Supporting Information – Characterization of the Effect of Drug Metabolism on the Gas-Phase Structures of Drugs Using Ion Mobility-Mass Spectrometry

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Contents

1 – Experimental Section

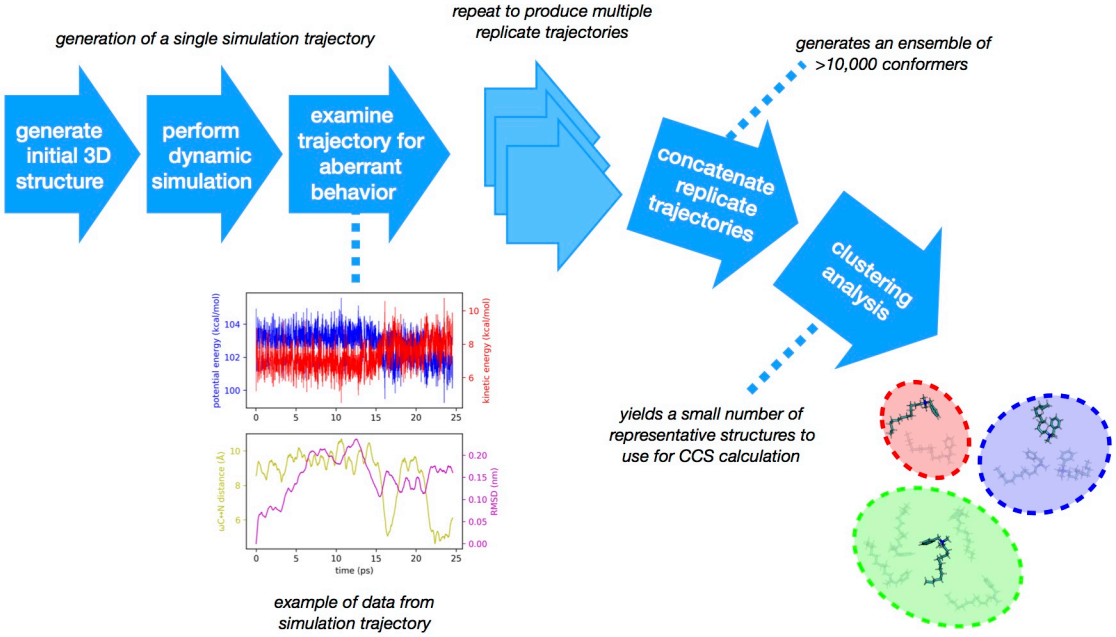
1.1 – Synthesis of Benzalkonium Chlorides (BACs) and ω-OH Metabolites

BACs were synthesized by nucleophilic coupling of N,N-dimethylbenzylamine with 1.4 equivalents of an alkyl chloride of the appropriate alkyl chain length in ethanol heated under reflux[1]. The corresponding ω-hydroxy versions of each BAC were prepared in the same manner by substituting the alkyl chloride with the appropriate ω-hydroxy alkyl chloride. Products were recrystallized from hot acetone and rinsed with cold diethyl ether. Chemical identities were confirmed by ^{1}H-NMR and/or high-resolution mass spectrometry (HRMS).

1.2 – Ion Mobility-Mass Spectrometry Electrospray Conditions

IM-MS analysis was performed on a Waters Synapt G2-Si HDMS (Waters Corp., Milford, MA) equipped with an electrospray ionization (ESI) source using nitrogen as the drift gas. ESI conditions were as follows: capillary, +2.5 kV; sampling cone, 40 V; source temperature, 100 °C; desolvation temperature, 250 °C; cone gas, 50 L/h; and desolvation gas, 600 L/h.

1.3 – Scheme S1 – Computational Modeling Workflow



2 – Results and Discussion

2.1 — ^{1}H-NMR and HRMS Characterization of Synthesized BACs and ω-OH Metabolites

C_4 BAC: HRMS [M-Cl+] ($C_{13}H_{22}N$): *observed, 192.1747; theoretical: 192.1752.*

C_6 BAC: HRMS [M-Cl+] ($C_{15}H_{26}N$): *observed, 220.2063; theoretical: 220.2065.*

C_8 BAC: HRMS [M-Cl+] ($C_{17}H_{30}N$): *observed, 248.2375; theoretical: 248.2378.*

C_{10} BAC: HRMS [M-Cl+] ($C_{19}H_{34}N$): *observed, 276.2694; theoretical: 276.2691*.

