Supporting Information

Large-scale Structural Characterization of Drug and Drug-Like Compounds by High-Throughput Ion Mobility-Mass Spectrometry

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Table SI-1. Comparison of CCS errors from calibration with PolyAla alone and PolyAla combined with the drug-like mixture.

Calibrant	$[M+H]^+$	N_2 DT CCS ($Å^2$)	Error of CCS_{cal} (%)	
			PolyAla	PolyAla+Drug-like
Ala_2	161.0926	136.1	0.1	0.4
Ala_3	232.1300	150.8	-0.3	0.0
Ala_4	303.1670	163.3	0.2	0.5
Ala_5	374.2040	177.6	-0.1	0.2
Ala_6	445.2410	190.8	0.2	0.5
Ala_7	516.2780	206.0	0.0	0.3
Ala_8	587.3150	222.7	-0.1	0.2
Ala_9	658.3520	236.4	0.1	0.4
Ala_{10}	729.3890	249.1	0.1	0.3
Ala_{11}	800.4260	262.6	0.1	0.4
Ala_{12}	871.4630	276.0	0.1	0.4
Ala_{13}	942.5010	289.3	0.1	0.3
Ala_{14}	1013.5370	300.8	0.2	0.4
Ala_{15}	1084.5740	312.8	0.4	0.6
Ala_{16}	1155.6110	327.1	-0.3	-0.1
Ala_{17}	1226.6480	338.2	-0.3	0.0
Ala_{18}	1297.6850	350.1	-0.5	-0.3
Ala_{19}	1368.7220	359.3	0.1	0.3
Ala_{20}	1439.7590	370.2	0.0	0.2
Ala_{21}	1510.8218	379.5	0.5	0.7
Betaine	118.0895	121.5 [a]	-0.2	0.0
Alprenolol	250.1952	157.5 [b]	0.1	0.4
Ondansetron	294.1745	172.7 [b]	-1.3	-1.0
Clozapine N-oxide	343.1425	180.6 [b]	-0.9	-0.6
Verapamil	455.3119	210.0 [b]	-1.1	-0.8
Reserpine	609.3069	254.3 [b]	-3.2	-2.9
Erythromycin	734.4884	257.3	0.8	1.1
Peptide K-8	1072.5385	320.7	-0.6	-0.4
Vancomycin	1448.5166	387.2	-1.4	-1.1

[a] Nitrogen drift tube CCS values from Agilent CCS Calibration Mix, Agilent Technologies.
[b] Nitrogen drift tube CCS values from Campuzano *et al.*, *Anal. Chem.* **2012**, *84*, 1026.

Table SI-2. Comparison of TWIM calibrated N_2 CCS values against DTIM N_2 CCS values from Zhou *et al.* for select compounds.

Compounds	*m/z*	Adduct	CCS_{CAL} ($Å^2$)	$CCS_{Zhou\ et\ al.}$ ($Å^2$) [a]	% Difference CCS_{CAL} : $CCS_{Zhou\ et\ al.}$
Choline	104.108	M^+	121.0	117.8	-2.7
Histamine	112.087	$[M+H]^+$	120.3	120.0	-0.2
Betaine	118.086	$[M+H]^+$	122.5	121.4	-0.9
Creatinine	114.066	$[M+H]^+$	120.2	123.4	2.6
Adenine	136.062	$[M+H]^+$	122.4	125.1	2.1
Hypoxanthine	137.046	$[M+H]^+$	123.1	127.1	3.7
Acetylcholine	146.118	$[M+H]^+$	130.1	129.7	-0.3
Carnitine	162.113	$[M+H]^+$	132.9	133.1	0.1
Arginine	175.119	$[M+H]^+$	135.5	136.4	0.6
Norepinephrine	152.077	$[M+H-H_2O]^+$	132.6	136.8	3.1
Theophylline	181.072	$[M+H]^+$	133.5	137.6	3.1
Cystine	241.031	$[M+H]^+$	148.1	149.4	0.8
Biotin	245.096	$[M+H]^+$	148.0	150.4	1.6
Melatonin	233.129	$[M+H]^+$	152.0	154.3	1.5
Thiamine	265.112	M^+	155.9	162.4	4.1
Glutathione	308.091	$[M+H]^+$	165.7	167.4	1.0
N-acetylneuramic acid	332.095	$[M+Na]^+$	166.6	168.6	1.2
Adenosine phosphate	348.070	$[M+H]^+$	167.6	171.4	2.2
Testosterone	289.216	$[M+H]^+$	170.6	172.7	1.2
Melibiose	365.105	$[M+Na]^+$	174.8	176.8	1.1
Cellobiose	365.105	$[M+Na]^+$	175.7	178.1	1.4
Progesterone	315.232	$[M+H]^+$	177.8	181.2	1.9
Riboflavin	377.146	$[M+H]^+$	184.9	188.3	1.8
Cortisone	361.201	$[M+H]^+$	184.4	188.6	2.2
Hydrocortisone	363.217	$[M+H]^+$	185.1	189.1	2.2
Cholic acid	431.277	$[M+Na]^+$	195.6	197.3	0.9
Curcumin	369.133	$[M+H]^+$	198.5	201.6	1.5
Glycholic acid	488.298	$[M+Na]^+$	200.8	202.0	0.6
Lathosterol	369.352	$[M+H-H_2O]^+$	204.0	204.5	0.2
Naringin	603.168	$[M+Na]^+$	233.5	237.5	1.7
Protoporphyrin IX	563.265	$[M+H]^+$	238.8	241.4	1.1

[a] Nitrogen drift tube CCS values from Zhou *et al.*, *Anal. Chem.* **2016**, *88*, 11084-11091.

Table SI-3. Comparison of TWIM calibrated N_2 CCS values against N_2 CCS values determined on the modified Waters Synapt G2 HDMS containing a radio-frequency confining drift cell for select compounds.

Compounds	*m/z*	Adduct	CCS_{CAL} ($Å^2$)	$CCS_{RF\text{-}DT}$ ($Å^2$)	%RSD $CCS_{RF\text{-}DT}$	% Difference CCS_{CAL} : $CCS_{RF\text{-}DT}$
Hypoxanthine	137.046	$[M+H]^+$	123.1	124.4	0.6	1.0
Norepinephrine	152.077	$[M+H-H_2O]^+$	132.6	133.5	0.0	0.7
Phenacetin	180.102	$[M+H]^+$	140.5	138.6	0.3	-1.4
Theophylline	181.072	$[M+H]^+$	133.5	133.7	0.1	0.2
Thiamine	265.112	M^+	155.9	160.0	0.5	2.6
Desipramine	267.186	$[M+H]^+$	162.1	160.6	0.3	-0.9
Dextromethorphan	272.201	$[M+H]^+$	165.2	162.8	0.2	-1.4
Fluoxetine	310.141	$[M+H]^+$	174.5	175.1	0.4	0.4
Chlorpromazine	319.103	$[M+H]^+$	169.8	168.5	0.2	-0.8
Ciprofloxacin	332.141	$[M+H]^+$	185.3	187.3	0.3	1.1
Thioridazine	371.161	$[M+H]^+$	185.0	183.6	0.2	-0.8
Tamoxifen	372.232	$[M+H]^+$	197.2	194.0	0.3	-1.7
Diltiazem	415.169	$[M+H]^+$	196.1	194.8	0.1	-0.7
Raloxifene	474.173	$[M+H]^+$	213.4	212.7	0.1	-0.3

Table SI-4. Comparison of N_2 CCS values reported by Zhou *et al.* against those determined on the modified Waters Synapt G2 HDMS containing a radio-frequency confining drift cell for select compounds.

Compounds	*m/z*	Adduct	$CCS_{RF\text{-}DT}$ ($Å^2$)	$CCS_{Zhou\ et\ al.}$ ($Å^2$) [a]	% Difference $CCS_{RF\text{-}DT}$: $CCS_{Zhou\ et\ al.}$
Hypoxanthine	137.046	$[M+H]^+$	124.4	127.8	-2.7
Norepinephrine	152.077	$[M+H-H_2O]^+$	133.5	136.8	-2.4
Theophylline	181.072	$[M+H]^+$	133.7	137.6	-2.9
Thiamine	265.112	M^+	160.0	162.4	-1.5

[a] Nitrogen drift tube CCS values from Zhou *et al.*, *Anal. Chem.* **2016**, *88*, 11084-11091.

Table SI-5. Comparison of TWIM calibrated CCS values from this work versus TWIM calibrated CCS values presented elsewhere for select compounds.

Compounds	*m/z*	Adduct	$CCS_{Hines\ et\ al.}$ ($Å^2$)	$CCS_{Aleylunas\ et\ al.}$ ($Å^2$) [a]	% Difference
Tacrine	199.123	$[M+H]^+$	141.4	141.2	0.2
Pilocarpine	209.129	$[M+H]^+$	146.4	146.7	-0.2
Altretamine	211.167	$[M+H]^+$	145.6	146.0	-0.3
Lidocaine	235.181	$[M+H]^+$	155.4	156.8	-0.8
Pindolol	249.160	$[M+H]^+$	157.6	159.2	-1.0
Lamtrigine	256.015	$[M+H]^+$	150.6	151.1	-0.3
Imipramine	281.201	$[M+H]^+$	165.1	165.5	-0.2
Promethazine	285.142	$[M+H]^+$	162.7	163.6	-0.6
Terbinafine	292.206	$[M+H]^+$	185.6	187.6	-1.1
Dobutamine	302.175	$[M+H]^+$	168.0	168.9	-0.6
Amoxapine	314.106	$[M+H]^+$	171.2	171.8	-0.3
Timolol	317.164	$[M+H]^+$	174.6	175.8	-0.7
Chlorpromazine	319.103	$[M+H]^+$	169.8	170.6	-0.5
Escitalopram	325.171	$[M+H]^+$	178.8	179.7	-0.5
Citalopram	325.171	$[M+H]^+$	178.9	179.1	-0.1
Clozapine	327.137	$[M+H]^+$	177.5	178.4	-0.5
Piroxicam	332.070	$[M+H]^+$	170.7	171.6	-0.5
Naltrexone	342.170	$[M+H]^+$	176.0	176.7	-0.4
Propafenone	342.206	$[M+H]^+$	177.2	178.1	-0.5
Meloxicam	352.043	$[M+H]^+$	174.5	175.5	-0.5
Pioglitazone	357.127	$[M+H]^+$	178.0	177.9	0.1
Bisacodyl	362.139	$[M+H]^+$	193.3	193.9	-0.3
Bumetanide	365.117	$[M+H]^+$	185.1	186.2	-0.6
Haloperidol	376.147	$[M+H]^+$	191.7	193.5	-0.9
Celecoxib	382.083	$[M+H]^+$	186.1	186.5	-0.2
Prazosin	384.167	$[M+H]^+$	193.4	193.6	-0.1
Quetiapine	384.174	$[M+H]^+$	191.5	191.8	-0.2
Buspirone	386.255	$[M+H]^+$	197.7	197.9	-0.1
Moxifloxacin	402.183	$[M+H]^+$	195.0	196.8	-0.9
Carvedilol	407.197	$[M+H]^+$	189.1	188.9	0.1
Lincomycin	407.221	$[M+H]^+$	199.1	200.6	-0.7
Diltiazem	415.169	$[M+H]^+$	196.1	196.1	0.0
Clindamycin	425.187	$[M+H]^+$	200.6	202.2	-0.8
Mycophenolate	434.217	$[M+H]^+$	197.3	196.6	0.4
Fluphenazine	438.182	$[M+H]^+$	197.5	197.2	0.1
Aripiprazole	448.155	$[M+H]^+$	203.5	204.5	-0.5
Loperamide	477.230	$[M+H]^+$	219.4	221.9	-1.1
Vardenafil	489.228	$[M+H]^+$	224.5	227.2	-1.2
Dipyridamole	505.325	$[M+H]^+$	223.9	225.3	-0.6
Ketoconazole	531.156	$[M+H]^+$	214.5	214.6	-0.1
Azithromycin	749.516	$[M+H]^+$	263.5	265.1	-0.6
Vinblastine	811.428	$[M+H]^+$	278.5	284.2	-2.0
Rifampicin	823.412	$[M+H]^+$	280.3	286.4	-2.1
Ivermectin	897.499	$[M+Na]^+$	292.7	299.9	-2.4
Sirolimus	936.544	$[M+Na]^+$	311.6	323.1	-3.6

[a] Nitrogen TWIM CCS values from Y. W. Alelyunas *et al.*, *Waters Application Note 720005903EN* **2017**, Milford, MA: Waters Corporation.

