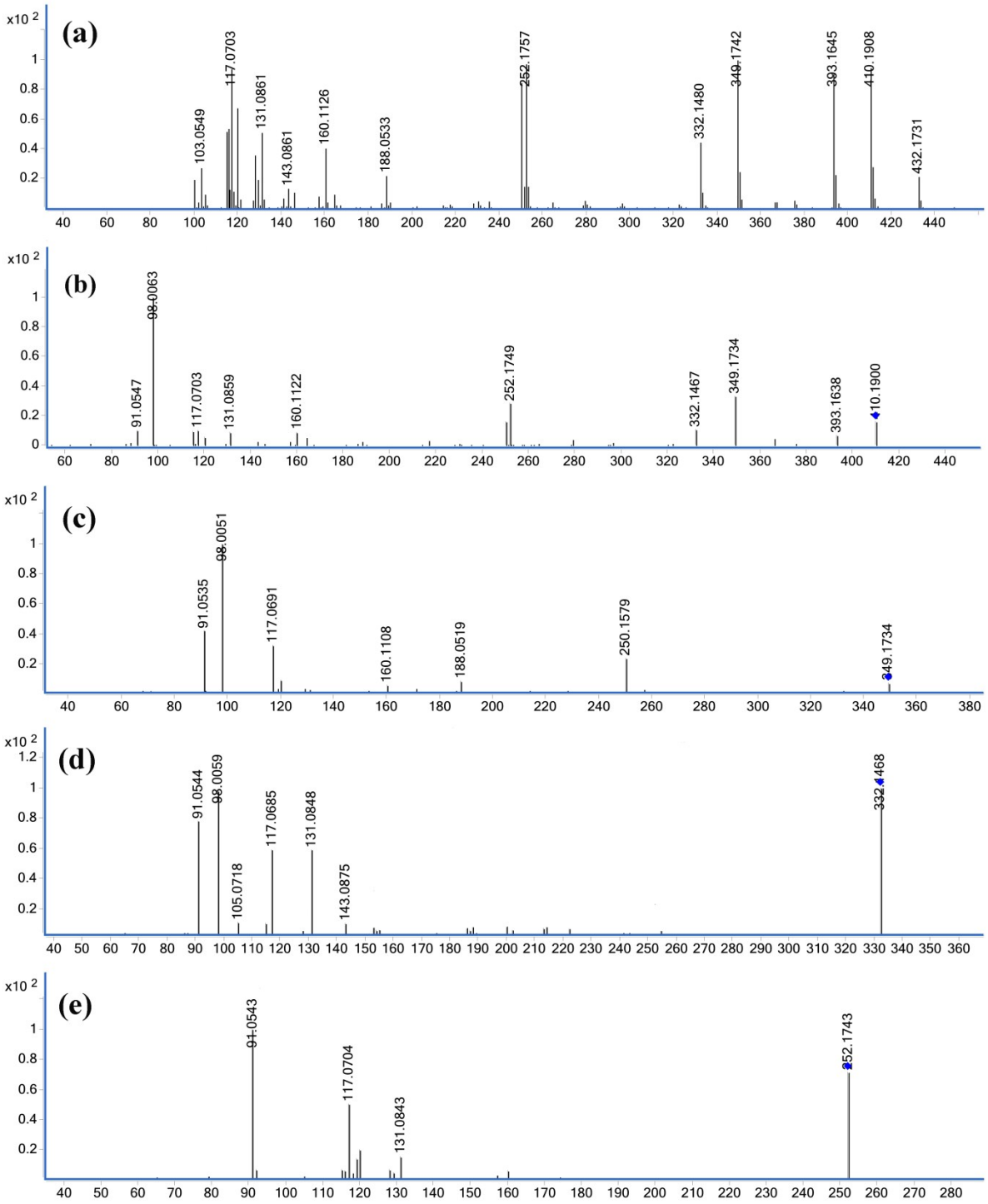


Fig. S5. (a) In-source fragmentation of C3 (*m/z* 410), (b) MS/MS spectra of $[M+H]^+$ ion of C3, (c) MS/MS spectra of $[M+H]^+$ ion of *m/z* 349, (d) MS/MS spectra of $[M+H]^+$ ion of *m/z* 332, (e) MS/MS spectra of $[M+H]^+$ ion of *m/z* 252