ω-OH C_4 BAC: HRMS [M-Cl+] ($C_{13}H_{22}NO$): *observed, 208.1694; theoretical: 208.1701.*

ω-OH C_6 BAC: HRMS [M-Cl+] ($C_{15}H_{26}NO$): *observed, 236.2010; theoretical: 236.2014.*

ω-OH C_8 BAC: HRMS [M-Cl+] ($C_{17}H_{30}NO$): *observed, 264.2327; theoretical: 264.2327.*

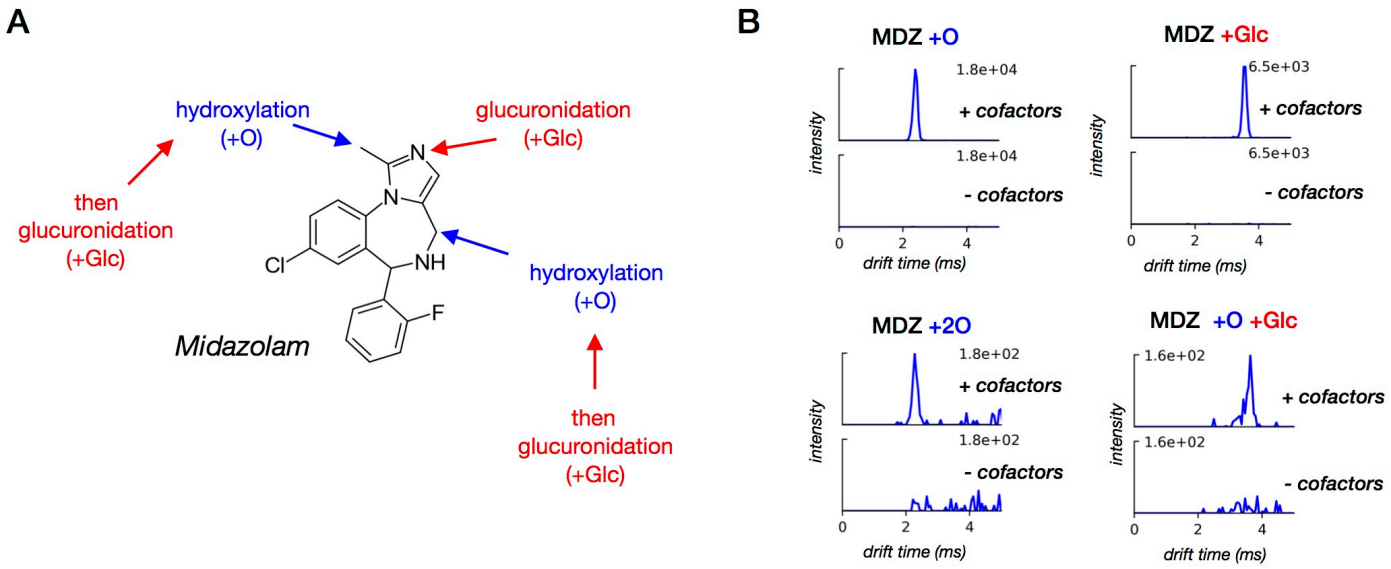
ω-OH C_{10} BAC: ^{1}H-NMR ($CDCl_3$, 500 MHz): *1.29 and 1.36 (br s, 12H), 1.56 (m, 2H), 1.81 (m, 2H), 3.30 (s, 6H), 3.53 (m, 2H), 3.64 (q, 2H, J = 6.1 Hz), 5.03 (s, 2H), 7.47 (m, 3H), 7.64 (d, 2H, J = 7.0 Hz);* MS [M-Cl^+] ($C_{19}H_{34}NO$): *observed, 292.2639; theoretical: 292.2640.*

2.2 – Computational Modeling on Sodiated Adduct of Quercetin Glucuronide

Both protonated and sodiated MS adducts of quercetin glucuronide displayed bimodal ATDs (Figure S1A), indicating conformational heterogeneity and/or isomerism. Sodiated adducts of all potential positional isomers of quercetin glucuronide (3-, 3'-, 4'-, 5-, 7-) were modeled using the same computational methodology as was applied to the protonated isomers. Representative minimum energy structures are presented in Figure S1B, and their corresponding theoretical CCS values in Figure S1C (measured values as dotted lines). The theoretical CCS values follow the same trend as was observed for the protonated isomers: the higher CCS peak likely corresponds to the 7-isomer (due to lack of formation of the 5-isomer in human hepatic metabolism[2]) while the lower CCS peak likely has contributions from the 3-, 3'-, and/or 4'-isomers.