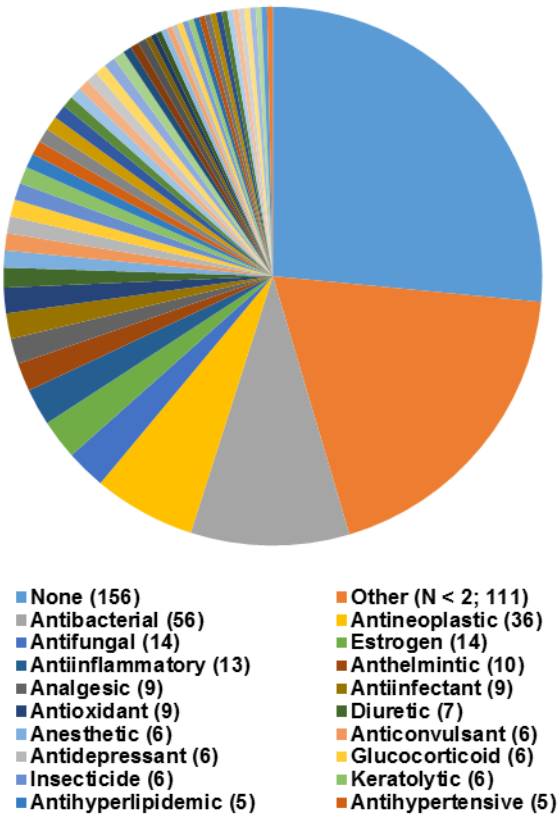

Figure SI-1. A summary of the bioactivities of the 560 drug and drug-like compounds that were not successfully determined in this analysis.

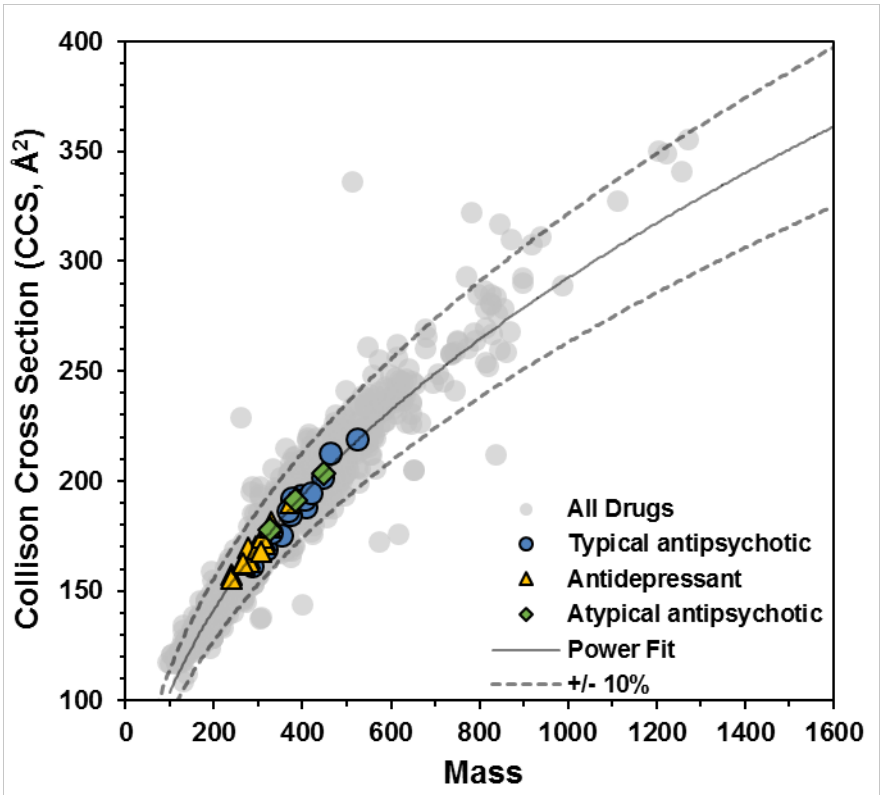

Figure SI-2. CCS versus mass trends of antidepressants, typical antipsychotics, and atypical antipsychotics. Antidepressants include SNRIs, SSRIs, NDRIs and NRIs.

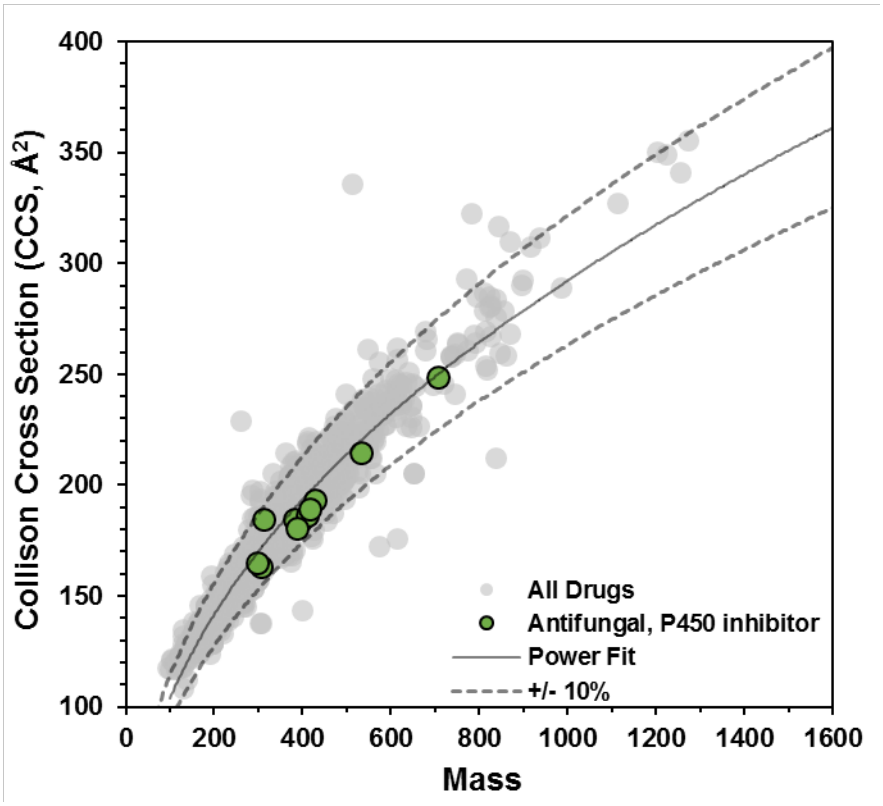

Figure SI-3. CCS versus mass trend for the antifungal P450 inhibitors.

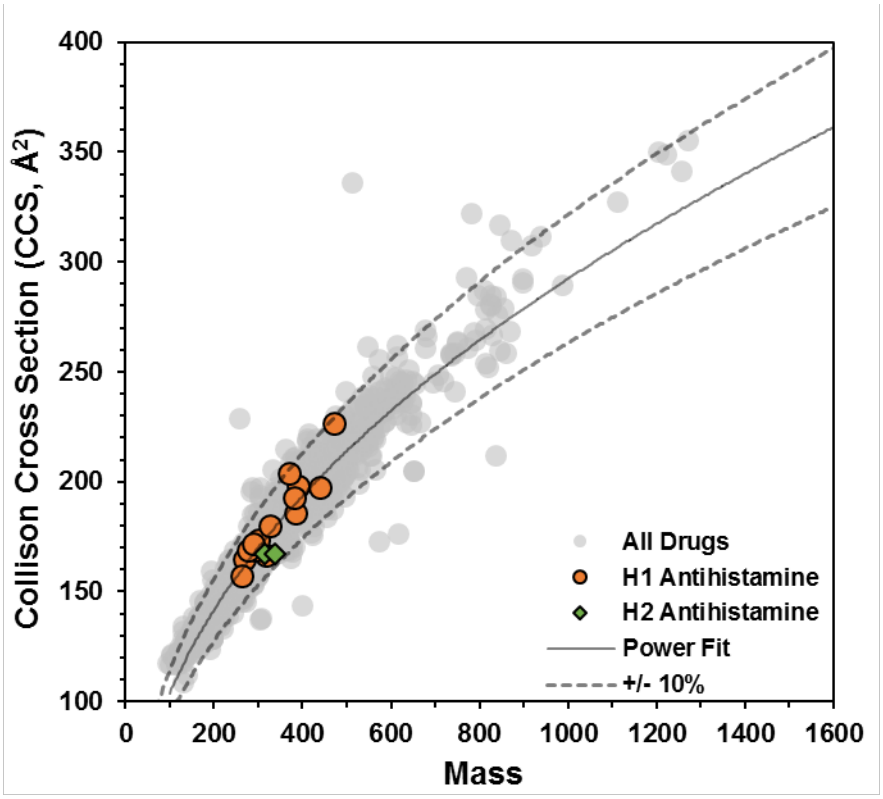

Figure SI-4. CCS versus mass trends for H1 and H2 antihistamines.

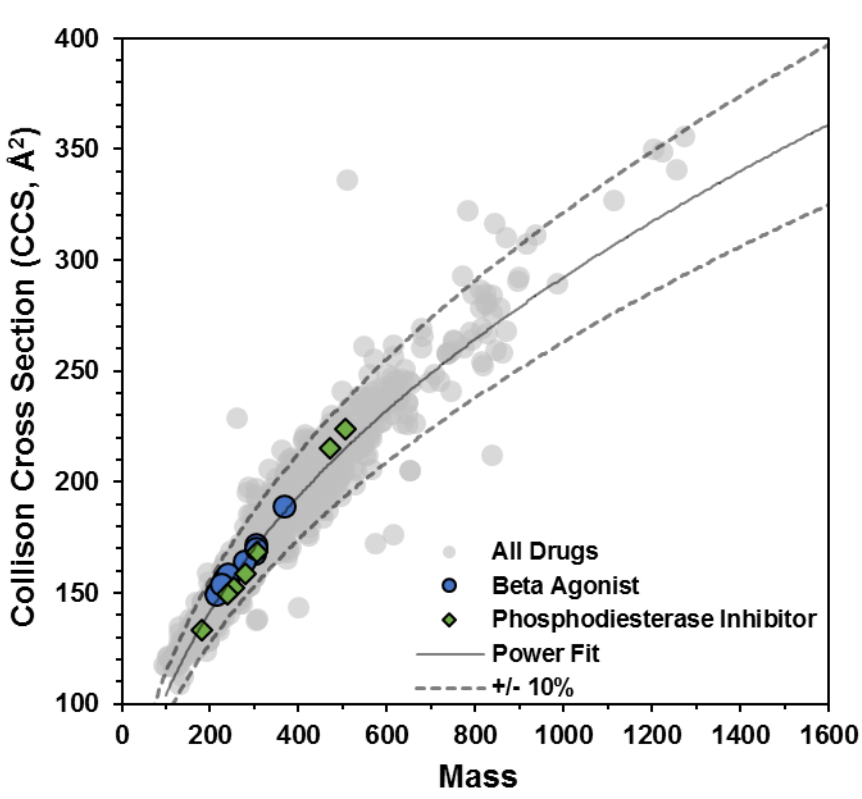

Figure SI-5. CCS versus mass trends for the beta agonist and phosphodiesterase inhibitors which are used as vasodilators and bronchodilators.

