Supporting Information

Collision Cross Section Conformational Analyses of Bile Acids via Ion Mobility-Mass Spectrometry

James C. Poland,*† Katrina L. Leaptrot,*† Stacy D. Sherrod,†
Charles Robb Flynn,‡ and John A. McLean*,†

†Center for Innovative Technology, Department of Chemistry, Institute of Chemical Biology, Institute for Integrative Biosystems Research and Education, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN 37235 USA

‡Department of Surgery; Vanderbilt University Medical Center, Nashville, TN 37235 USA

*Corresponding Author Email: john.a.mclean@vanderbilt.edu

Comments on Untargeted Metabolomics Presented in this Work

In this Supporting Information we include the arrival time distributions of four β-muricholic acid ion forms (**Figure S1a**) and comparative arrival time of distributions for chenodeoxycholic acid and ursodeoxycholic acid **(Figure S1b)**. **Figure S2** shows three examples of ion mobility-mass spectrometry spectra with potential metastable adduct behavior. **Figure S3** shows the trendlines for three bile acid subclasses. **Figure S4** shows how previously unidentified features from a metabolomics sample fall on the bile acid trendline. In addition, we include tables of all measured bile acids and their drift tube collision cross section values measured in nitrogen ($^{DT}CCS_{N2}$) in both positive and negative ionization mode (**Tables S1-S3**).

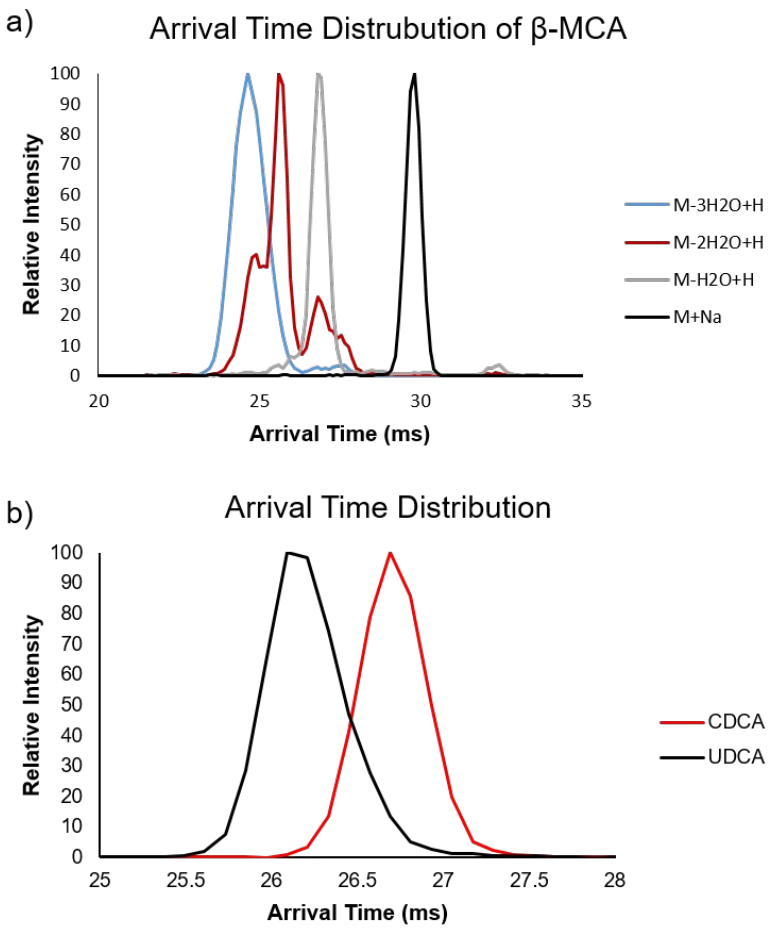


Figure S1. Drift tube ion mobility arrival time distributions (ATD). a) ATDs of the four highest abundance ion forms of β-muricholic acid, taken in positive ionization mode. b) ATDs of the $[M-H]^-$ ion forms of chenodeoxycholic acid and ursodeoxycholic acid, two bile acid isomers, analyzed individually as pure standards.