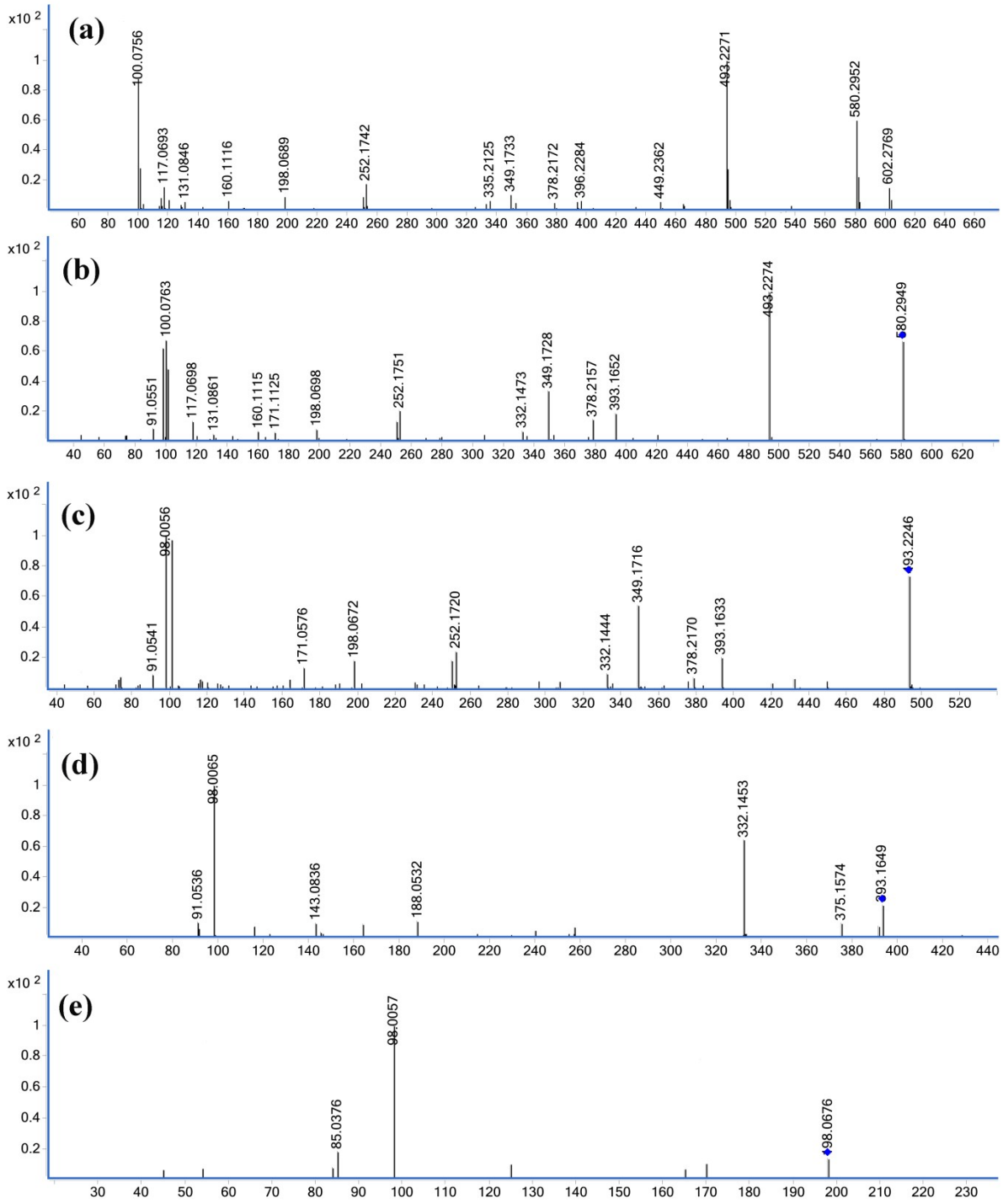


Fig. S6. (a) In-source fragmentation of C5 (*m/z* 580), (b) MS/MS spectra of $[M+H]^+$ ion of C5, (c) MS/MS spectra of $[M+H]^+$ ion of *m/z* 493, (d) MS/MS spectra of $[M+H]^+$ ion of *m/z* 393, (e) MS/MS spectra of $[M+H]^+$ ion of *m/z* 198

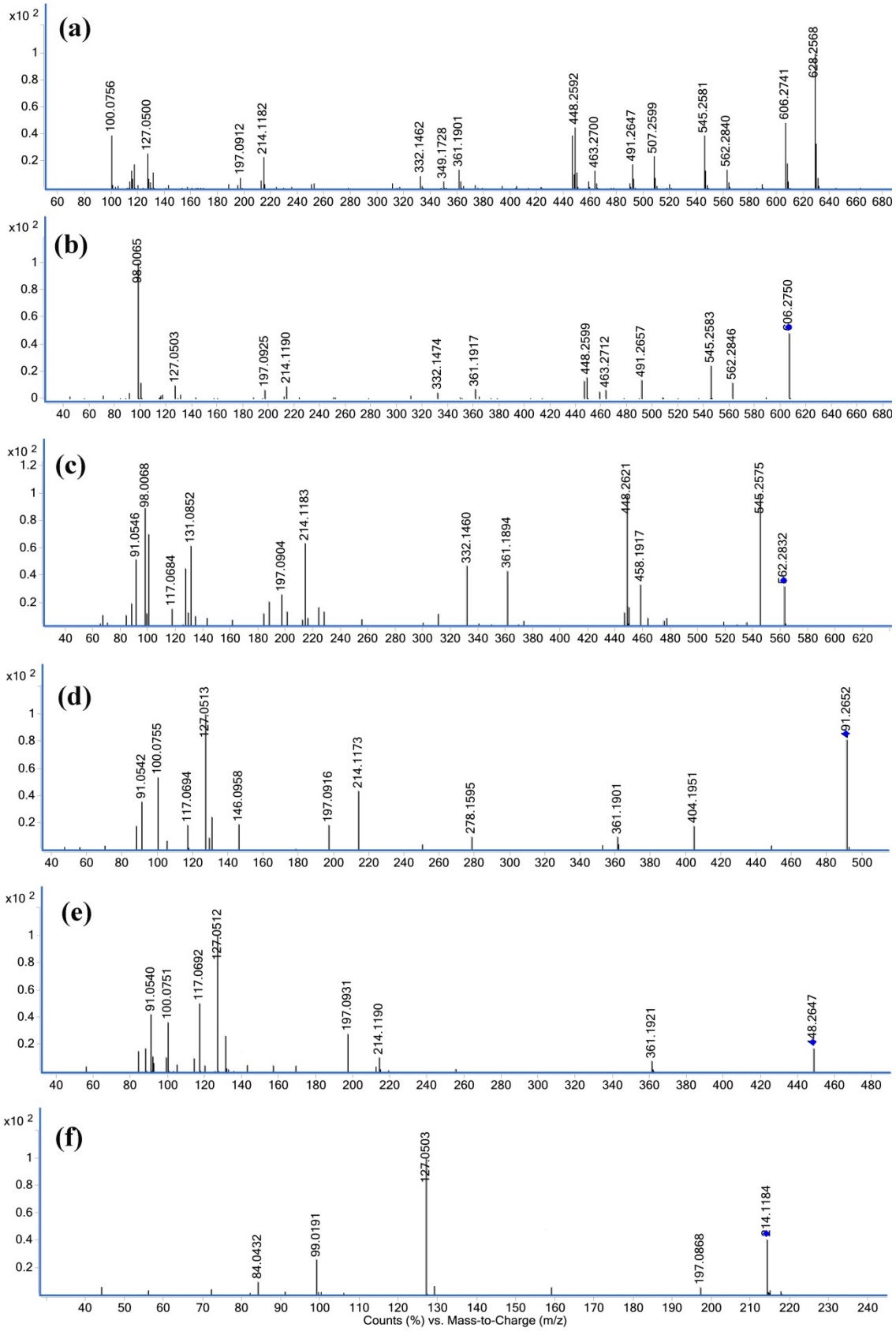


Fig. S7. (a) In-source fragmentation of C6/C8 (*m/z* 606), (b) MS/MS spectra of $[M+H]^+$ ion of C6/C8, (c) MS/MS spectra of $[M+H]^+$ ion of *m/z* 562, (d) MS/MS spectra of $[M+H]^+$ ion of *m/z* 491, (e) MS/MS spectra of $[M+H]^+$ ion of *m/z* 448, (f) MS/MS spectra of $[M+H]^+$ ion of *m/z* 214