3 – Tables and Figures

3.1 – Figure S1 – Initial Characterization of Midazolam Metabolites



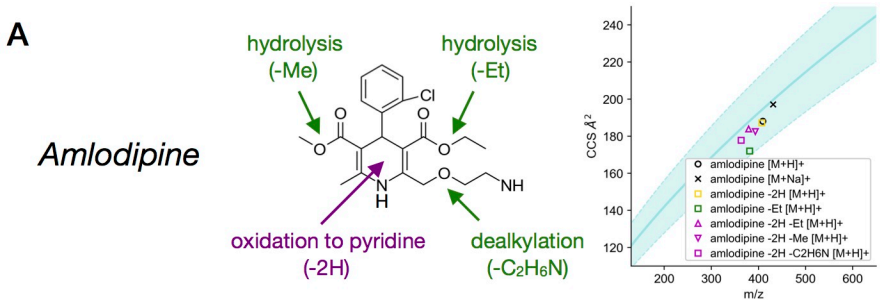
A Expected metabolism for midazolam[3,4] (MDZ). **B** Arrival time distributions (ATDs) for observed primary and secondary metabolites of MDZ showing cofactor-dependent formation.

3.2 – Table S1 – Experimental CCS Values for Drugs and Observed Metabolites

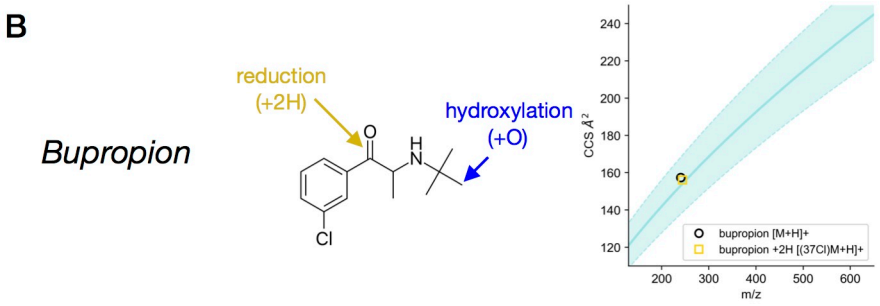
compound	metabolite	adduct	m/z	CCS (Å²)	CCS RSD (%)	Compaction factor (C)
C_4 BAC		$[M]^+$	192.1838	144.96	0.52	
C_4 BAC	ω-OH	$[M]^+$	208.1795	148.19	0.22	1.032
C_6 BAC		$[M]^+$	220.2167	157.07	0.51	
C_6 BAC	ω-OH	$[M]^+$	236.2128	158.92	0.26	1.036
C_8 BAC		$[M]^+$	248.2505	170.09	0.55	
C_8 BAC	ω-OH	$[M]^+$	264.2128	170.14	0.31	1.042
C_{10} BAC		$[M]^+$	276.2869	182.24	0.60	
C_{10} BAC	ω-OH	$[M]^+$	292.2815	178.99	0.23	1.057
C_{12} BAC		$[M]^+$	304.3187	193.19	0.21	
C_{12} BAC	ω-OH	$[M]^+$	320.3119	187.55	0.20	1.066
C_{14} BAC		$[M]^+$	332.3427	203.67	0.27	
C_{14} BAC	ω-OH	$[M]^+$	348.3444	196.33	0.24	1.070
C_{16} BAC		$[M]^+$	360.3843	212.76	0.16	
C_{16} BAC	ω-OH	$[M]^+$	376.3800	205.00	0.53	1.068
amlodipine		$[M+H]^+$	409.1586	188.14	0.53	
amlodipine		$[M+Na]^+$	431.1457	197.21	0.24	
amlodipine	-2H	$[M+H]^+$	407.1475	187.24	0.04	1.002
amlodipine	-2H, -Me	$[M+H]^+$	393.1390	182.61	0.11	1.003
amlodipine	-2H, -Et	$[M+H]^+$	379.1376	183.95	0.10	0.972
amlodipine	-2H, $-C_2H_6N$	$[M+H]^+$	363.1180	177.84	0.08	0.977
amlodipine	-Et	$[M+H]^+$	381.0955	172.06	0.30	1.043
amlodipine	-Et	$[M+Na]^+$	403.1226	207.14	0.20	0.910
bupropion		$[M+H]^+$	240.1217	157.37	0.24	