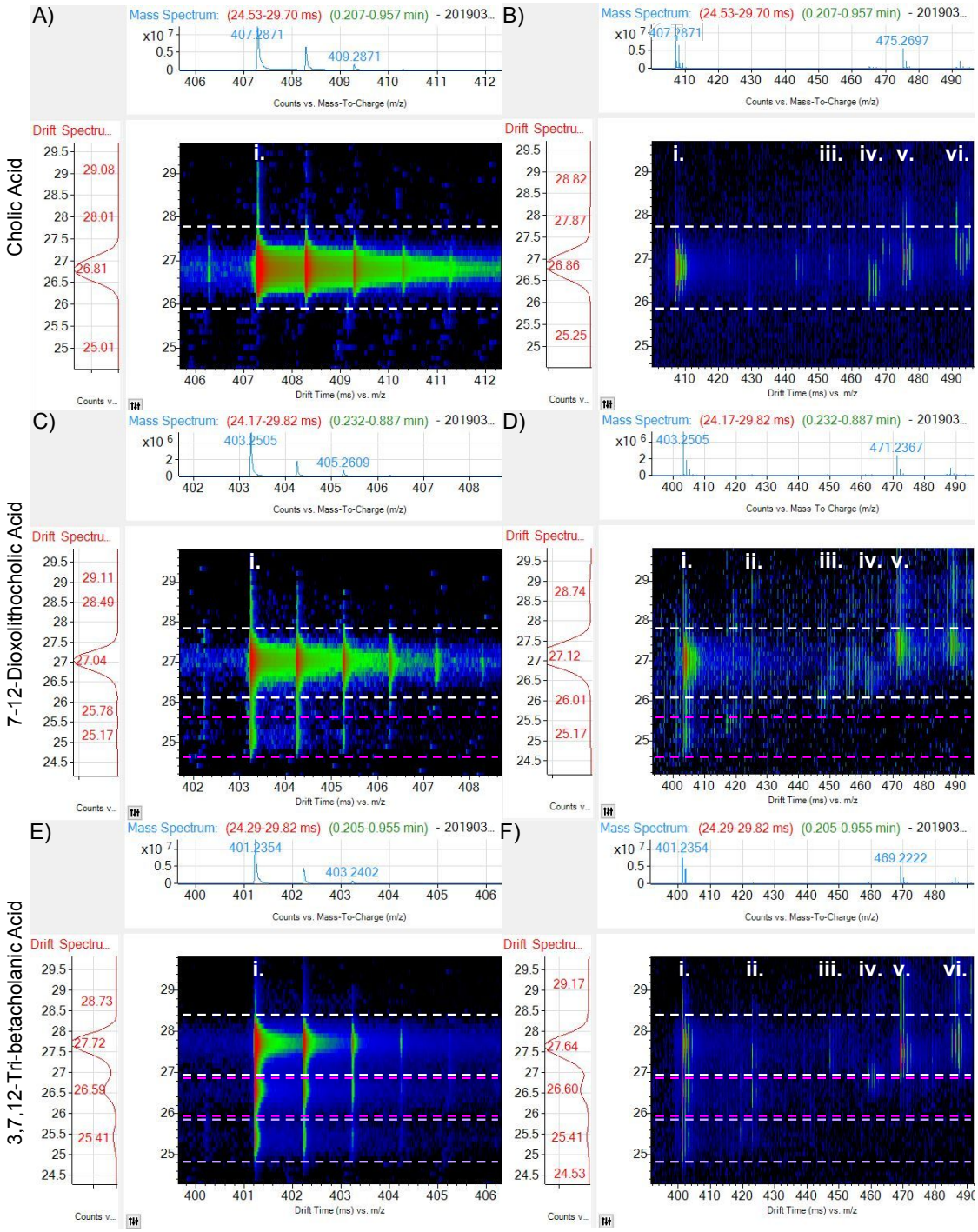


Figure S2. Screenshots from Agilent MassHunter IM-MS Browser showing IM-MS spectra of 3 bile acids analyzed in negative ionization mode. Roman numerals are mass-aligned in each spectra to the observed ion forms: i. $[M-H]^-$, ii. $[M+Na-2H]^-$, iii. $[M+HCOO]^-$, iv. $[M+Na-H+Cl]^-$, v. $[M+Na-H+HCOO]^-$, and vi. $[M+K-H+HCOO]^-$. A) Cholic acid had one observed $[M-H]^-$ ion mobility feature B) which was mobility-aligned with higher *m/z* ion forms (inside the white dashed lines). C) 7-12-Dioxolithocholic acid had two observed $[M-H]^-$ ion mobility features D) of which the lower mobility feature did not align with higher *m/z* ion forms (inside the pink dashed lines), and the higher mobility feature was mobility-aligned with higher *m/z* ion forms (inside the white dashed lines). E) 3,7,12-Tri-betacholanic acid had three observed $[M-H]^-$ ion mobility features F) of which the lowest mobility feature did not align with higher *m/z* ion forms (inside the lavendar dashed lines), the middle mobility feature was mobility-aligned with the lower mobility feature of the $[M+Na-2H]^-$ ion form (inside the pink dashed lines), and the highest mobility feature was mobility-aligned with higher *m/z* ion forms (inside the white dashed lines).