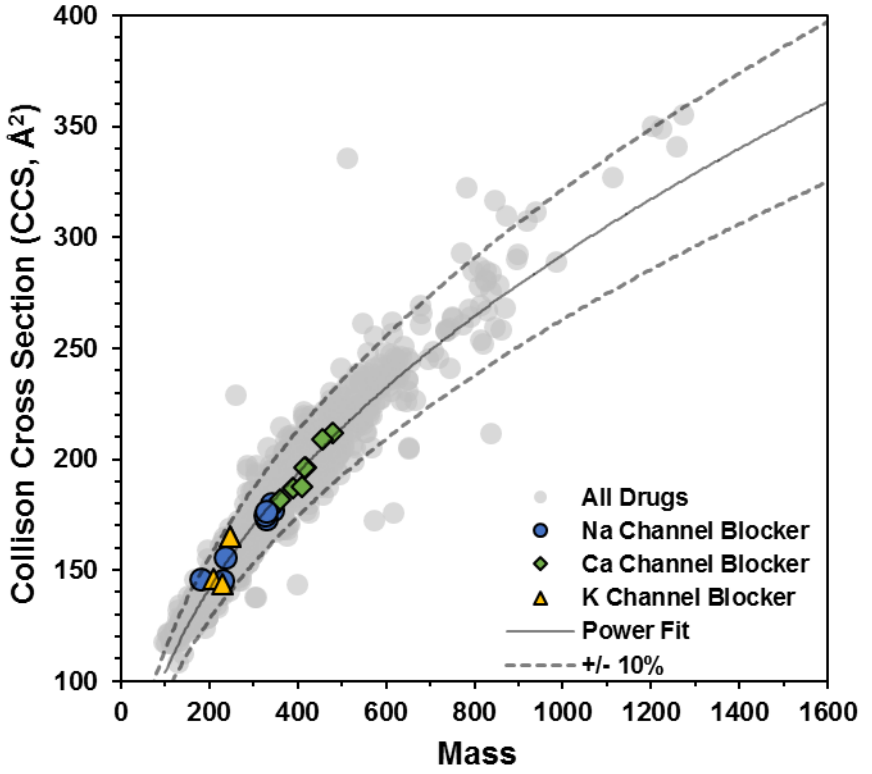

Figure SI-6. CCS versus mass trends for various ion channel blockers. Sodium channel blockers are mostly antiarrhythmic drugs. Calcium channel blockers are mostly vasodilators. Potassium channel blockers are mostly antihypertensive drugs.

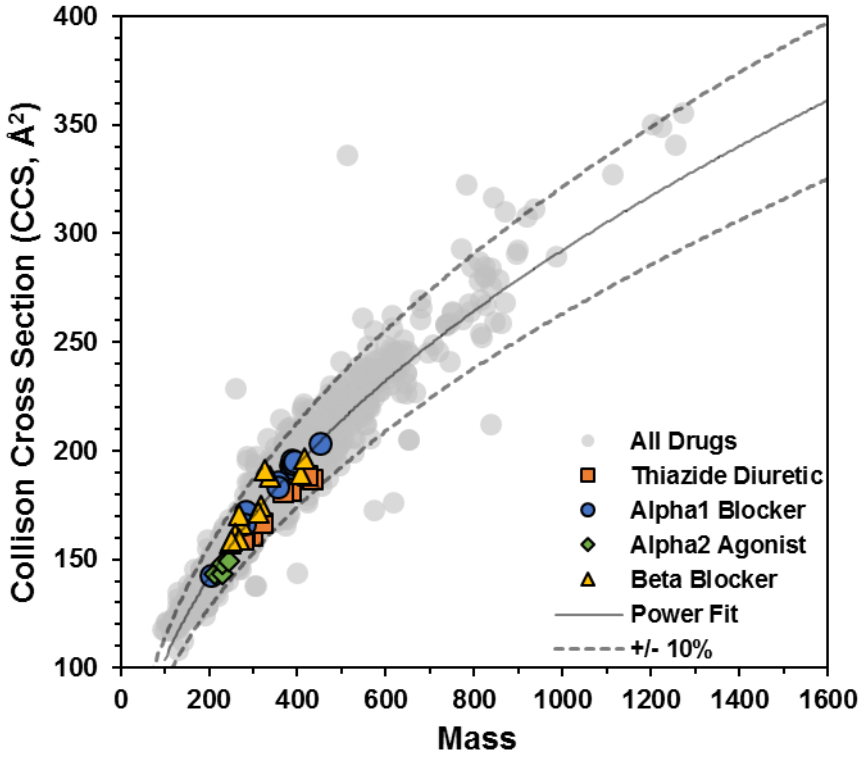

Figure SI-7. CCS versus mass trends for thiazide diuretics, alpha1 blockers, and alpha2 agonists. Alpha 1 blockers and alpha 2 agonists are antihypertensive drugs.

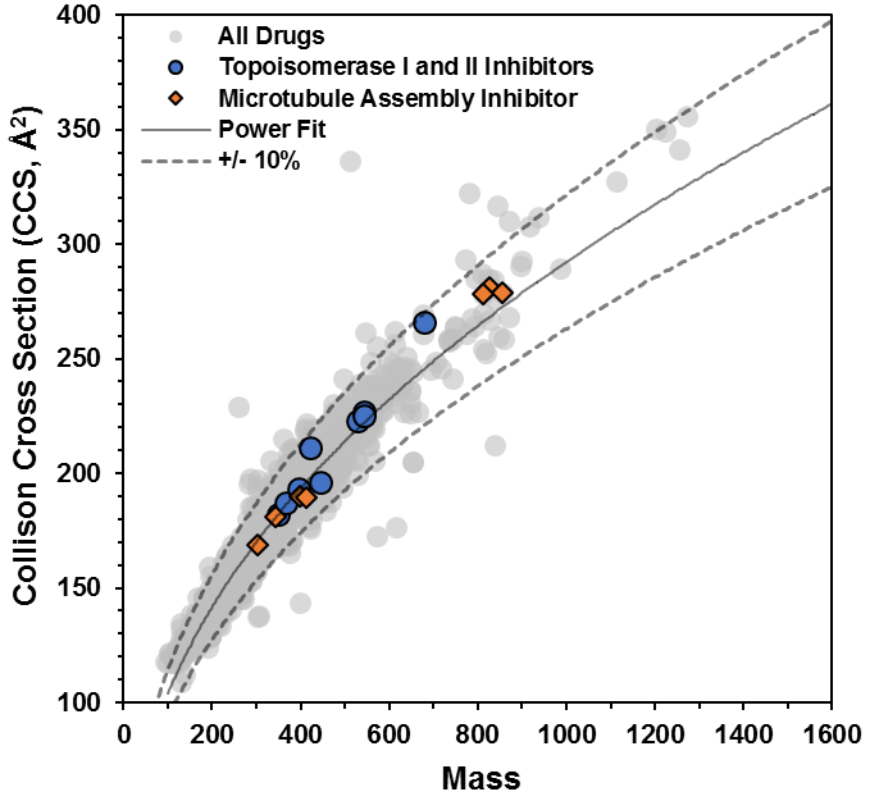

Figure SI-8. CCS versus mass trends for antineoplastic topoisomerase I and II inhibitors and microtubule assembly inhibitors.

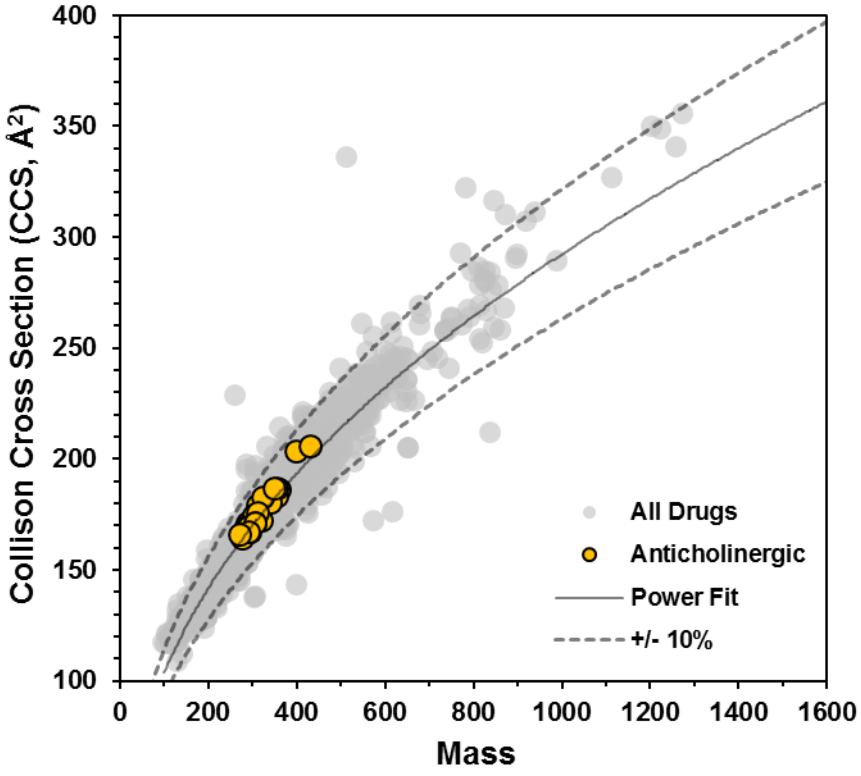

Figure SI-9. CCS versus mass trend for the anticholinergic drugs.