bupropion	+2H	$[(^{37}Cl)M+H]^+$	244.1346	156.07	0.31	1.020
chlorpromazine		$[M+H]^+$	319.1169	169.58	0.32	
chlorpromazine		$[M+Na]^+$	341.1305	170.83	0.42	
chlorpromazine	-Me	$[M+H]^+$	305.0947	167.66	0.47	0.982
chlorpromazine	+O	$[M+H]^+$	335.1066	170.74	0.55	1.026
clomifene		$[M+H]^+$	406.2054	203.68	0.60	
clomifene	+O	$[M+H]^+$	422.1923	209.10	0.11	0.999
clomifene	-Et	$[M+H]^+$	378.1691	197.10	0.11	0.985
clomifene	+O, -Et	$[M+H]^+$	394.1640	200.20	0.12	0.997
clopidogrel		$[M+H]^+$	322.0720	167.32	0.27	
clopidogrel	+O	$[M+H]^+$	338.0700	172.49	0.26	1.002
clozapine		$[M+H]^+$	327.1458	177.67	0.27	
clozapine		$[M+Na]^+$	349.1254	178.03	0.17	
clozapine	+O	$[M+H]^+$	343.1388	179.97	0.23	1.019
clozapine	+GSH	$[M+H]^+$	632.2207	238.91	0.11	1.154
dextromethorphan		$[M+H]^+$	272.2093	164.54	0.38	
dextromethorphan		$[M+Na]^+$	294.2091	174.13	0.91	
dextromethorphan	-Me	$[M+H]^+$	258.1915	159.93	0.43	0.993
diclofenac		$[M+H]^+$	296.0769	159.05	0.45	
diclofenac		$[M+Na]^+$	318.0157	159.32	1.19	
diclofenac	+O	$[M+Na]^+$	334.0125	167.11	1.07	0.985
diclofenac	+Glc	$[M+Na]^+$	494.0488	200.79	0.50	1.064
diclofenac	+O, +Glc	$[M+Na]^+$	510.0204	202.54	0.38	1.078
midazolam		$[M+H]^+$	326.0963	171.68	0.32	
midazolam	+O	$[M+H]^+$	342.0881	175.13	0.26	1.012
midazolam	+Glc	$[M]^+$	502.1203	211.85	1.45	1.081
progesterone		$[M+H]^+$	315.2368	178.43	0.26	
progesterone		$[M+Na]^+$	337.2199	202.53	0.16	
progesterone		$[M+H-H_2O]^+$	297.2274	172.96	0.58	
progesterone	+O	$[M+H]^+$	331.2334	181.35	0.15	1.017
progesterone	+2H	$[M+H]^+$	317.2529	178.82	0.17	1.002
progesterone	+4H, +Glc	$[M+H]^+$	495.2497	215.41	0.22	1.119
quercetin		$[M+H]^+$	303.0568	162.96	0.44	
quercetin		$[M+Na]^+$	325.1876	173.17	1.16	
quercetin (peak 1)	+Glc	$[M+H]^+$	479.0892	204.10	0.20	1.084
quercetin (peak 2)	+Glc	$[M+H]^+$	479.0892	209.87	0.02	1.054
quercetin (peak 1)	+Glc	$[M+Na]^+$	501.0968	207.30	0.21	1.114
quercetin (peak 2)	+Glc	$[M+Na]^+$	501.0968	216.62	0.43	1.067
quinidine		$[M+H]^+$	325.2060	174.69	0.29	
quinidine		$[M+Na]^+$	347.1915	177.11	0.30	
quinidine	+O	$[M+H]^+$	341.1923	177.48	0.31	1.016
terfenadine		$[M+H]^+$	472.3376	227.04	0.13	
terfenadine		$[M+Na]^+$	494.3174	223.48	0.07	
terfenadine	+O	$[M+H]^+$	488.3268	222.82	0.12	1.042
thioridazine		$[M+H]^+$	371.1743	184.60	0.09	
thioridazine		$[M+Na]^+$	393.1823	188.74	0.13	
thioridazine	+O	$[M+H]^+$	387.1683	185.91	0.36	1.021
thioridazine	-Me	$[M+H]^+$	357.1578	184.20	0.23	0.977
thioridazine	+O, -Me	$[M+H]^+$	373.1677	184.83	0.16	1.002
thioridazine	+2O	$[M+H]^+$	403.1605	190.50	0.19	1.024
thioridazine	+2O, -Me	$[M+H]^+$	389.1668	186.11	0.35	1.024
triclosan		$[M+H]^+$	288.9481	153.08	0.30	
triclosan		$[M+Na]^+$	310.9798	163.04	0.28	
triclosan	+Glc	$[M+H]^+$	464.9557	179.03	0.28	1.174
triclosan	+O, +Glc	$[M+Na]^+$	502.9555	203.88	0.10	1.102