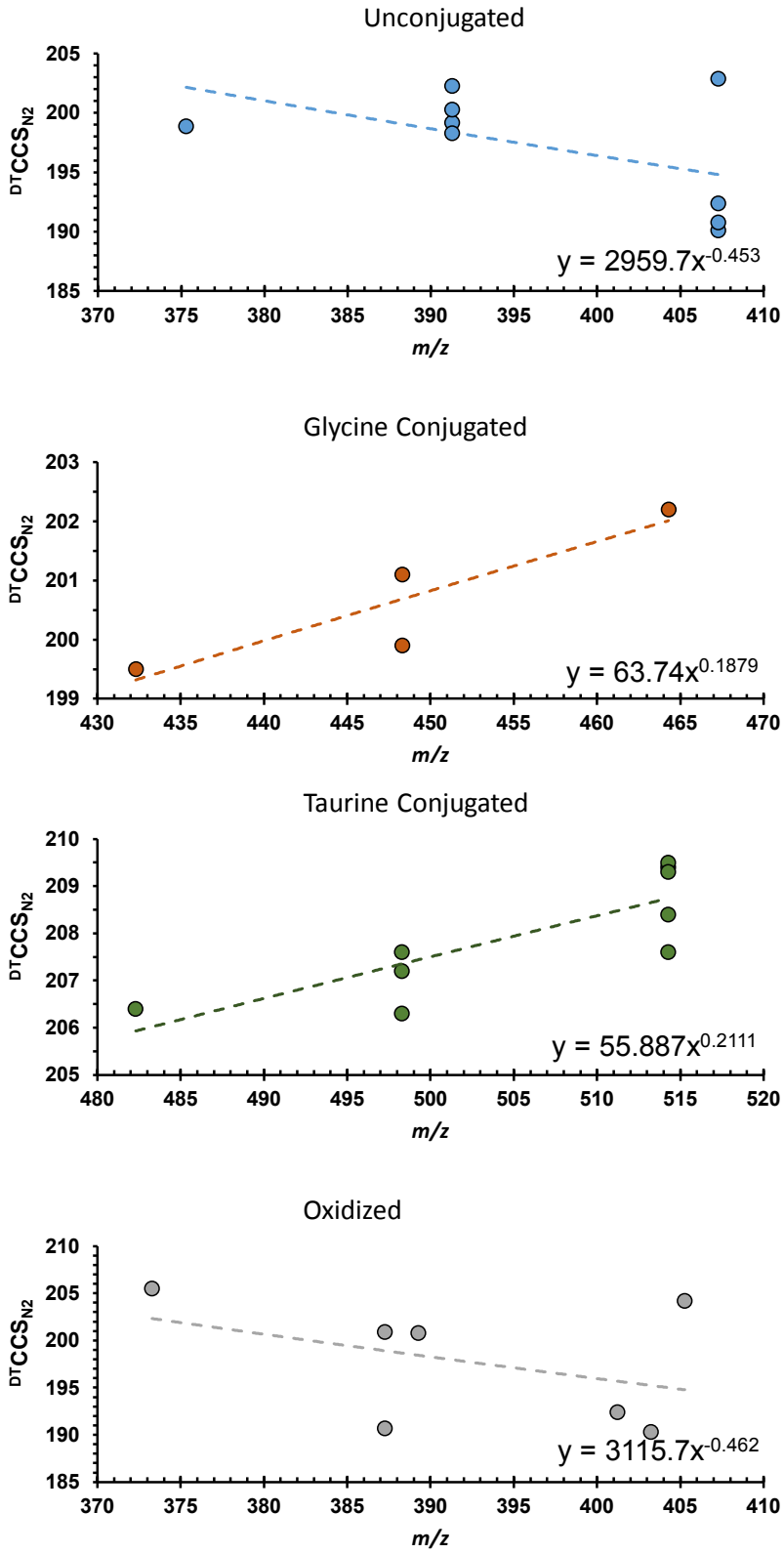
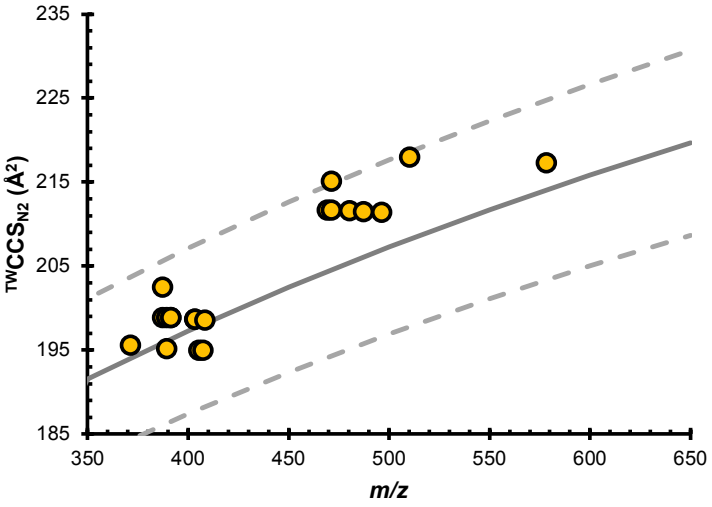


Figure S3. This figure demonstrates the trendlines of the different subclasses. In addition to the general bile acid trendline, the subclasses can be fit to their own individual trendlines, although the sample numbers may not currently be sufficient to accurately represent individual subclass trends.