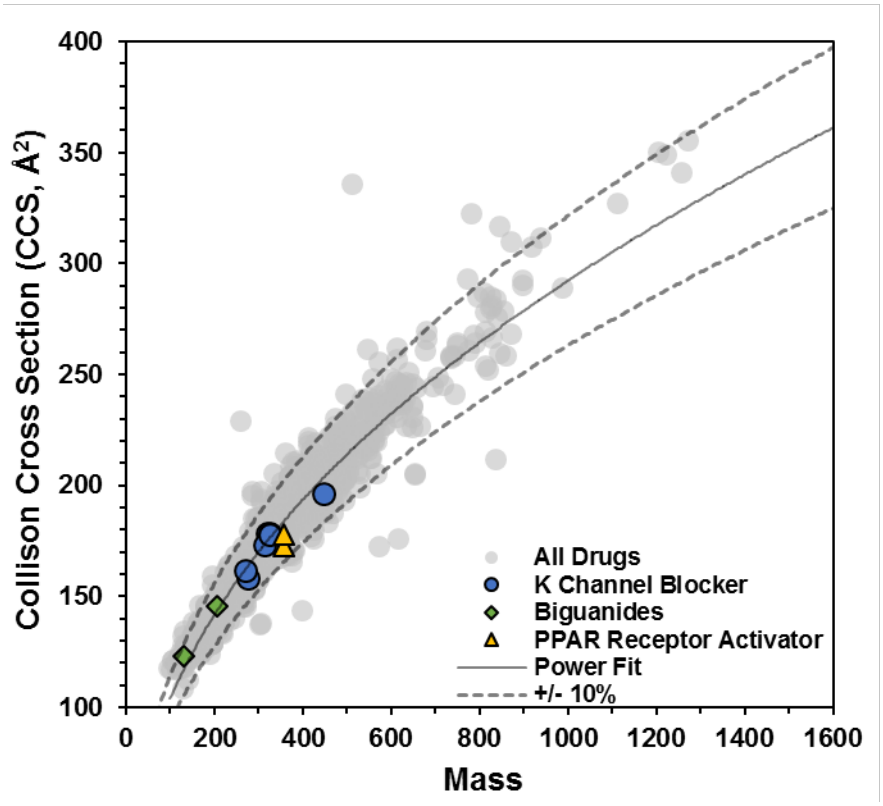

Figure SI-10. CCS versus mass trends for K channel blockers, biguanides, and PPAR receptor activators which are used in the treatment of diabetes.

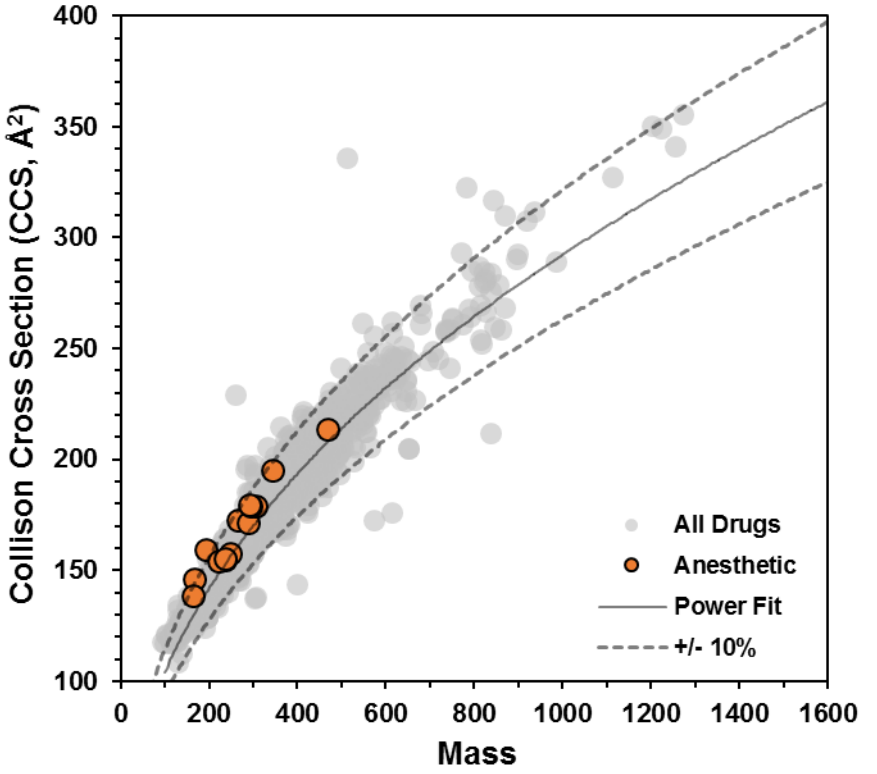

Figure SI-11. CCS versus mass trend for the local and topical anesthetics.

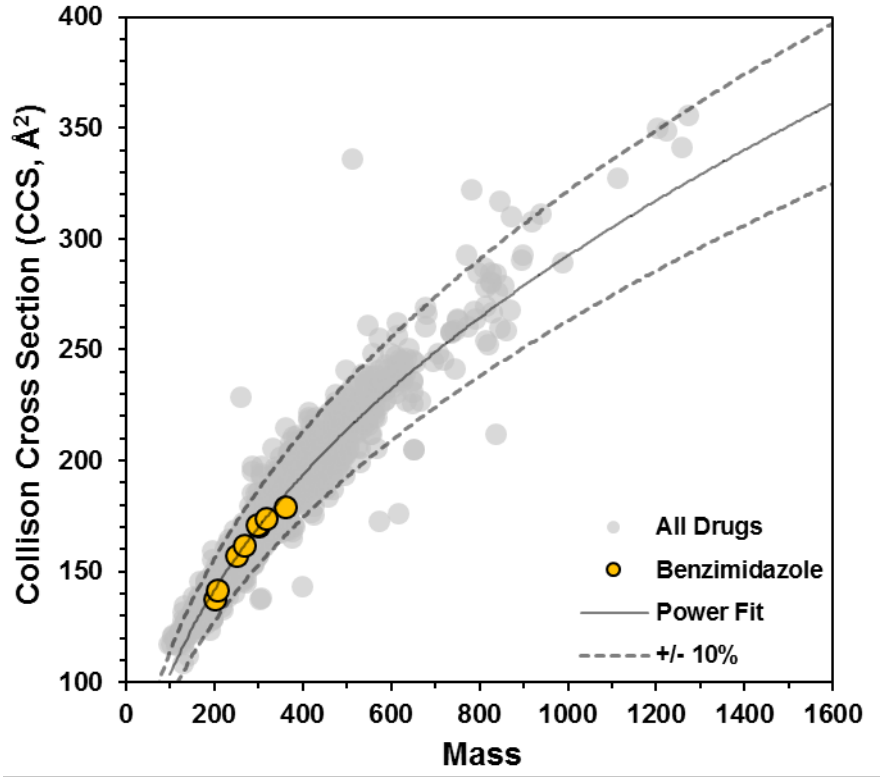

Figure SI-12. CCS versus mass trend for the benzimidazole anthelmintic drugs.

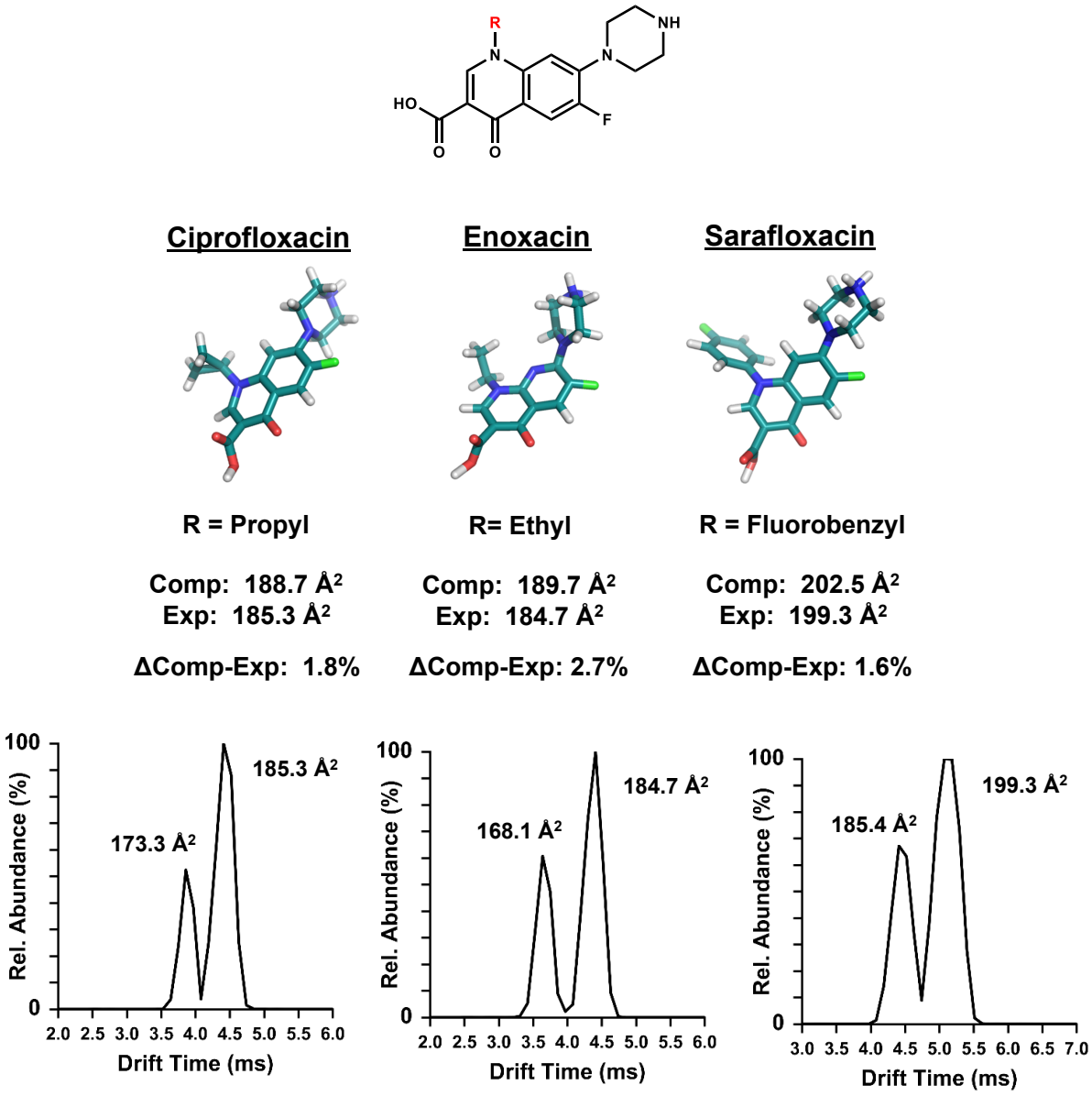

Figure SI-13. Several fluoroquinolones showed bimodal arrival time distributions. Molecular modeling by MMFF94 and CCS calculation by MobCal were able to match the larger of the two conformations for each of the fluoroquinolones with 3%. The CCS values of fluoroquinolones scale with increasing size of the R group. Note that the aromatic ring for enoxacin is a pyridine ring, instead of a phenyl ring.