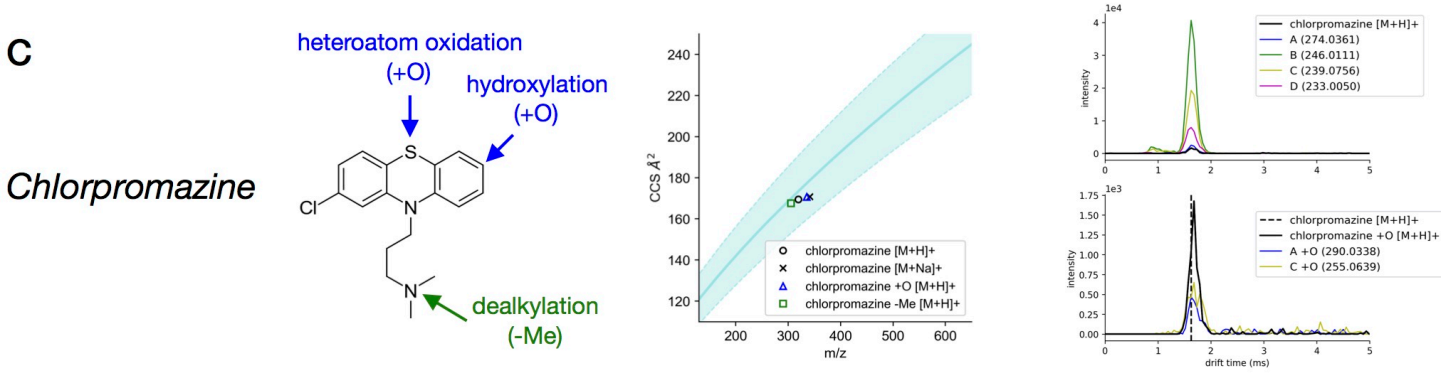
3.3 – Figure S2 – Expected Metabolites from Literature, Observed Metabolites, and Fragmentation Data for Drugs



Expected metabolism for amlodipine[5] (left) and observed metabolites (right).



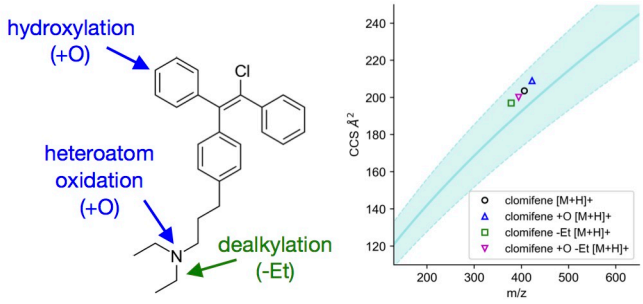
Expected metabolism for bupropion[6] (left) and observed metabolites (right).