Feature	*m/z*	$^{TW}CCS_{N2}$ (Å^2)
18.26_510.2526m/z	510.25	218.0
8.06_578.2464m/z	578.25	217.3
20.71_472.2495n	471.24	215.1
18.66_469.2261m/z	469.23	211.7
18.95_472.2506n	471.24	211.7
23.03_480.2791m/z	480.28	211.6
8.04_488.2438n	487.24	211.5
20.93_496.2734m/z	496.27	211.4
22.69_387.2546m/z	387.25	202.5
23.05_387.2537m/z	387.25	198.9
20.91_390.2770n	389.27	198.9
23.98_392.2943n	391.29	198.9
23.98_391.3341m/z	391.33	198.9
15.72_403.2489m/z	403.25	198.7
13.02_403.2983m/z	403.30	198.7
19.62_408.2839n	408.28	198.6
24.13_371.2549m/z	371.25	195.6
23.29_389.2693m/z	389.27	195.2
14.52_405.2646m/z	405.26	195.0
16.61_407.3288m/z	407.33	195.0

Figure S4. Graph and associated data table showing previously unidentified metabolites and where they fall on the reported $[M-H]^-$ bile acid trendline.

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å^2)						
			$[M-2SO_3-2H_2O+H]^+$	$[M-SO_3-3H_2O+H]^+$	$[M-SO_3-2H_2O+H]^+$	$[M-SO_3-H_2O+H]^+$	$[M-2H_2O+H]^+$	$[M-H_2O+H]^+$	$[M+Na]^+$
Dehydrolithocholic acid	C24H38O3	373.28	--	--	--	--	--	--	201.4±0.1
Lithocholic acid	C24H40O3	376.3	--	--	--	--	--	191.6±0.3	214.8±0.3
3,6-diketocholic acid	C24H36O4	388.26	--	--	--	--	184.7±0.3	188.8±0.3	218.8±0.3
3,7-dioxy-5β-choanoic acid	C24H36O4	388.26	--	--	--	--	182.2±0.1	185.0±0.2	201.8±0.3
Dehydrodeoxycholic acid	C24H36O4	388.26	--	--	--	--	182.2±0.2	188.0±0.2	197.5±0.1
3-oxo-12α-cholic acid	C24H38O4	390.28	--	--	--	--	182.1±0.3	191.5±0.3	192.1±0.2
Apocholic acid	C24H38O4	390.28	--	--	--	--	184.5±0.3	190.7±0.2	202.4±0.2
Chenodeoxycholic acid	C24H40O4	392.29	--	--	--	--	186.9±0.2	--	202.8±0.2
Hyodeoxycholic acid	C24H40O4	392.29	--	--	--	--	186.8±0.1	--	219.1±0.2
Murideoxycholic acid	C24H40O4	392.29	--	--	--	--	--	--	222.5±0.3
Ursodeoxycholic acid	C24H40O4	392.29	--	--	--	--	186.8±0.1	--	--
7α, 12α, dihydroxy-5β-cholanic acid	C24H40O4	392.3	--	--	--	--	186.4±0.1	--	203.7±0.2
3,7,12-tri-betacholanic acid	C24H34O5	402.24	--	--	--	--	185.3±0.3	188.5±0.2	201.2±0.2
7,12-dioxolithocholic acid	C24H36O5	404.26	--	--	--	--	185.5±0.2	194.9±0.3	204.8±0.1
12-dehydrocholic acid	C24H38O5	406.27	--	--	--	--	186.6±0.0	191.2±0.3	204.6±0.3
3-oxocholic acid	C24H38O5	406.27	--	--	--	--	182.5±0.2	186.2±0.2	201.5±0.1
3β, 7α, dihydroxy-5β-cholanic acid	C24H39DO4	406.27	--	--	--	--	186.5±0.0	--	210.0±0.2
7-ketodeoxycholic acid	C24H38O5	406.27	--	--	--	--	186.6±0.2	204.8±0.3	204.5±0.2
Hyocholic acid	C24H40O5	408.29	--	--	--	--	203.3±0.1	199.6	218.4±0.2
α-Muricholic acid	C24H40O5	408.29	--	--	--	--	187.4±0.2	--	222.8±0.1
β-Muricholic acid	C24H40O5	408.29	--	--	--	--	192.4±0.1	201.3±0.2	222.60±.1
Glycolithocholic acid	C26H43NO4	433.32	--	--	--	--	--	209.2±0.2	211.6±0.2
Glycodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	204.2±0.2	207.2±0.2	207.6±0.4
Glycoursodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	202.8±0.5	--	211.1±0.3
Lithocholic acid 3-sulfate	C24H40O6S	456.25	--	--	--	191.6±0.1	--	--	--
Glycocholic acid	C26H43NO6	465.31	--	--	--	--	196.9±0.2	197.4±0.2	202.8±0.1
Chenodeoxycholic acid 3-sulfate	C24H40O7S	472.25	--	--	186.7±0.2	--	--	--	--
Chenodeoxycholic acid 7-sulfate	C24H40O7S	472.25	--	--	186.6±0.1	--	--	--	--
Deoxycholic acid 3-sulfate	C24H40O7S	472.25	--	--	187.4±0.2	--	--	--	--
Deoxycholic acid 17-sulfate	C24H40O7S	472.25	--	--	187.4±0.2	--	--	--	--
Taurolithocholic acid	C26H45NO5S	483.3	--	--	--	--	--	219.3±0.3	214.2±0.3