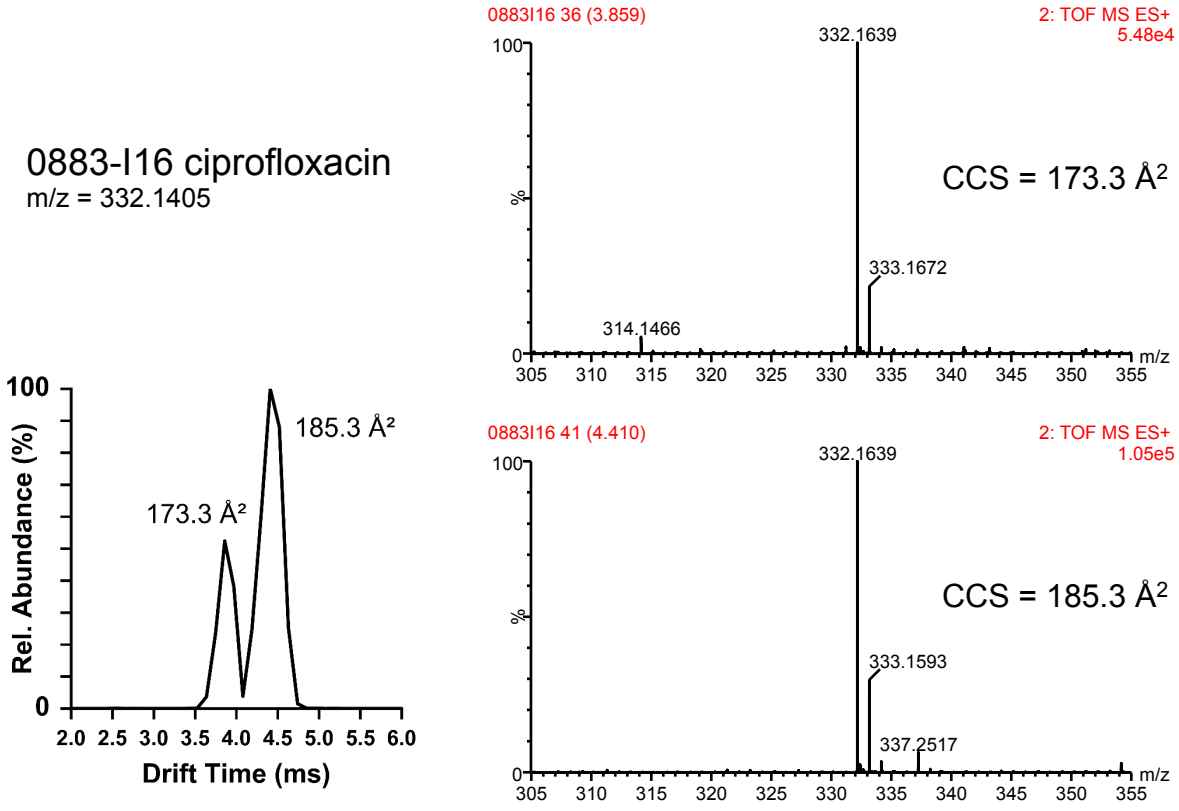

Figure SI-14. Drift time chromatogram and mass spectra of ciprofloxacin.

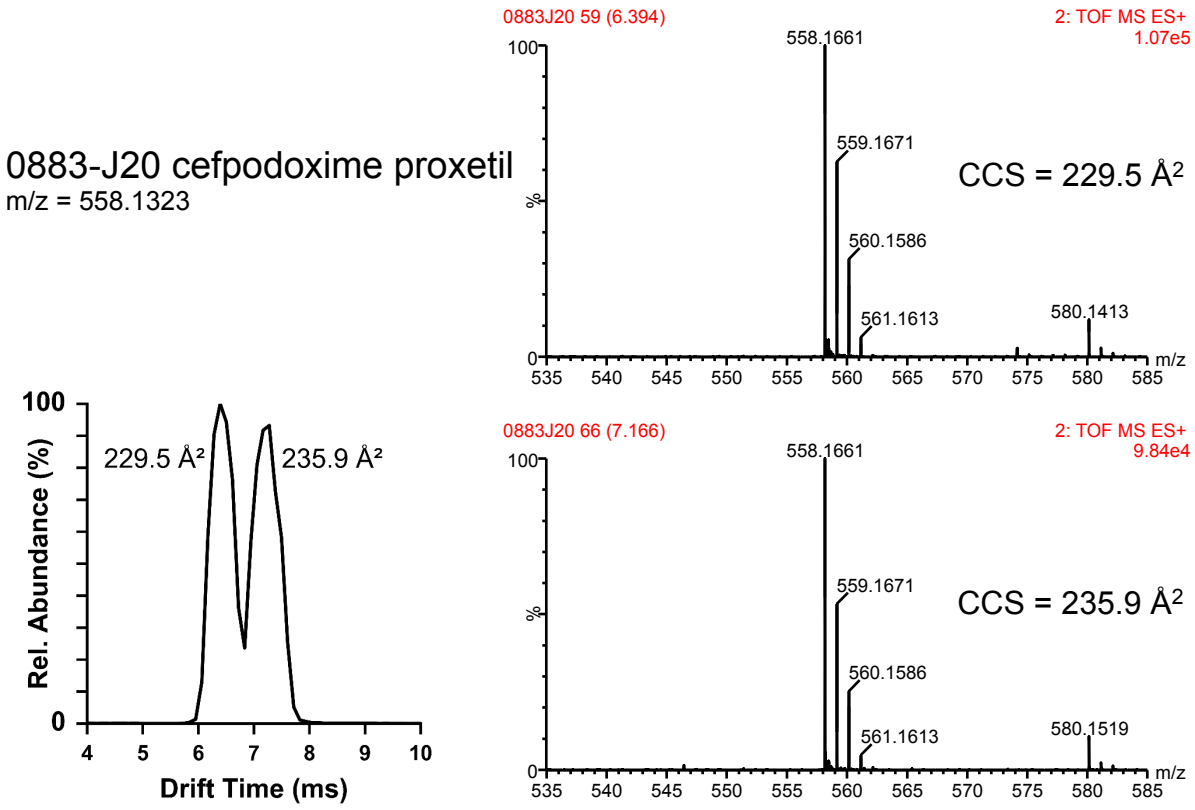

Figure SI-15. Drift time chromatogram and mass spectra of cefpodoxime proxetil.

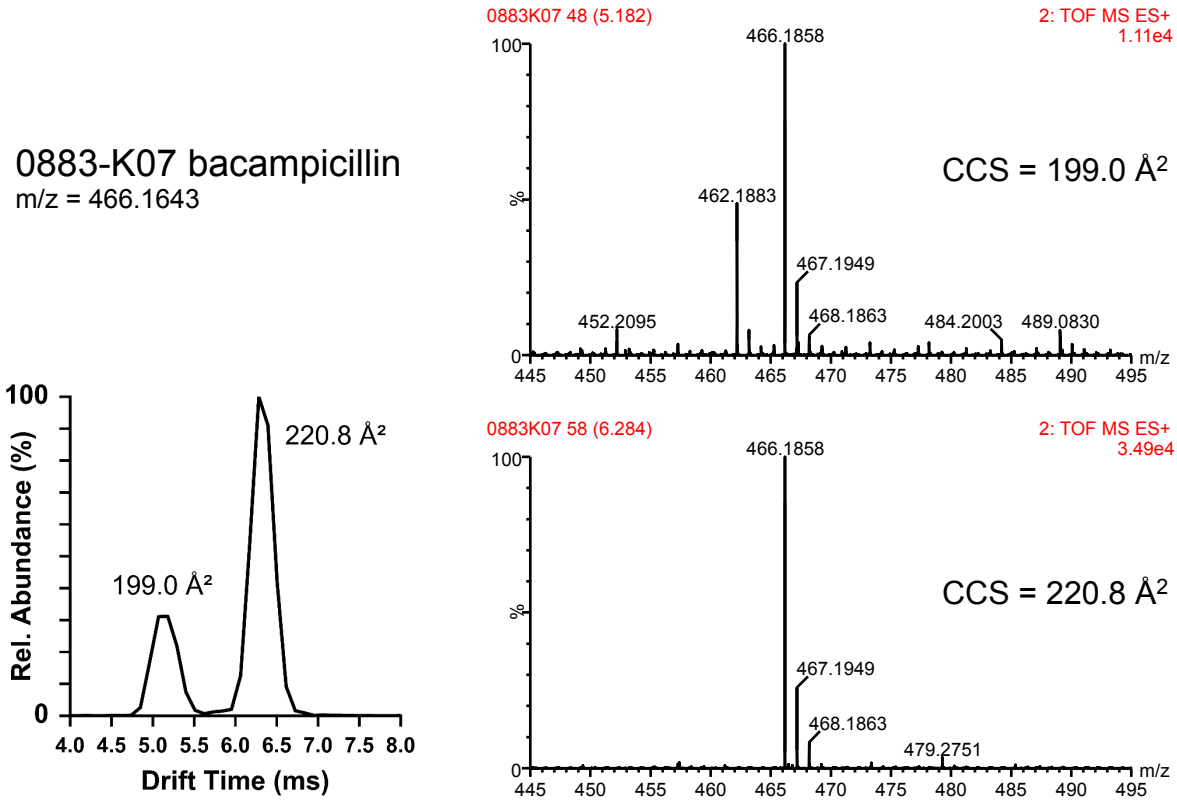

Figure SI-16. Drift time chromatogram and mass spectra of bacampicillin.

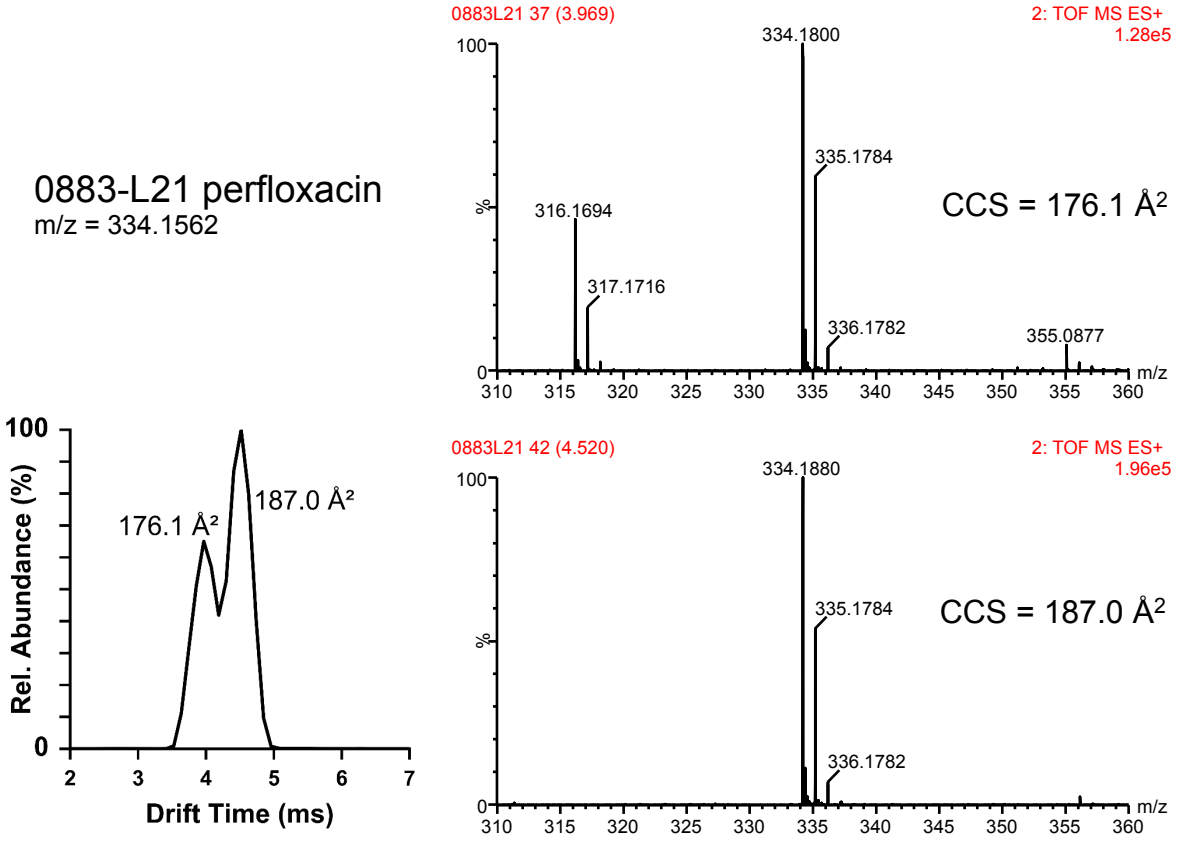

Figure SI-17. Drift time chromatogram and mass spectra of perfloxacin.