Expected metabolism for chlorpromazine[7] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

D

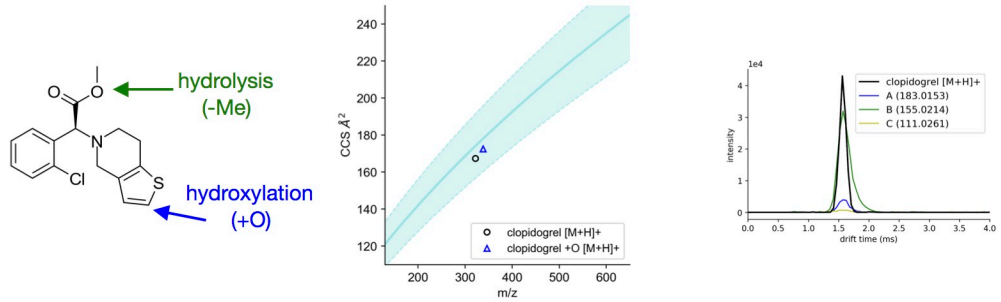
Clomifene



Expected metabolism for clomifene[8] (left) and observed metabolites (right).

E

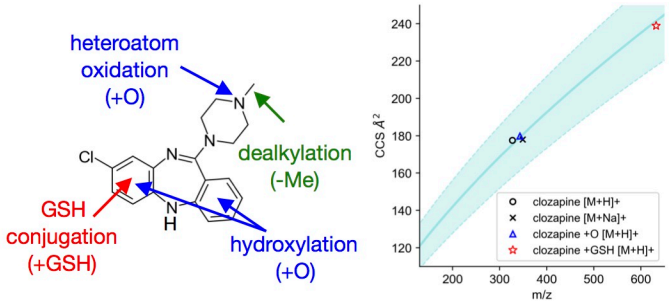
Clopidogrel



Expected metabolism for clopidogrel[9] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

F

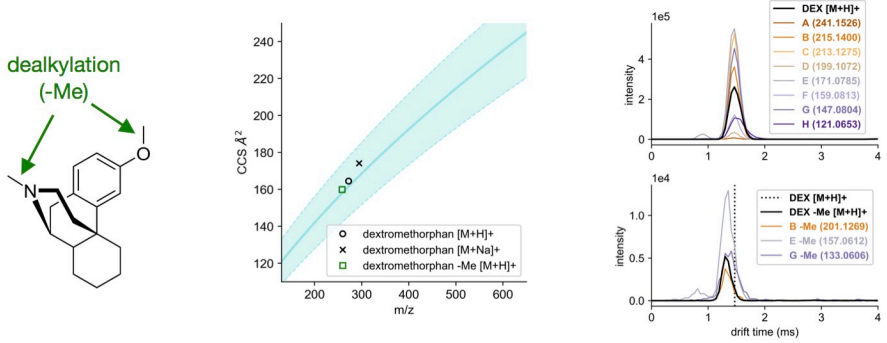
Clozapine



Expected metabolism for clozapine[10] (left) and observed metabolites (right).

G

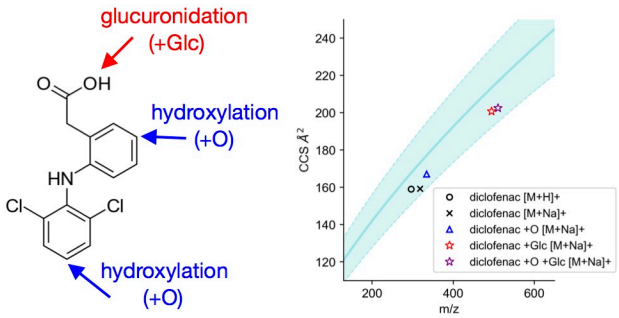
Dextromethorphan



Expected metabolism for dextromethorphan[11] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

H

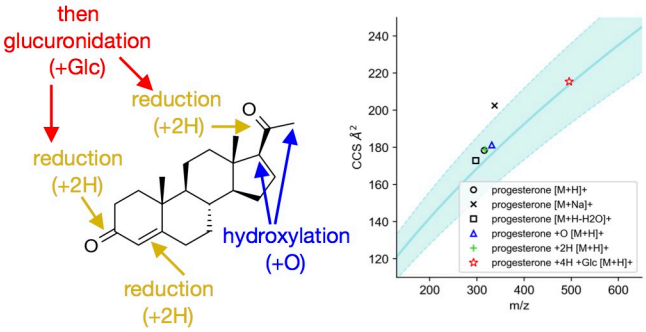
Diclofenac



Expected metabolism for diclofenac[12,13] (left) and observed metabolites (right).