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å^2)						
			$[M-2SO_3-2H_2O+H]^+$	$[M-SO_3-3H_2O+H]^+$	$[M-SO_3-2H_2O+H]^+$	$[M-SO_3-H_2O+H]^+$	$[M-2H_2O+H]^+$	$[M-H_2O+H]^+$	$[M+Na]^+$
Cholic acid 3-sulfate	C24H40O8S	488.244	--	186.1±0.2	189.6±0.2	--	--	--	--
Cholic acid 12-sulfate	C24H40O8S	488.24	--	186.4±0.1	190.3±0.1	--	--	--	--
Taurochenodeoxycholic acid	C26H45NO6S	499.3	--	--	--	--	218.7±0.1	--	--
Taurohyodeoxycholic acid	C26H45NO6S	499.3	--	--	--	--	204.5±0.2	204.5±0.1	206.7±0.2
Tauroursodeoxycholic acid	C26H45NO6S	499.3	--	--	--	--	202.6±0.2	--	216.4±0.3
Glycolithocholic acid 3-sulfate	C26H43NO7S	513.28	--	--	--	209.0±0.1	--	--	--
Taurocholic acid	C26H45NO7S	515.29	--	--	--	--	200.7±0.1	204.6±0.2	211.1±0.2
Taurohyocholic acid	C26H45NO7S	515.29	--	--	--	--	206.7±0.3	206.2±0.1	213.3±0.2
Tauro-α-muricholic acid	C26H45NO7S	515.29	--	--	--	--	201.2±0.3	--	213.1±0.4
Tauro-β-muricholic acid	C26H45NO7S	515.29	--	--	--	--	203.3±0.3	203.2±0.2	218.3±0.3
Tauro-ω-muricholic acid	C26H45NO7S	515.29	--	--	--	--	201.9±0.2	209.2±0.2	221.9±3.1
Glycochenodeoxycholic acid 3-sulfate	C26H43NO8S	529.27	--	--	201.9±0.3	203.6±0.3	--	--	--
Glycodeoxycholic acid 12-sulfate	C26H43NO8S	529.27	--	--	204.5±0.5	202.8±1.2	--	--	--
Glycocholic acid 3-sulfate	C26H43NO9S	545.27	--	196.6±0.2	200.6±0.2	211.4±0.2	--	--	--
Glycocholic acid 12-sulfate	C26H43NO9S	545.27	--	196.9±0.1	204.5±0.2	--	--	--	207.8±0.2
Chenodeoxycholic acid, disulfate	C24H40O10S2	552.21	186.9±0.1	--	--	--	--	--	--
Taurolithocholic acid 3-sulfate	C26H45NO8S2	563.26	--	--	--	219.4±0.1	--	--	219.5±0.2
Taurochenodeoxycholic acid 3-sulfate	C26H45NO9S2	579.25	--	--	202.5±0.3	--	--	--	--
Glycochenodeoxycholic acid disulfate	C26H43NO11S2	609.23	206.5±1.1	--	--	--	--	--	--

Table S1. Bile acid collision cross section values measured via drift tube ion mobility with nitrogen ($^{DT}CCS_{N_2}$) in positive ionization mode in this work. Standard deviations for $^{DT}CCS_{N_2}$ values are reported from three replicate measurements (n=3) performed on three separate days. Entries are sorted by molecular weight (MW).