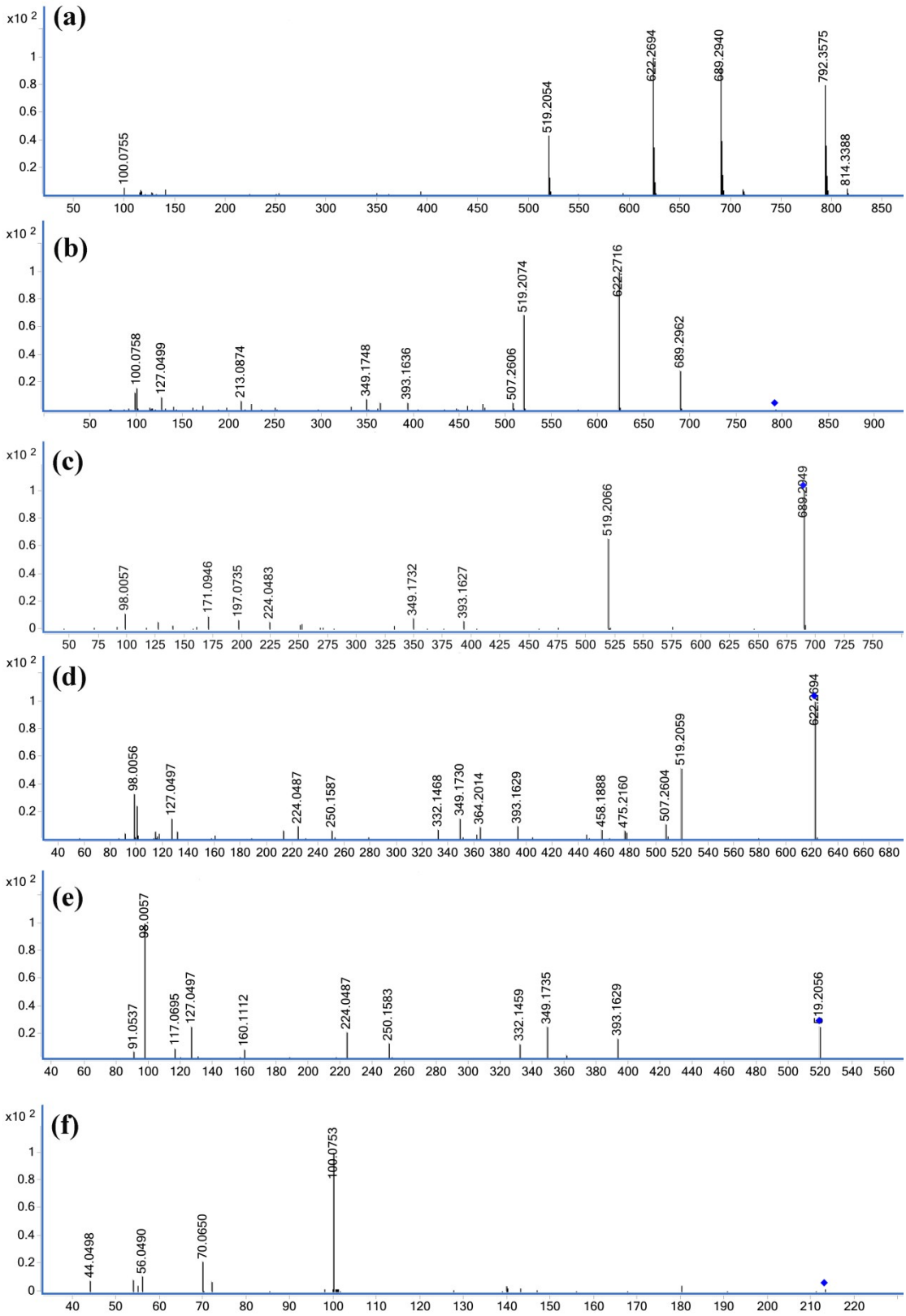


Fig. S8. (a) In-source fragmentation of C9 (*m/z* 792), (b) MS/MS spectra of $[M+H]^+$ ion of C9, (c) MS/MS spectra of $[M+H]^+$ ion of *m/z* 689, (d) MS/MS spectra of $[M+H]^+$ ion of *m/z* 622, (e) MS/MS spectra of $[M+H]^+$ ion of *m/z* 519, (f) MS/MS spectra of $[M+H]^+$ ion of *m/z* 213