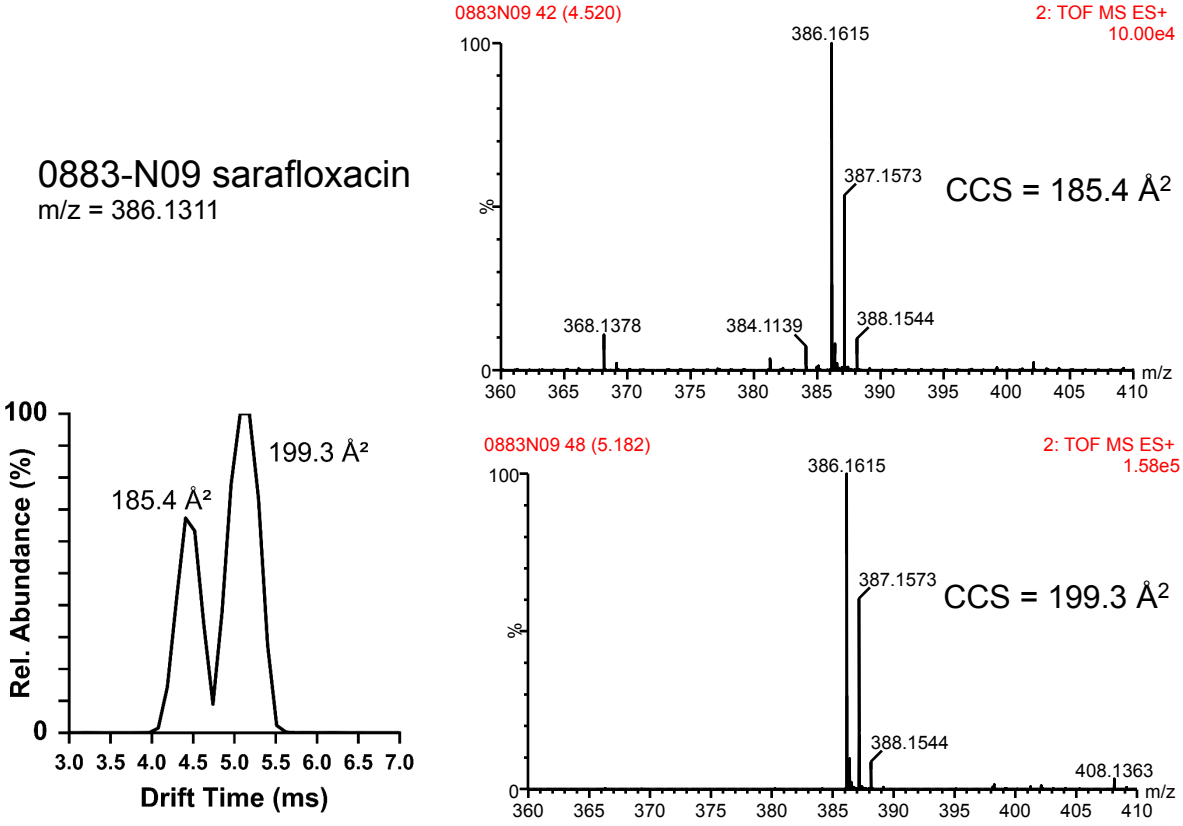

Figure SI-18. Drift time chromatogram and mass spectra of sarafloxacin.

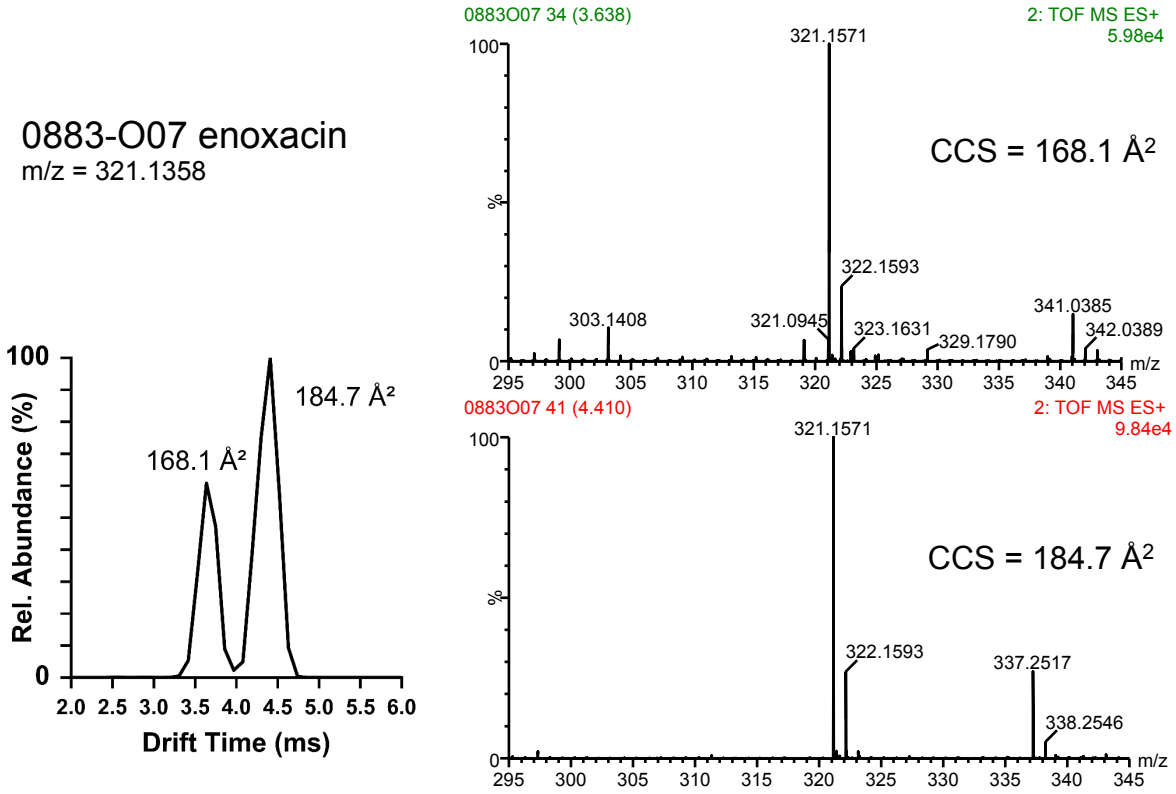

Figure SI-19. Drift time chromatogram and mass spectra of enoxacin.

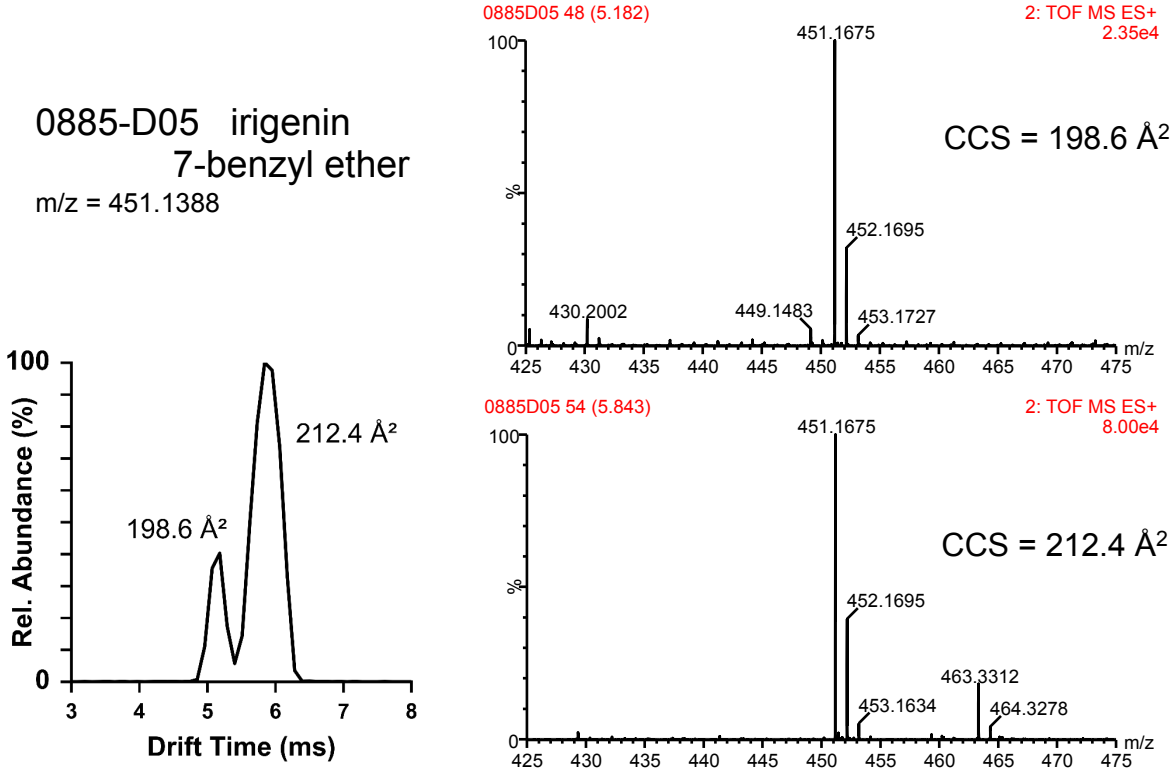

Figure SI-20. Drift time chromatogram and mass spectra of irigenin 7-benzyl ether.

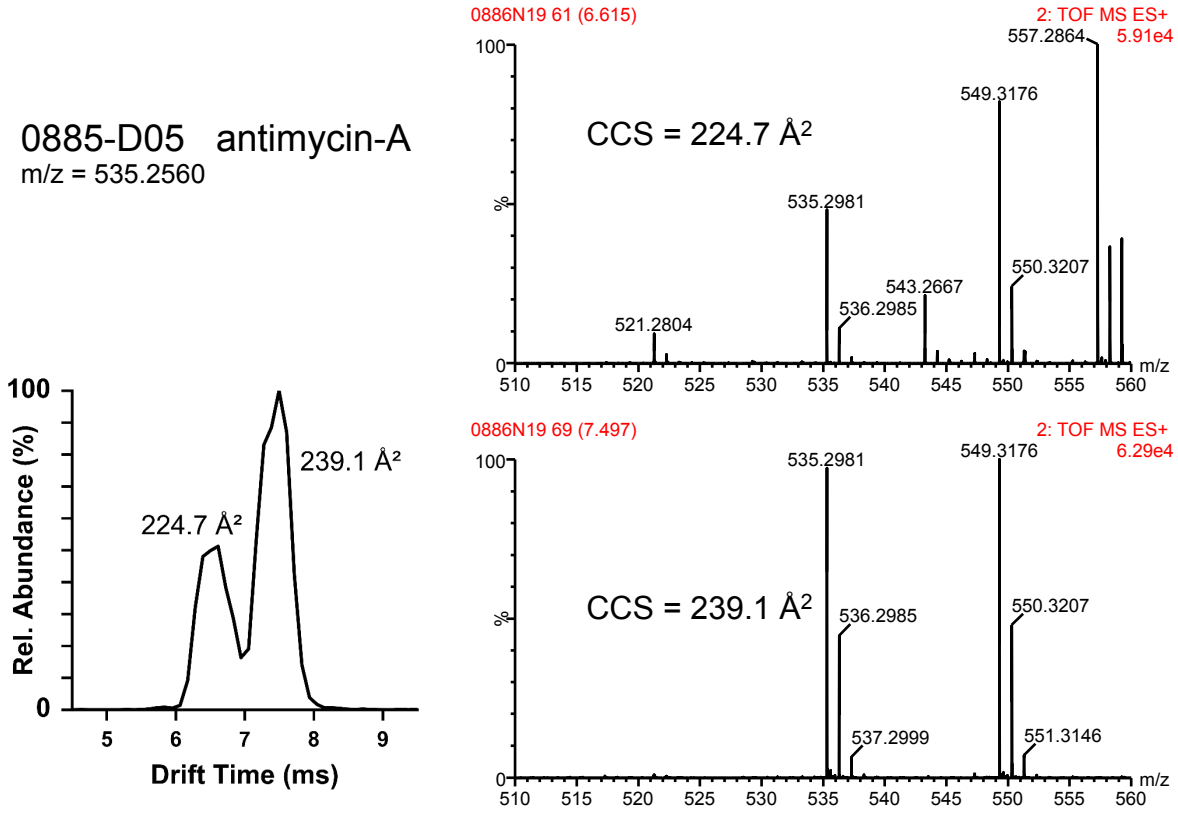

Figure SI-21. Drift time chromatogram and mass spectra of antimycin-A.