I

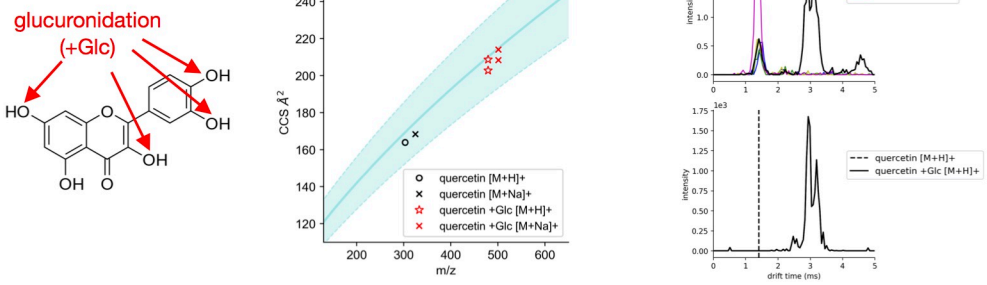
Progesterone



Expected metabolism for progesterone[14] (left) and observed metabolites (right).

J

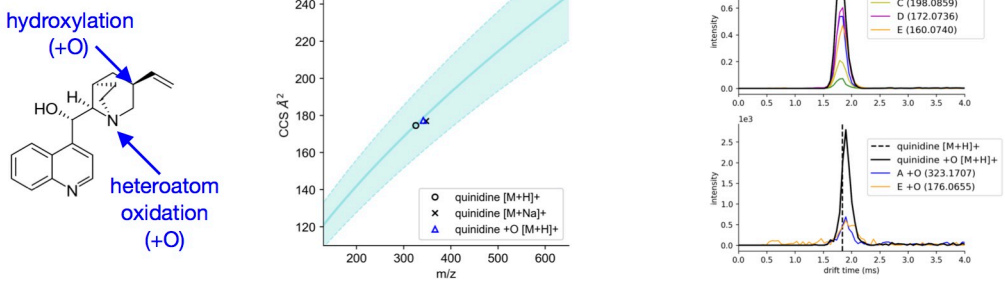
Quercetin



Expected metabolism for quercetin[2] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

K

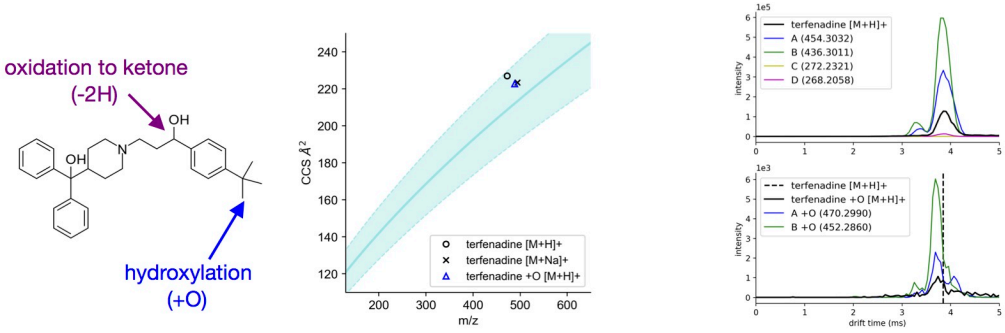
Quinidine



Expected metabolism for quinidine[15] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