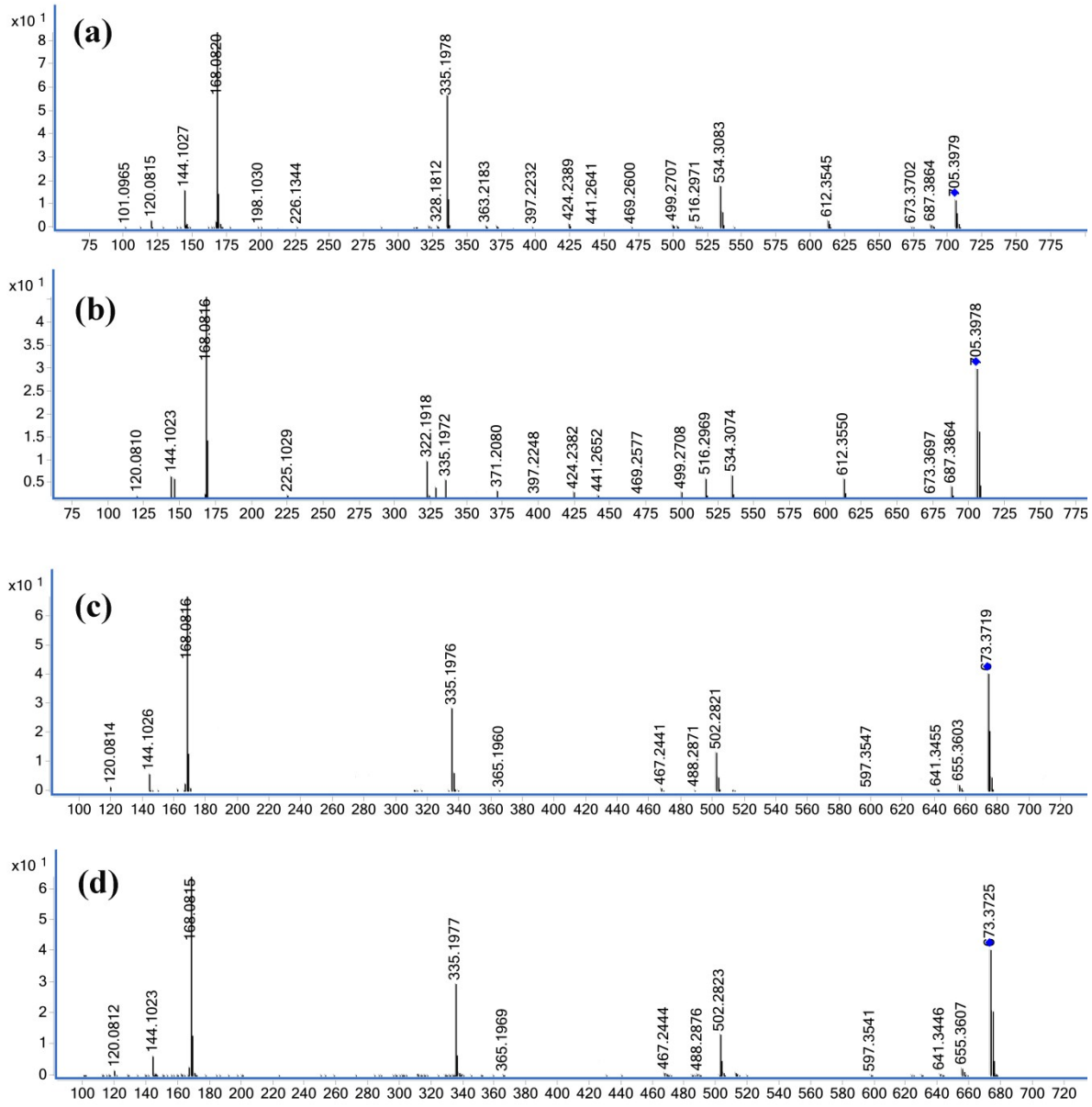


Fig. S9. LC/MS/MS spectra (Count (%) vs. mass-to-charge (*m/z*)) of the $[M+H]^+$ ions of (a) ATZ, (b) A1, (c) A2, and (d) A3