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å^2)						
			$[M\text{-}SO_3\text{-}H_2O\text{-}H]^-$	$[M\text{-}SO_3\text{-}H]^-$	$[M\text{-}SO_3\text{-}H_2O+Cl]^-$	$[M\text{-}SO_3\text{-}H_2O+HCOO]^-$	$[M\text{-}SO_3+Cl]^-$	$[M\text{-}H_2O\text{-}H]^-$	$[M\text{-}H]^-$
Dehydrolithocholic acid	C24H38O3	374.28	--	--	--	--	--	--	205.5±0.2
Lithocholic acid	C24H40O3	376.30	--	--	--	--	--	--	198.9±0.6
3,6-diketocholic acid	C24H36O4	388.26	--	--	--	--	--	--	200.9±0.4
3,7-dioxy-5β-choanoic acid	C24H36O4	388.26	--	--	--	--	--	--	--
Dehydrodeoxycholic acid	C24H36O4	388.26	--	--	--	--	--	--	190.7±1.6
3-oxo-12α-cholic acid	C24H38O4	390.28	--	--	--	--	--	193.2±0.0	200.8±0.1
Apocholic acid	C24H38O4	390.28	--	--	--	--	--	197.3±0.5	189.1±0.5
7α, 12α, dihydroxy-5β-cholanic acid	C24H40O4	392.29	--	--	--	--	--	194.9±0.3	200.6±0.2
Chenodeoxycholic acid	C24H40O4	392.29	--	--	--	--	--	--	202.3±0.2
Hyodeoxycholic acid	C24H40O4	392.29	--	--	--	--	--	--	199.2±0.5
Murideoxycholic acid	C24H40O4	392.29	--	--	--	--	--	--	200.3±0.5
Ursodeoxycholic acid	C24H40O4	392.29	--	--	--	--	--	--	198.3±0.4
3β, 7α, dihydroxy-5β-cholanic acid	C24H39DO4	393.30	--	--	--	--	--	--	202.7±0.1
3,7,12-tri-betacholanic acid	C24H34O5	402.24	--	--	--	--	--	191.9±0.4	192.4±0.1
7,12-dioxolithocholic acid	C24H36O5	404.26	--	--	--	--	--	194.8±0.7	190.3±0.4
7-ketodeoxycholic acid	C24H38O5	406.27	--	--	--	--	--	--	204.2±0.1
Cholic acid	C24H40O5	408.29	--	--	--	--	--	--	202.9±0.3
Hyocholic acid	C24H40O5	408.29	--	--	--	--	--	196.4±0.2	190.1±0.3
α-Muricholic acid	C24H40O5	408.29	--	--	--	--	--	200.8±0.3	192.4±0.2
β-Muricholic acid	C24H40O5	408.29	--	--	--	--	--	205.7±0.3	190.8±0.1
Glycolithocholic acid	C26H43NO4	433.32	--	--	--	--	--	--	199.5±0.2
Glycodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	--	--	199.9±0.2
Glycoursodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	--	--	201.1±0.1
Lithocholic acid 3-sulfate	C24H40O6S	456.25	--	--	--	--	--	--	196.6±0.6
Glycocholic acid	C26H43NO6	465.31	--	--	--	--	--	--	202.2±0.1
Chenodeoxycholic acid 3-sulfate	C24H40O7S	472.25	199.1±0.5	202.7±0.2	--	--	--	--	194.9±0.0
Chenodeoxycholic acid 7-sulfate	C24H40O7S	472.25	197.6±0.7	--	--	--	--	--	202.4±2.0
Deoxycholic acid 3-sulfate	C24H40O7S	472.25	198.1±0.1	202.2±0.2	--	--	--	202.5±0.2	196.3±0.6
Deoxycholic acid 17-sulfate	C24H40O7S	472.25	--	201.9±0.1	--	--	--	--	206.1±0.1
Taurolithocholic acid	C26H45NO5S	483.30	--	--	--	--	--	--	206.4±0.1
Cholic acid 3-sulfate	C24H40O8S	488.24	199.3±0.1	202.6±0.2	204.3±0.2	210.3±0.2	203.6±0.1	212.0±0.1	192.4±0.2

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å^2)						
			$[M-SO_3-H_2O-H]^-$	$[M-SO_3-H]^-$	$[M-SO_3-H_2O+Cl]^-$	$[M-SO_3-H_2O+HCOO]^-$	$[M-SO_3+Cl]^-$	$[M-H_2O-H]^-$	$[M-H]^-$
Cholic acid 12-sulfate	C24H40O8S	488.24	--	--	--	--	--	--	207.7±0.2
Taurochenodeoxycholic acid	C26H45NO6S	499.30	--	--	--	--	--	--	207.2±0.2
Taurohyodeoxycholic acid	C26H45NO6S	499.30	--	--	--	--	--	--	206.3±0.2
Tauroursodeoxycholic acid	C26H45NO6S	499.30	--	--	--	--	--	--	207.6±0.1
Glycolithocholic acid 3-sulfate	C26H43NO7S	513.28	200.1±0.4	199.0±0.2	--	--	--	--	215.6±0.2
Taurocholic acid	C26H45NO7S	515.29	--	--	--	--	--	--	207.6±0.2
Taurohyocholic acid	C26H45NO7S	515.29	--	--	--	--	--	210.2±0.4	208.4±0.2
Tauro-α-muricholic acid	C26H45NO7S	515.29	--	--	--	--	--	--	209.4±0.1
Tauro-β-muricholic acid	C26H45NO7S	515.29	--	--	--	--	--	211.9±0.3	209.5±0.2
Tauro-ω-muricholic acid	C26H45NO7S	515.29	--	--	--	--	--	--	209.3±0.6
Glycochenodeoxycholic acid 3-sulfate	C26H43NO8S	529.27	200.3±0.1	200.3±0.1	--	204.7±0.1	--	214.8±0.1	215.2±0.1
Glycodeoxycholic acid 12-sulfate	C26H43NO8S	529.27	--	199.5±0.0	--	--	--	--	213.0±0.2
Glycocholic acid 3-sulfate	C26H43NO9S	545.27	200.2±0.1	201.9±0.1	--	--	--	215.0±0.1	215.8±0.1
Glycocholic acid 12-sulfate	C26H43NO9S	545.27	--	--	--	--	--	--	212.6±0.1
Chenodeoxycholic acid, disulfate	C24H40O10S2	552.21	203.1±0.0	203.9±0.0	--	219.1±0.1	--	--	207.6±0.2
Taurolithocholic acid 3-sulfate	C26H45NO8S2	563.26	206.9±0.0	206.3±0.1	--	--	--	--	217.1±0.1
Glycochenodeoxycholic acid disulfate	C26H43NO11S2	609.23	214.4±0.0	212.8±0.1	--	--	--	--	223.7±0.0