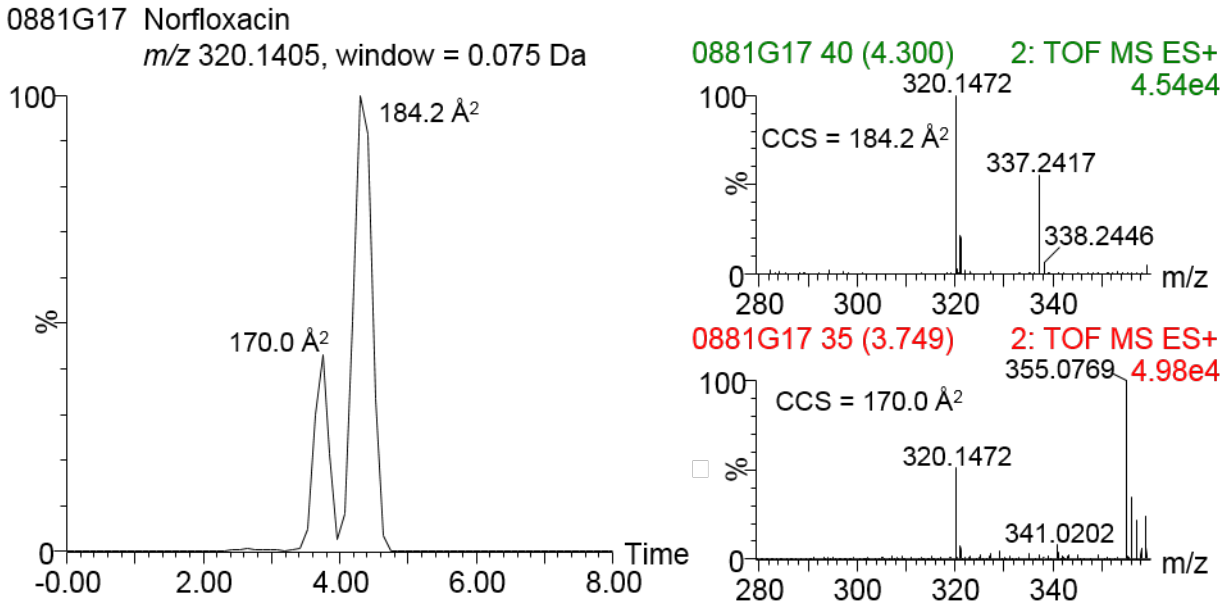

Figure SI-22. Drift time chromatogram and mass spectra of norfloxacin.

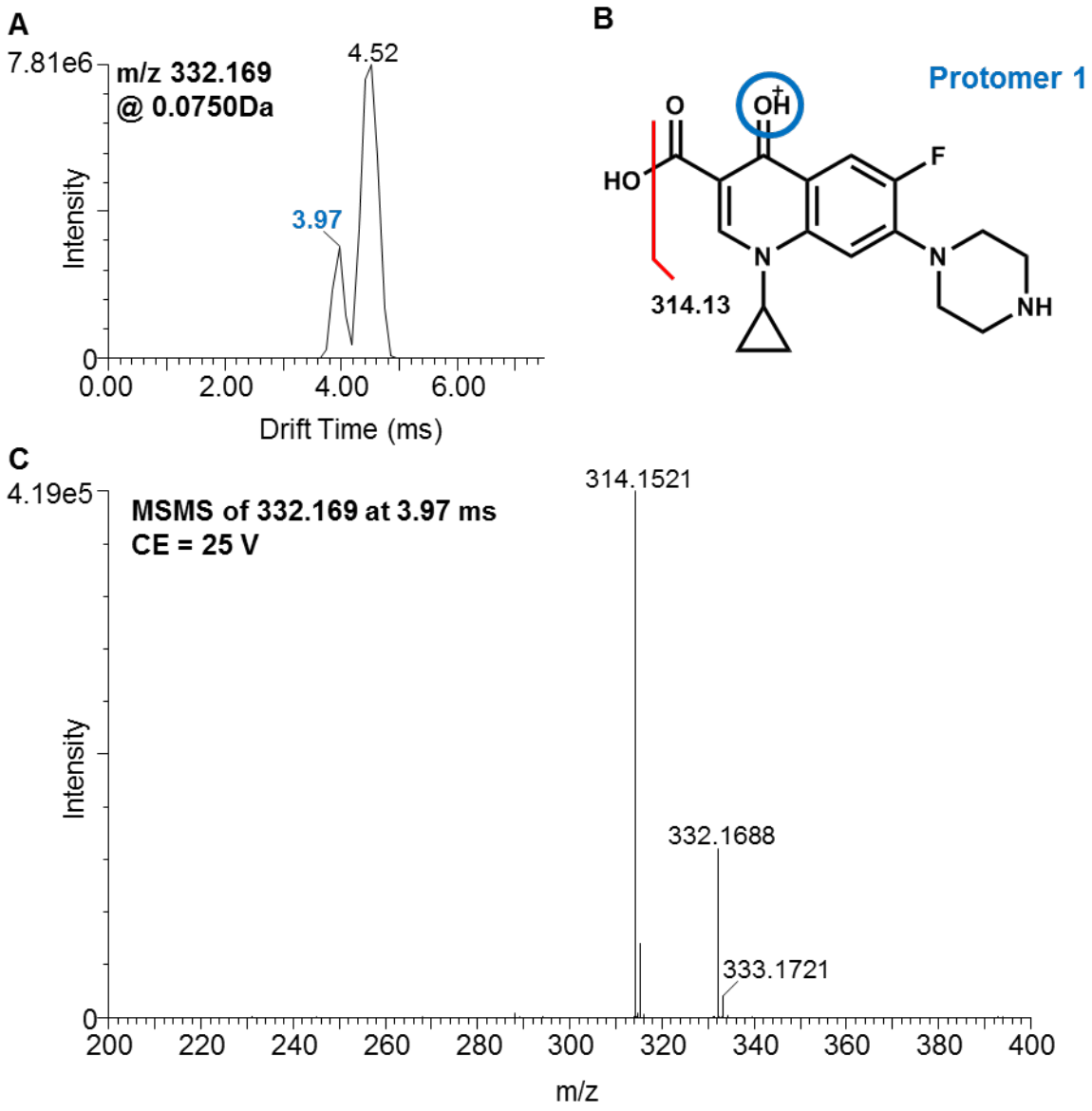

Figure SI-23. Post-ion mobility MS/MS analysis of the ciprofloxacin protomer with (A) a drift time of 3.97 ms corresponding to protonation of the ring carbonyl oxygen (B). The major fragments observed (C) are displayed on the structure shown in (B).

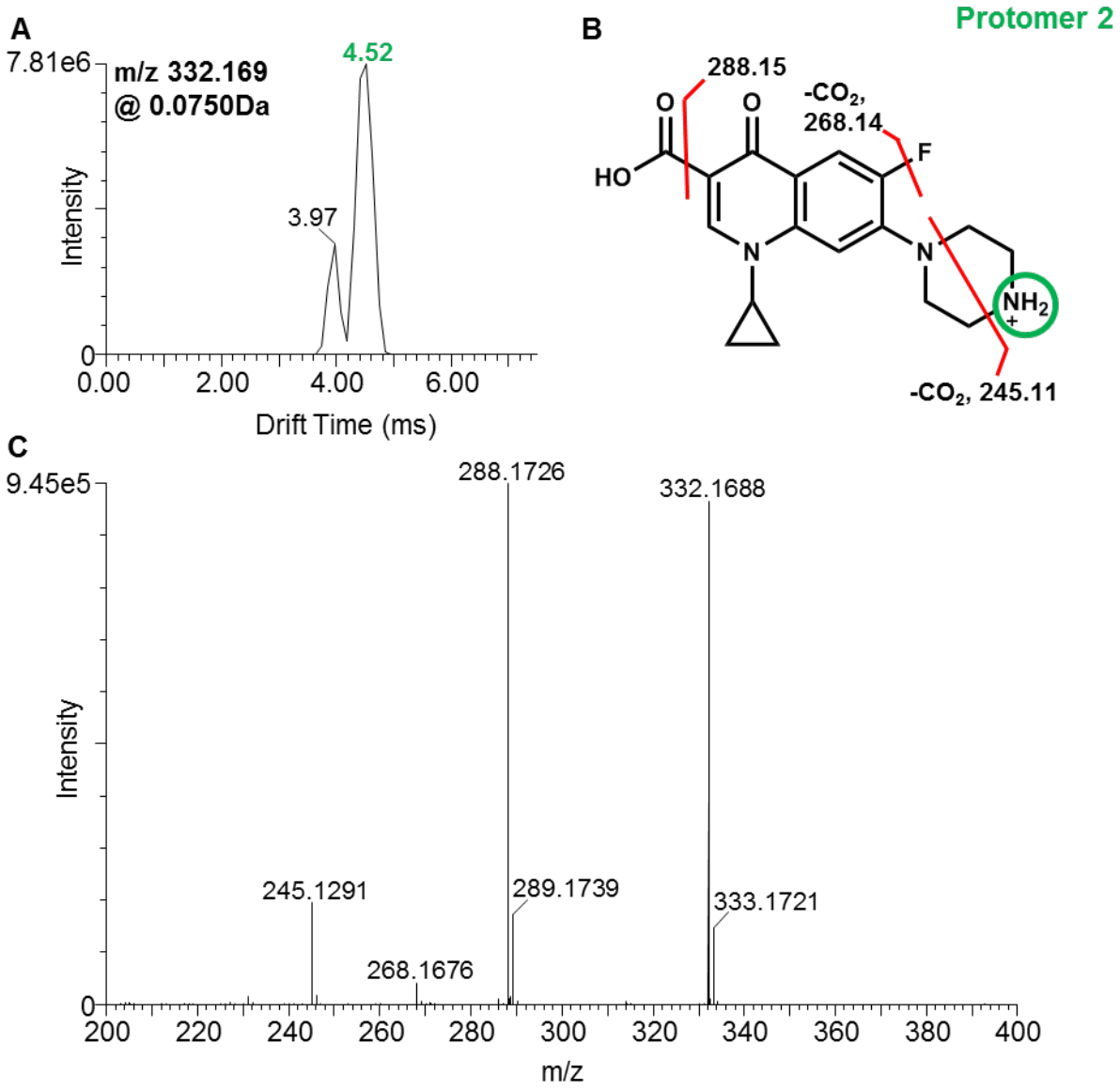

Figure SI-24. Post-ion mobility MS/MS analysis of the ciprofloxacin protomer with (A) a drift time of 4.52 ms corresponding to protonation of the terminal piperazine nitrogen (B). The major fragments observed (C) are displayed on the structure shown in (B).