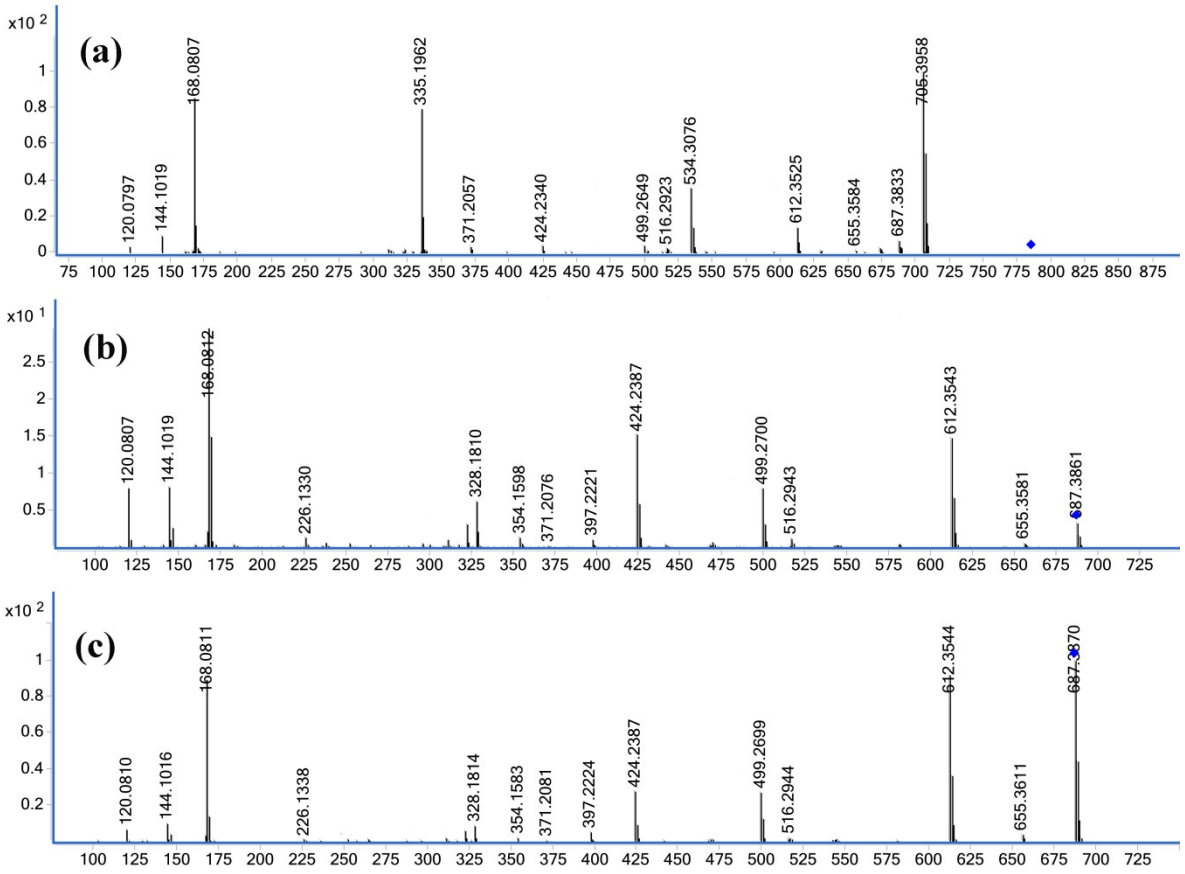
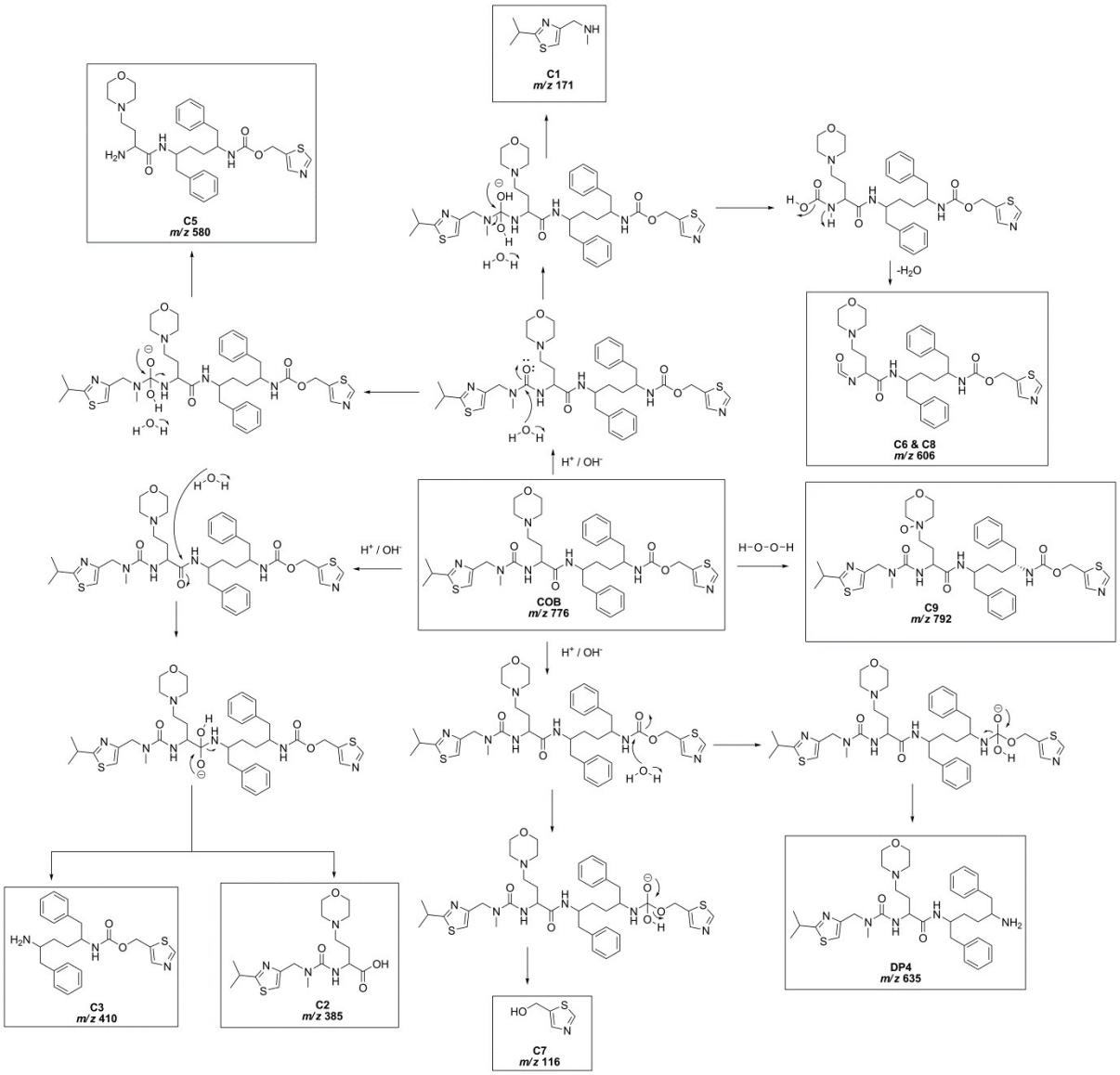
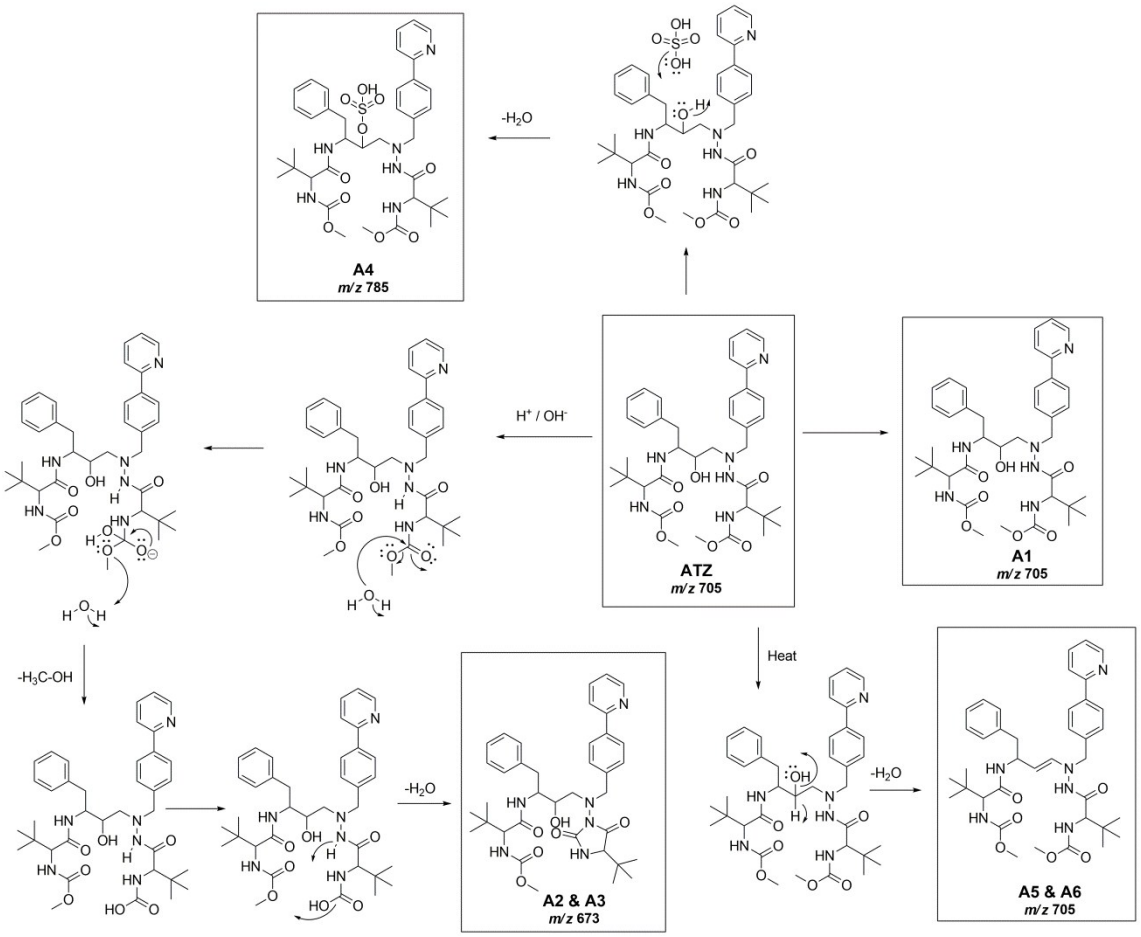


Fig. S10. LC/MS/MS spectra (Count (%) vs. mass-to-charge (*m/z*)) of the $[M+H]^+$ ions of (a) A4, (b) A5, and (c) A6



Scheme S1. Probable mechanisms of the formation of C1-C9



Scheme S2. Probable mechanisms of the formation of A1-A6

Table S1 Data of trials carried out to separate the critical pair (A2 & A3) of ATZ