Table S2. Bile acid collision cross section values measured via drift tube ion mobility with nitrogen ($^{DT}CCS_{N_2}$) in negative ionization mode in this work, including ion forms with *m/z* values less than the neutral mass. Additional ion forms observed in negative ionization mode are reported in Table S3. Standard deviations for $^{DT}CCS_{N_2}$ values are reported from three replicate measurements (n=3) performed on three separate days. Entries are sorted by molecular weight (MW).

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å^2)							
			$[M+Na-2H]^-$	$[M-H2O+HCOO]^-$	$[M+K-2H]^-$	$[M+HCOO]^-$	$[M+Na-H+Cl]^-$	$[M+Na-H+HCOO]^-$	$[M+K-H+Cl]^-$	$[M+K-H+HCOO]^-$
Dehydrolithocholic acid	C24H38O3	374.28	190.6±0.3	--	--	201.1±0.1	197.5±0.1	200.6±0.1	--	203.2±0.2
Lithocholic acid	C24H40O3	376.30	--	--	--	198.8±0.2	197.9±0.0	201.6±0.1	--	204.1±0.2
3,6-diketocholic acid	C24H36O4	388.26	190.9±0.1	--	203.4±0.0	199.6±0.1	197.5±0.1	200.6±0.1	--	--
3,7-dioxy-5β-choanoic acid	C24H36O4	388.26	191.3±0.1	--	--	202.2±0.1	199.4±0.3	205.2±2.8	203.1±0.2	205.7±0.4
Dehydrodeoxycholic acid	C24H36O4	388.26	--	--	--	206.2±0.3	200.1±0.0	208.4±0.1	--	205.2±0.0
3-oxo-12α-cholic acid	C24H38O4	390.28	200.9±0.9	--	--	201.2±1.8	199.0±0.1	202.5±0.1	202.4±0.1	--
Apocholic acid	C24H38O4	390.28	--	--	--	201.4±0.1	198.8±0.1	202.4±0.1	201.8±0.3	--
7α, 12α, dihydroxy-5β-cholanic acid	C24H40O4	392.29	199.5±0.3	--	--	202.2±0.5	199.1±0.2	202.4±0.1	202.1±0.2	--
Chenodeoxycholic acid	C24H40O4	392.29	--	--	--	201.9±0.1	199.2±0.0	205.8±0.1	--	208.5±0.1
Hyodeoxycholic acid	C24H40O4	392.29	194.8±0.6	--	--	200.4±0.3	199.5±0.2	203.6±0.2	203.1±1.5	206.5±0.3
Murideoxycholic acid	C24H40O4	392.29	--	--	--	200.6±0.5	200.0±0.1	204.0±0.1	203.7±0.1	206.1±0.9
Ursodeoxycholic acid	C24H40O4	392.29	--	--	--	200.2±0.2	199.4±0.2	203.2±0.1	--	205.7±0.2
3β, 7α, dihydroxy-5β-cholanic acid	C24H39DO4	393.30	--	--	--	204.1±0.2	202.4±0.0	206.3±0.1	--	209.1±0.2
3,7,12-tri-betacholanic acid	C24H34O5	402.24	192.8±0.3	--	207.2±1.2	206.1±0.1	202.7±0.1	206.9±0.1	--	208.7±0.1
7,12-dioxolithocholic acid	C24H36O5	404.26	203.2±0.2	--	--	201.3±0.3	200.4±0.1	206.1±0.1	--	--
7-ketodeoxycholic acid	C24H38O5	406.27	--	--	203.2±0.6	202.1±0.4	200.4±0.1	204.1±0.1	--	205.6±0.7
Cholic acid	C24H40O5	408.29	--	--	--	203.0±0.2	199.8±0.1	204.5±2.1	--	205.7±1.0
Hyocholic acid	C24H40O5	408.29	197.0±0.6	197.8±0.3	--	202.5±0.2	200.1±0.2	206.8±0.2	--	209.2±0.3
α-Muricholic acid	C24H40O5	408.29	194.5±0.2	--	--	203.7±0.2	201.0±0.2	207.8±0.2	--	209.8±0.1
β-Muricholic acid	C24H40O5	408.29	202.8±0.3	--	--	201.9±0.3	201.3±0.1	206.0±0.2	--	209.2±0.3
Glycolithocholic acid	C26H43NO4	433.32	--	--	--	--	211.8±0.2	214.5±0.2	--	216.9±0.2
Glycodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	212.3±0.2	214.0±0.2	--	214.2±1.4
Glycoursodeoxycholic acid	C26H43NO5	449.31	--	--	--	--	213.5±0.4	215.5±0.1	--	218.0±0.1
Lithocholic acid 3-sulfate	C24H40O6S	456.25	202.1±0.1	208.9±0.3	205.5±0.1	--	--	--	--	--
Glycocholic acid	C26H43NO6	465.31	204.8±0.4	--	--	--	210.7±0.1	212.2±0.2	--	214.2±0.4
Dehydrolithocholic acid	C24H38O4	445.47	190.6±0.4	--	--	201.1±0.2	197.5±0.2	200.6±0.2	214.3±1.1	215.4±0.1
Lithocholic acid	C24H40O4	448.50	--	--	--	198.8±0.3	197.9±0.1	201.6±0.2	--	217.6±0.2
3,6-diketocholic acid	C24H36O4	451.54	190.9±0.2	--	203.4±0.1	199.6±0.2	197.5±0.2	200.6±0.2	212.0±1.2	212.4±0.5
3,7-dioxy-5β-choanoic acid	C24H36O4	454.57	191.3±0.2	--	--	202.2±0.2	199.4±0.4	205.2±2.9	--	--
Dehydrodeoxycholic acid	C24H36O4	457.61	--	--	--	206.2±0.4	200.1±0.1	208.4±0.2	--	221.4±0.3
3-oxo-12α-cholic acid	C24H38O4	460.64	200.9±0.10	--	--	201.2±1.9	199.0±0.2	202.5±0.2	213.4±0.3	212.7±0.2