L

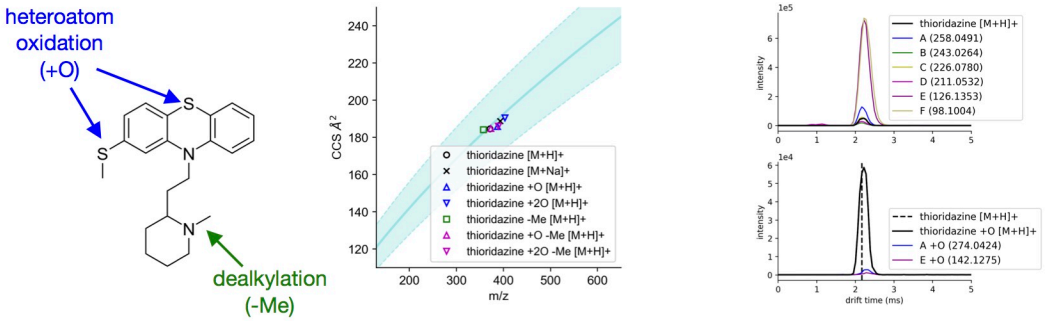
Terfenadine



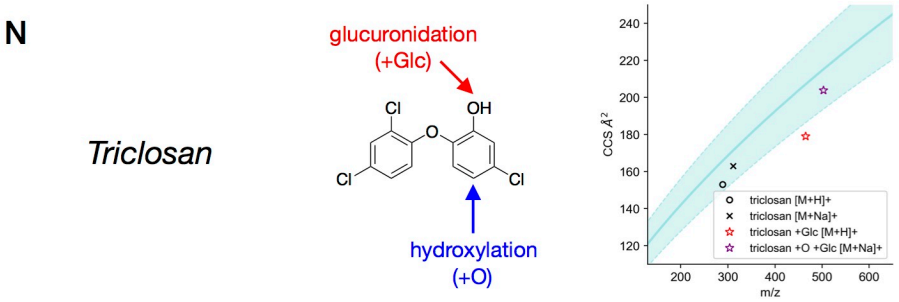
Expected metabolism for terfenadine[16] (left), observed metabolites (center), and drift time aligned fragmentation data (right).

M

Thioridazine

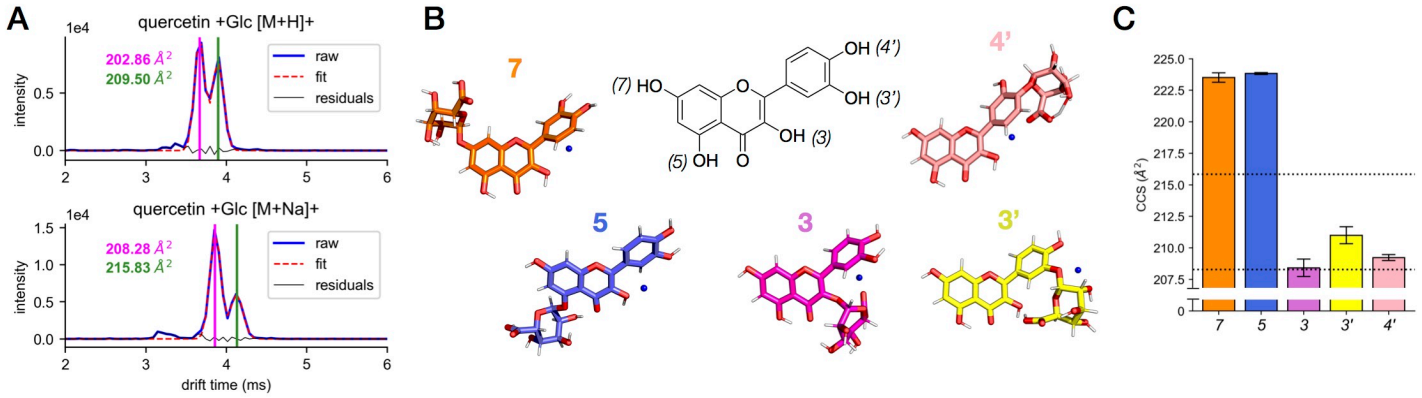


Expected metabolism for thioridazine[17] (left), observed metabolites (center), and drift time aligned fragmentation data (right).



Expected metabolism for triclosan[18] (left) and observed metabolites (right).

3.4 – Figure S3 – Theoretical CCS of Sodiated Quercetin Glucuronide Isomers



A Bimodal arrival time distributions (ATDs) for protonated (top) and sodiated (bottom) adducts of quercetin glucuronide. **B** Minimum energy structures for sodiated isomers of quercetin glucuronide. **C** Theoretical CCS of sodiated isomers of quercetin glucuronide, compared against experimental values (dotted lines).

4 – References

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