Fig No	Column	Mobile phase		USP Resolution between the A2&A3
		Aqueous phase (A)	Organic phase (B)	
S1a	BEH C18	10mM Ammonium acetate	Acetonitrile	1.10
S1b	BEH C18	10mM Ammonium acetate	Acetonitrile: methanol (70: 30, %v/v)	1.25
S1c	BEH C18	10mM Ammonium acetate, pH 4	Acetonitrile: methanol (70: 30, %v/v)	1.35
S1d	BEH C18	10mM Ammonium acetate, pH 5	Acetonitrile: methanol (70: 30, %v/v)	1.19
S1e	CSH C18	10mM Ammonium acetate	Acetonitrile	1.26
S1f	CSH C18	10mM Ammonium acetate	Acetonitrile: methanol (70: 30, %v/v)	1.24
S1g	HSS Cyano	10mM Ammonium acetate	Acetonitrile: methanol (70: 30, %v/v)	Merged peaks- no separation
S1h	CSH Flouro phenyl	10mM Ammonium acetate	Acetonitrile: methanol (50: 50, %v/v) + 10% water	1.11
S1i	CSH phenyl hexyl	10mM Ammonium acetate	Acetonitrile	1.11
S1j	CSH phenyl hexyl	10mM Ammonium acetate	Acetonitrile: methanol (70: 30, %v/v)	1.09
S1k	CSH phenyl hexyl	0.1% Trifloro acetic acid	Acetonitrile: methanol (80: 20, %v/v)	1.34
S1l	CSH phenyl hexyl	0.1% Formic acid	Acetonitrile: methanol (80: 20, %v/v)	1.70

Table S2

High resolution mass spectrometry data of all fragment ions of drug and its DPs

Name of drug/DP	Molecular Formula	Theoretical mass	Observed mass	Error ppm
COB	$C_{40}H_{54}N_7O_5S_2^+$	776.3622	776.3629	-0.90
	$C_{36}H_{45}N_6O_4S_2^+$	689.2938	689.2928	1.45
	$C_{32}H_{40}N_5O_5S^+$	606.2745	606.2746	-0.16
	$C_{31}H_{40}N_5O_3S^+$	562.2846	562.2845	0.18
	$C_{31}H_{37}N_4O_3S^+$	545.2581	545.2579	0.37
	$C_{28}H_{31}N_4O_4S^+$	519.2061	519.2059	0.39
	$C_{28}H_{35}N_4O_4^+$	491.2653	491.2651	0.41
	$C_{27}H_{35}N_4O_3^+$	463.2704	463.2699	1.08
	$C_{27}H_{34}N_3O_3^+$	448.2595	448.2592	0.67
	$C_{23}H_{25}N_2O_2^+$	361.1911	361.191	0.28
	$C_9H_{16}N_3O_3^+$	214.1186	214.1182	1.87
	$C_9H_{13}N_2O_3^+$	197.0921	197.0916	2.54
	$C_8H_{15}N_2S^+$	171.095	171.0948	1.17
	$C_5H_7N_2O_2^+$	127.0502	127.0498	3.15
	$C_4H_4NS^+$	98.0059	98.006	-1.02
C1	$C_8H_{15}N_2S^+$	171.095	171.0951	-0.58
	$C_7H_{10}NS^+$	140.0528	140.0526	1.43
	$C_5H_6NS^+$	112.0215	112.0229	-12.50
	$C_3H_3S^+$	70.995	70.9951	-1.41
	$C_2H_3S^+$	58.995	58.9947	5.09
	$C_3H_4N^+$	54.0338	54.0332	11.10
C2	$C_{17}H_{29}N_4O_4S^+$	385.1904	385.1912	-2.08
	$C_{13}H_{20}N_3O_3S^+$	298.122	298.1214	2.01
	$C_9H_{15}N_2O_4^+$	215.1026	215.1027	-0.46
	$C_9H_{13}N_2O_3^+$	197.0921	197.0907	7.10
	$C_8H_{15}N_2S^+$	171.095	171.095	0.00
	$C_7H_{10}NS^+$	140.0528	140.0527	0.71
	$C_5H_6NO_3^+$	128.0342	128.0343	-0.78
	$C_5H_{10}NO^+$	100.0757	100.0758	-1.00
	$C_4H_{10}NO^+$	88.0757	88.0759	-2.27
	$C_3H_6N^+$	56.0495	56.0498	-5.35
C3	$C_{23}H_{28}N_3O_2S^+$	410.1897	410.1899	-0.49
	$C_{23}H_{25}N_2O_2S^+$	393.1631	393.1638	-1.78
	$C_{22}H_{25}N_2S^+$	349.1733	349.1734	-0.29
	$C_{22}H_{22}NS^+$	332.1467	332.1467	0.00
	$C_{18}H_{22}N^+$	252.1747	252.1749	-0.79
	$C_{11}H_{14}N^+$	160.1121	160.1121	0.00
	$C_{10}H_{11}^+$	131.0855	131.0858	-2.29
	$C_9H_9^+$	117.0699	117.0702	-2.56
	$C_4H_4NS^+$	98.0059	98.0063	-4.08
	$C_7H_7^+$	91.0542	91.0547	-5.49