Name	Molecular Formula	MW (Da)	$^{DT}CCS_{N2}$ (Å²)							
			$[M+Na-2H]^-$	$[M-H2O+HCOO]^-$	$[M+K-2H]^-$	$[M+HCOO]^-$	$[M+Na-H+Cl]^-$	$[M+Na-H+HCOO]^-$	$[M+K-H+Cl]^-$	$[M+K-H+HCOO]^-$
Apocholic acid	C24H38O4	463.68	--	--	--	201.4±0.2	198.8±0.2	202.4±0.1	--	217.7±0.2
7α, 12α, dihydroxy-5β-cholanic acid	C24H40O4	466.72	199.5±0.4	--	--	202.2±0.6	199.1±0.3	202.4±0.1	--	223.3±0.3
Chenodeoxycholic acid	C24H40O4	469.75	--	--	--	201.9±0.2	199.2±0.1	205.8±0.2	--	224.5±0.1
Hyodeoxycholic acid	C24H40O4	472.79	194.8±0.7	--	--	200.4±0.4	199.5±0.3	203.6±0.3	--	223.9±0.2
Murideoxycholic acid	C24H40O4	475.82	--	--	--	200.6±0.6	200.0±0.2	204.0±0.2	--	--
Ursodeoxycholic acid	C24H40O4	478.86	--	--	--	200.2±0.3	199.4±0.3	203.2±0.2	--	--
3β, 7α, dihydroxy-5β-cholanic acid	C24H39DO5	481.89	--	--	--	204.1±0.3	202.4±0.1	206.3±0.2	--	224.6±1.1
3,7,12-tri-betacholanic acid	C24H34O6	484.93	192.8±0.4	--	207.2±1.3	206.1±0.2	202.7±0.2	206.9±0.2	--	225.0±1.0
7,12-dioxolithocholic acid	C24H36O6	487.96	203.2±0.3	--	--	201.3±0.4	200.4±0.1	206.1±0.2	--	--
7-ketodeoxycholic acid	C24H38O6	491.00	--	--	203.2±0.7	202.1±0.5	200.4±0.1	204.1±0.2	--	--
Glycochenodeoxycholic acid 3-sulfate	C26H43NO8S	529.27	216.5±0.1	219.5±0.1	217.8±0.8	--	--	--	223.3±0.2	224.2±0.2
Glycodeoxycholic acid 12-sulfate	C26H43NO8S	529.27	219.0±0.2	220.3±1.3	215.5±0.3	--	--	--	--	--
Glycocholic acid 3-sulfate	C26H43NO9S	545.27	217.1±0.1	--	--	--	--	--	--	--
Glycocholic acid 12-sulfate	C26H43NO9S	545.27	212.7±0.1	--	215.3±0.1	--	--	--	--	--
Chenodeoxycholic acid, disulfate	C24H40O10S2	552.21	228.1±0.1	--	--	--	--	--	--	--
Taurolithocholic acid 3-sulfate	C26H45NO8S2	563.26	222.8±0.1	--	225.2±0.0	--	--	--	--	--
Glycochenodeoxycholic acid disulfate	C26H43NO11S2	609.23	223.6±0.0	--	--	--	--	--	--	--

Table S3. Bile acid collision cross section values measured via drift tube ion mobility with nitrogen ($^{DT}CCS_{N_2}$) in negative ionization mode in this work, including ion forms with *m/z* values greater than the neutral mass. Additional ion forms observed in negative ionization mode are reported in Table S2. Standard deviations for $^{DT}CCS_{N_2}$ values are reported from three replicate measurements (n=3) performed on three separate days. Entries are sorted by molecular weight (MW).