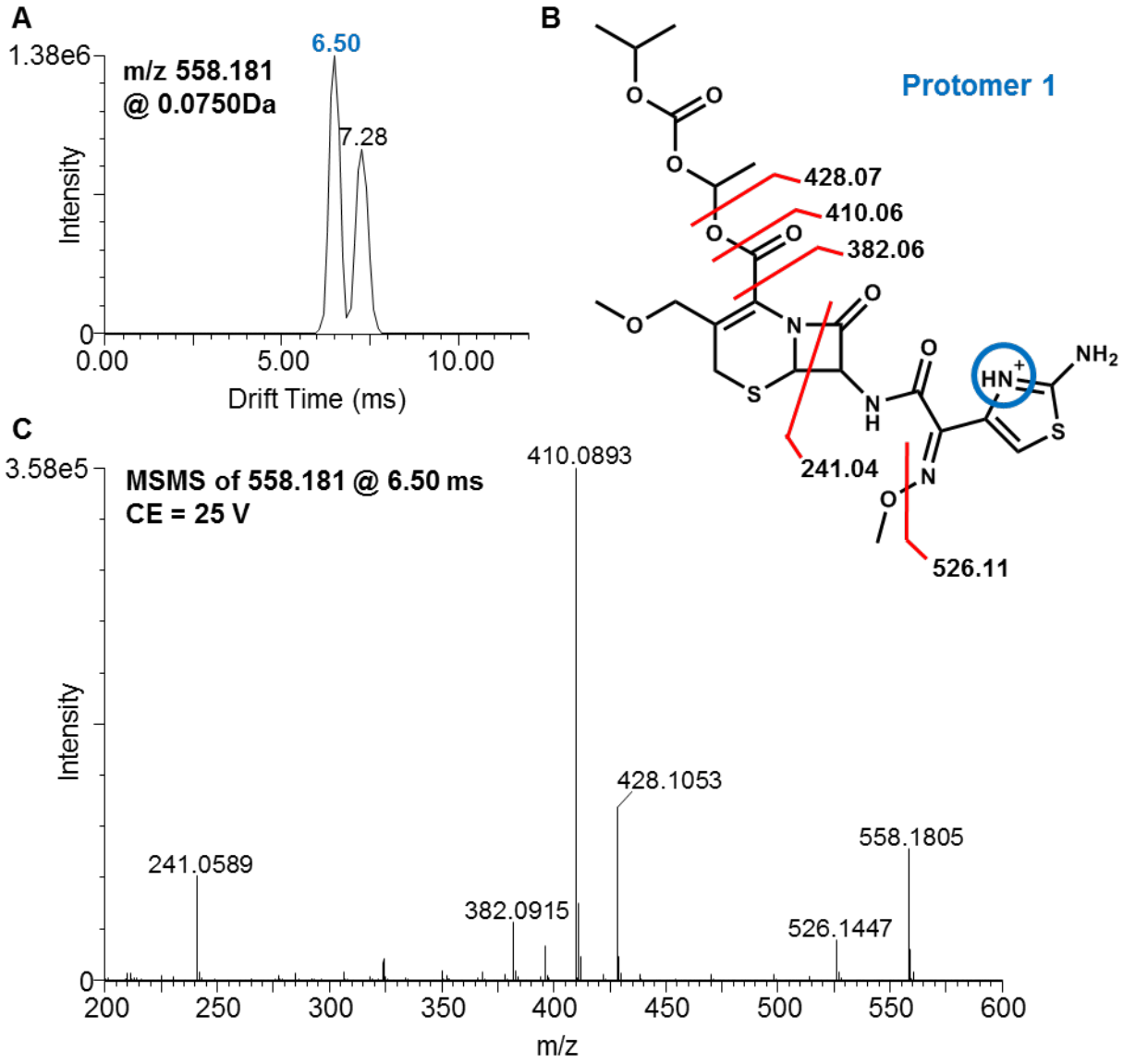

Figure SI-25. Post-ion mobility MS/MS analysis of the cefpodoxime proxetil protomer with (A) a drift time of 6.5 ms corresponding to protonation of the thiazole nitrogen (B). The major fragments observed (C) are displayed on the structure shown in (B).

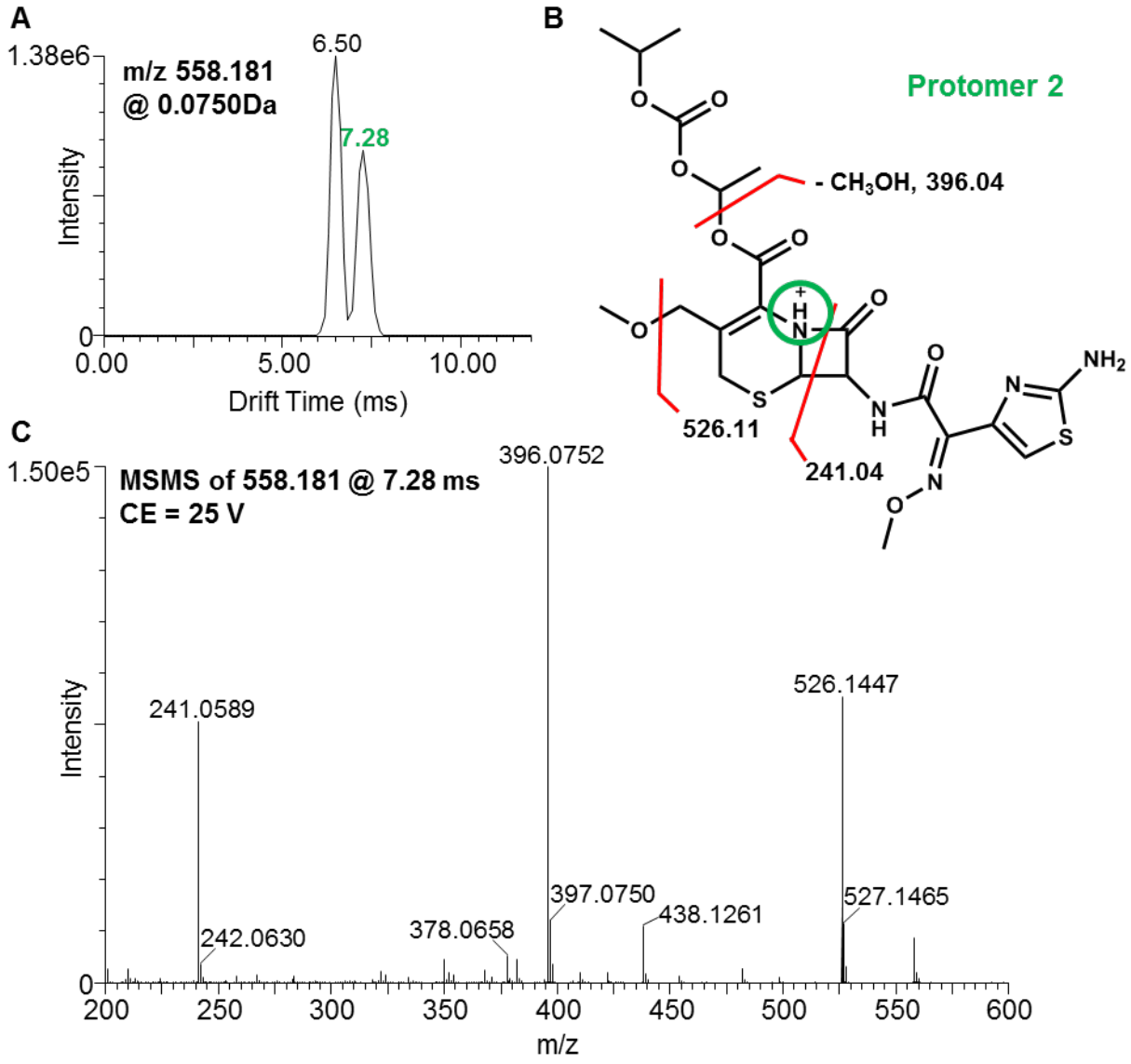

Figure SI-26. Post-ion mobility MS/MS analysis of the cefpodoxime proxetil protomer with (A) a drift time of 7.28 ms corresponding to protonation of the β-lactam nitrogen (B). The major fragments observed (C) are displayed on the structure shown in (B).

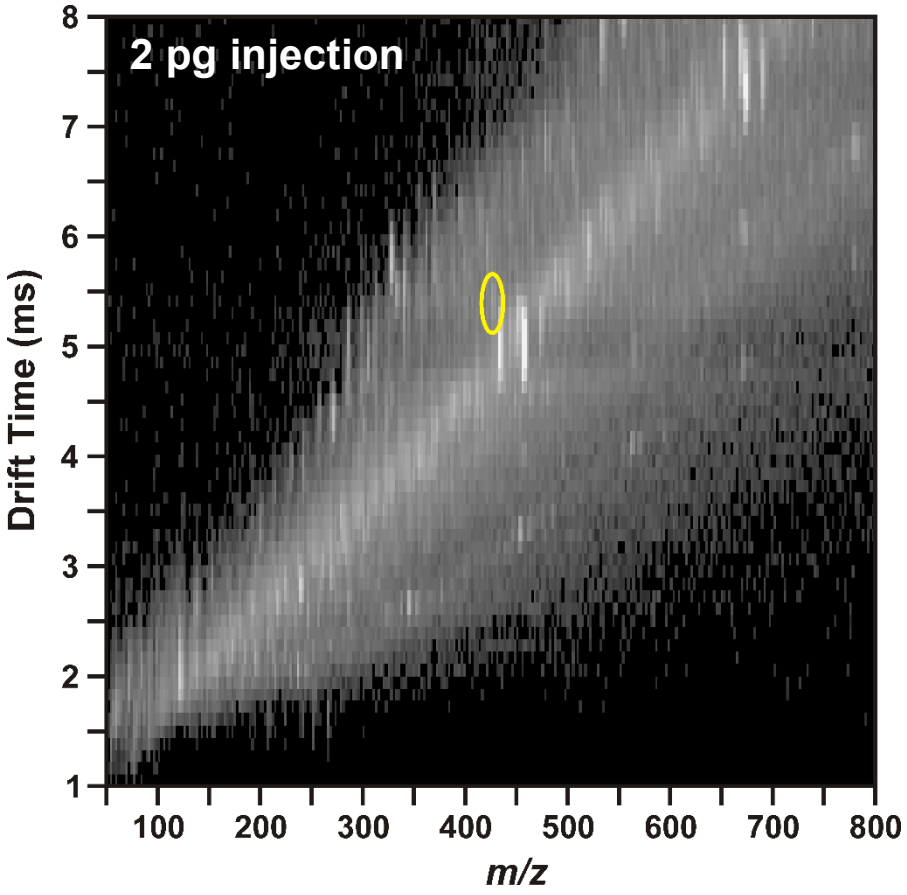

Figure SI-27. A 2D IM-MS plot from the analysis of a 300 mg capsule of clindamycin, which accompanies Figure 5 in the main text. The signal corresponding to protonated clindamycin, *m/z* 425.18, is highlighted in the yellow oval.