C4	$C_{35}H_{51}N_6O_3S^+$	635.3738	635.3728	1.57
	$C_{27}H_{37}N_4O_3^+$	465.286	465.2855	1.07
	$C_{27}H_{34}N_3O_3^+$	448.2595	448.2597	-0.45
	$C_{23}H_{28}N_3O_2^+$	378.2176	378.2175	0.26
	$C_{23}H_{25}N_2O_2^+$	361.1911	361.1936	-6.92
	$C_{18}H_{22}N^+$	252.1747	252.1725	8.72
	$C_9H_{16}N_3O_3^+$	214.1186	214.1193	-3.27
	$C_9H_{13}N_2O_3^+$	197.0921	197.0916	2.54
	$C_8H_{15}N_2S^+$	171.095	171.0944	3.51
	$C_5H_7N_2O_2^+$	127.0502	127.0509	-5.51
	$C_5H_{10}NO^+$	100.0754	100.0754	0.00
C5	$C_{31}H_{42}N_5O_4S^+$	580.2952	580.2949	0.52
	$C_{27}H_{33}N_4O_3S^+$	493.2268	493.2274	-1.22
	$C_{23}H_{25}N_2O_2S^+$	393.1631	393.1652	-5.34
	$C_{23}H_{28}N_3O_2^+$	378.2176	378.2157	5.02
	$C_{22}H_{25}N_2S^+$	349.1733	349.1728	1.43
	$C_{22}H_{22}NS^+$	332.1467	332.1473	-1.81
	$C_{18}H_{22}N^+$	252.1747	252.1751	-1.59
	$C_8H_{12}N_3OS^+$	198.0696	198.0698	-1.01
	$C_8H_{15}N_2O_2^+$	171.1128	171.1125	1.75
	$C_{11}H_{14}N^+$	160.1121	160.1115	3.75
	$C_{10}H_{11}^+$	131.0855	131.0861	-4.58
	$C_9H_9^+$	117.0699	117.0698	0.85
	$C_5H_{10}NO^+$	100.0757	100.0755	2.00
	$C_7H_7^+$	91.0542	91.0551	-9.88
C6	$C_{32}H_{40}N_5O_5S^+$	606.2745	606.275	-0.82
	$C_{31}H_{40}N_5O_3S^+$	562.2846	562.2846	0.00
	$C_{31}H_{37}N_4O_3S^+$	545.2581	545.2583	-0.37
	$C_{28}H_{35}N_4O_4^+$	491.2653	491.2657	-0.81
	$C_{27}H_{35}N_4O_3^+$	463.2704	463.2712	-1.73
	$C_{27}H_{34}N_3O_3^+$	448.2595	448.2599	-0.89
	$C_{23}H_{25}N_2O_2^+$	361.1911	361.1917	-1.66
	$C_{22}H_{22}NS^+$	332.1467	332.1474	-2.11
	$C_9H_{16}N_3O_3^+$	214.1186	214.119	-1.87
	$C_9H_{13}N_2O_3^+$	197.0921	197.0925	-2.03
	$C_5H_7N_2O_2^+$	127.0502	127.0503	-0.79
	$C_4H_4NS^+$	98.0059	98.0065	-6.12
C7	$C_4H_6NOS^+$	116.0165	116.0174	-7.76
	$C_3H_5OS^+$	89.0056	89.0055	1.12
	$C_3H_4NS^+$	86.0059	86.0058	1.16
	$C_3H_3S^+$	70.995	70.9948	2.82
	$C_2H_3S^+$	58.995	58.9948	3.39
C9	$C_{40}H_{54}N_7O_6S_2^+$	792.3572	792.3575	-0.38
	$C_{36}H_{45}N_6O_4S_2^+$	689.2938	689.2962	-3.48

	$C_{32}H_{40}N_5O_6S^+$	622.2694	622.2716	-3.54
	$C_{28}H_{31}N_4O_4S^+$	519.2061	519.2074	-2.50
	$C_{28}H_{35}N_4O_5^+$	507.2602	507.2606	-0.79
	$C_{23}H_{25}N_2O_2S^+$	393.1631	393.1636	-1.27
	$C_{22}H_{25}N_2S^+$	349.1733	349.1748	-4.30
	$C_9H_{13}N_2O_4^+$	213.087	213.0874	-1.88
	$C_5H_7N_2O_2^+$	127.0502	127.0499	2.36
	$C_5H_{10}NO^+$	100.0757	100.0758	-1.00
ATZ	$C_{38}H_{53}N_6O_7^+$	705.397	705.3978	-1.13
	$C_{38}H_{51}N_6O_6^+$	687.3865	687.3863	0.29
	$C_{36}H_{46}N_5O_4^+$	612.3544	612.3544	0.00
	$C_{30}H_{40}N_5O_4^+$	534.3075	534.3082	-1.31
	$C_{30}H_{38}N_5O_3^+$	516.2969	516.2971	-0.39
	$C_{30}H_{35}N_4O_3^+$	499.2704	499.2706	-0.40
	$C_{28}H_{33}N_4O^+$	441.2649	441.264	2.04
	$C_{28}H_{30}N_3O^+$	424.2383	424.2388	-1.18
	$C_{22}H_{29}N_4O_3^+$	397.2234	397.2231	0.76
	$C_{20}H_{27}N_4O_3^+$	371.2078	371.2084	-1.62
	$C_{18}H_{27}N_2O_4^+$	335.1965	335.1977	-3.58
	$C_{20}H_{24}N_3O^+$	322.1914	322.1918	-1.24
	$C_{12}H_{10}N^+$	168.0808	168.082	-7.14
	$C_7H_{14}NO_2^+$	144.1019	144.1027	-5.55
A1	$C_{38}H_{53}N_6O_7^+$	705.397	705.3979	-1.28
	$C_{38}H_{51}N_6O_6^+$	687.3865	687.3864	0.15
	$C_{36}H_{46}N_5O_4^+$	612.3544	612.355	-0.98
	$C_{30}H_{40}N_5O_4^+$	534.3075	534.3075	0.00
	$C_{30}H_{38}N_5O_3^+$	516.2969	516.2969	0.00
	$C_{30}H_{35}N_4O_3^+$	499.2704	499.2709	-1.00
	$C_{28}H_{33}N_4O^+$	441.2649	441.2653	-0.91
	$C_{28}H_{30}N_3O^+$	424.2383	424.2383	0.00
	$C_{22}H_{29}N_4O_3^+$	397.2234	397.2249	-3.78
	$C_{20}H_{27}N_4O_3^+$	371.2078	371.2081	-0.81
	$C_{18}H_{27}N_2O_4^+$	335.1965	335.1972	-2.09
	$C_{20}H_{24}N_3O^+$	322.1914	322.1918	-1.24
	$C_{12}H_{10}N^+$	168.0808	168.0817	-5.35
	$C_7H_{14}NO_2^+$	144.1019	144.1024	-3.47
	$C_8H_{10}N^+$	120.0808	120.081	-1.67
A2	$C_{37}H_{49}N_6O_6^+$	673.3708	673.3719	-1.63
	$C_{37}H_{47}N_6O_5^+$	655.3602	655.3603	-0.15
	$C_{36}H_{45}N_6O_5^+$	641.3446	641.3455	-1.40
	$C_{35}H_{45}N_6O_3^+$	597.3548	597.3547	0.17
	$C_{29}H_{36}N_5O_3^+$	502.2813	502.2821	-1.59
	$C_{25}H_{38}N_5O_5^+$	488.2867	488.2871	-0.82
	$C_{29}H_{31}N_4O_2^+$	467.2442	467.2441	0.21
	$C_{21}H_{25}N_4O_2^+$	365.1972	365.196	3.29

	$C_{18}H_{27}N_2O_4^+$	335.1965	335.1976	-3.28
	$C_{12}H_{10}N^+$	168.0808	168.0816	-4.76
	$C_7H_{14}NO_2^+$	144.1019	144.1026	-4.86
	$C8H10N^+$	120.0808	120.0814	-5.00
A3	$C_{37}H_{49}N_6O_6^+$	673.3708	673.3725	-2.52
	$C_{37}H_{47}N_6O_5^+$	655.3602	655.3607	-0.76
	$C_{36}H_{45}N_6O_5^+$	641.3446	641.3446	0.00
	$C_{35}H_{45}N_6O_3^+$	597.3548	597.3541	1.17
	$C_{29}H_{36}N_5O_3^+$	502.2813	502.2823	-1.99
	$C_{25}H_{38}N_5O_5^+$	488.2867	488.2876	-1.84
	$C_{29}H_{31}N_4O_2^+$	467.2442	467.2444	-0.43
	$C_{21}H_{25}N_4O_2^+$	365.1972	365.1969	0.82
	$C_{18}H_{27}N_2O_4^+$	335.1965	335.1977	-3.58
	$C_{12}H_{10}N^+$	168.0808	168.0816	-4.76
	$C_7H_{14}NO_2^+$	144.1019	144.1023	-2.78
	$C_8H_{10}N^+$	120.0808	120.0812	-3.33
A4	$C_{38}H_{53}N_6O_{10}S^+$	785.3538	785.3534	0.51
	$C_{38}H_{53}N_6O_7^+$	705.397	705.3958	1.70
	$C_{38}H_{51}N_6O_6^+$	687.3865	687.3833	4.66
	$C_{37}H_{47}N_6O_5^+$	655.3602	655.3584	2.75
	$C_{36}H_{46}N_5O_4^+$	612.3544	612.3525	3.10
	$C_{30}H_{40}N_5O_4^+$	534.3075	534.3076	-0.19
	$C_{30}H_{38}N_5O_3^+$	516.2969	516.2923	8.91
	$C_{30}H_{35}N_4O_3^+$	499.2704	499.2649	11.02
	$C_{28}H_{30}N_3O^+$	424.2383	424.234	10.14
	$C_{20}H_{27}N_4O_3^+$	371.2078	371.2057	5.66
	$C_{18}H_{27}N_2O_4^+$	335.1965	335.1962	0.89
	$C_{12}H_{10}N^+$	168.0808	168.0807	0.59
	$C_7H_{14}NO_2^+$	144.1019	144.1019	0.00
	$C_8H_{10}N^+$	120.0808	120.0797	9.16
A5	$C_{38}H_{51}N_6O_6^+$	687.3865	687.3861	0.58
	$C_{37}H_{47}N_6O_5^+$	655.3602	655.3581	3.20
	$C_{36}H_{46}N_5O_4^+$	612.3544	612.3543	0.16
	$C_{30}H_{38}N_5O_3^+$	516.2969	516.2943	5.04
	$C_{30}H_{35}N_4O_3^+$	499.2704	499.27	0.80
	$C_{28}H_{30}N_3O^+$	424.2383	424.2387	-0.94
	$C_{22}H_{29}N_4O_3^+$	397.2234	397.2221	3.27
	$C_{20}H_{27}N_4O_3^+$	371.2078	371.2076	0.54
	$C_{23}H_{20}N_3O^+$	354.1601	354.1598	0.85
	$C_{22}H_{22}N_3^+$	328.1808	328.181	-0.61
	$C_{14}H_{16}N_3^+$	226.1339	226.133	3.98
	$C_{12}H_{10}N^+$	168.0808	168.0812	-2.38
	$C_7H_{14}NO_2^+$	144.1019	144.1019	0.00
	$C_8H_{10}N^+$	120.0808	120.0807	0.83

A6	$C_{38}H_{51}N_6O_6^+$	687.3865	687.387	-0.73
	$C_{37}H_{47}N_6O_5^+$	655.3602	655.3611	-1.37
	$C_{36}H_{46}N_5O_4^+$	612.3544	612.3544	0.00
	$C_{30}H_{38}N_5O_3^+$	516.2969	516.2944	4.84
	$C_{30}H_{35}N_4O_3^+$	499.2704	499.2699	1.00
	$C_{28}H_{30}N_3O^+$	424.2383	424.2387	-0.94
	$C_{22}H_{29}N_4O_3^+$	397.2234	397.2224	2.52
	$C_{20}H_{27}N_4O_3^+$	371.2078	371.2081	-0.81
	$C_{23}H_{20}N_3O^+$	354.1601	354.1583	5.08
	$C_{22}H_{22}N_3^+$	328.1808	328.1814	-1.83
	$C_{14}H_{16}N_3^+$	226.1339	226.1338	0.44
	$C_{12}H_{10}N^+$	168.0808	168.0811	-1.78
	$C_7H_{14}NO_2^+$	144.1019	144.1016	2.08
	$C_8H_{10}N^+$	120.0808	120.081	-1.67

Table S3

Accuracy data of UPLC method for COB and ATZ

Spiked concentration(µg/ mL)	**concentration found (in µg/mL) (mean ± SD; RSD)**	**Mean recovery (%)**
COB		
120 (at 80%)	119.96 ± 0.41;0.41	99.97
150 (at 100%)	150.13 ± 0.10;0.10	100.08
180 (at 120%)	180.17 ± 0.20;0.20	100.09
ATZ		
240 (at 80%)	240.37 ± 0.34;0.33	100.15
300 (at 100%)	300.24 ± 0.13;0.13	100.08
360 (at 120%)	359.97 ± 0.31;0.31	99.99

Table S4

Precision data of UPLC method for COB and ATZ

Concentration (**µg**/mL) n = 6	Method precision Mean% Assay ± SD;%RSD	Intra-day precision Mean% Assay ± SD;%RSD	Inter-day precision Mean% Assay ± SD;%RSD
COB			
150	99.81 ± 0.57; 0.57	99.83± 0.53; 0.53	99.99± 0.34; 0.34
ATZ			

300	99.97± 0.46; 0.46	99.96± 0.42; 0.42	99.81± 0.44; 0.44

Table S5

Robustness data of UPLC method for COB and ATZ

Conditions	Variation of parameters	Tailing		Retention time (min)		% Assay	
		COB	**ATZ**	**COB**	**ATZ**	**COB**	**ATZ**
Flow rate (ml/min)	0.2	1.72	1.59	10.50	11.58	98.12	98.54
	0.3	1.72	1.45	9.26	10.13	99.05	99.6
	0.4	1.87	1.64	8.62	9.36	100.4	100.2
Column oven temperature (°C)	25	1.69	1.56	9.22	10.05	98.09	98.12
	30	1.70	1.49	9.22	10.09	99.4	99.6
	35	1.69	1.50	9.13	10.03	100.06	100.12
Wavelength (nm)	243	1.68	1.45	9.26	10.13	98.01	100.06
	245	1.66	1.44	9.26	10.13	99.23	100.15
	247	1.66	1.45	9.26	10.13	99.67	100.19