Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for:

Implementation of Charged Microdroplet-Based Derivatization of Bile Acids on a Cyclic Ion Mobility Spectrometry-Mass Spectrometry Platform

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EXPERIMENTAL SECTION:

L-tyrosine hydrazide & N-boc-L-histidine hydrazide were purchased from Sigma Aldrich (St. Louis, MO, USA). All bile acids were purchased from AvantiLipids (Alabaster, AL, USA), except for Δ^4-Dafachronic acid and Δ^7-Dafachronic acid which were purchased from Cayman Chemicals (Ann Arbor, MI, USA), and 3-oxo allocholic acid which was purchased from Santa Cruz Biotechnology, Inc (Dallas, TX, USA), Girard's reagent T was purchased from TCI Chemicals (Portland, OR, USA). LC–MS grade solvents were purchased from Fischer Scientific (Pittsburgh, PA, USA). Stock solutions for were prepared in methanol and then diluted to final concentrations of 10 μM (unless noted otherwise), respectively, in either 90/10 (v/v) water/methanol with 0.5% (v/v) formic acid or 80/20 (v/v) water/methanol with 0.5% (v/v) formic acid.

The commercial Waters cIMS−MS platform (Wilmslow, UK) has been previously described in detail elsewhere. Briefly, a quadrupole is located before the separation region and was used to filter out the desired *m/z* values to be subjected to cIMS-MS separations. The 1 m cIMS traveling wave separation region (the total separation of each separation pathlength corresponding to the number of cycles multiplied by 1 m) was operated at a pressure of 1.74 mbar in nitrogen. The time-of-flight MS was operated in 'V' mode and 50 to 1200 m/z range. Signal averaging was performed for 2-10 minutes for each arrival time distribution and is listed in each of the respective figure captions. Data was acquired and processed with the MassLynx and Quartz software.

For the droplet-based experiments, a dual syringe setup with a T-in junction was used. Bile acid solutions were loaded in one syringe, while the derivatization reagent solutions were loaded into the other syringe. Both syringes were set to a flow rate of 5 μL/min. The distance between the exit of the capillary and inlet of the cIMS-MS was ~10 mm (i.e., the charged microdroplet reaction region). The instrument was equipped with a high-flow ESI probe with an internal diameter of 125 μm (Waters; P/N: 700011242). Optimization of operating parameters for a single experiment is described in the Table S1. For a schematic of our dual syringe setup, please see the main text of the Manuscript.

For the bulk reactions the product solutions were made according to the following procedure:

1. Micropipette 10 μL of a 1mM bile acid solution in methanol into the microcentrifuge tube, add 45 μL glacial acetic acid, add 10 μL of a 1 mg/mL tagging reagent solution in methanol, add 35 μL of methanol.
2. Vortex the microcentrifuge tube for 1 minute
3. Place the sample in heat block at 37 °C for 2 hours.
4. Dry the samples down.
5. Reconstitute sample with either 90/10 (v/v) water/methanol with 0.5% (v/v) formic acid or 80/20 (v/v) water/methanol with 0.5% (v/v) formic acid solution.

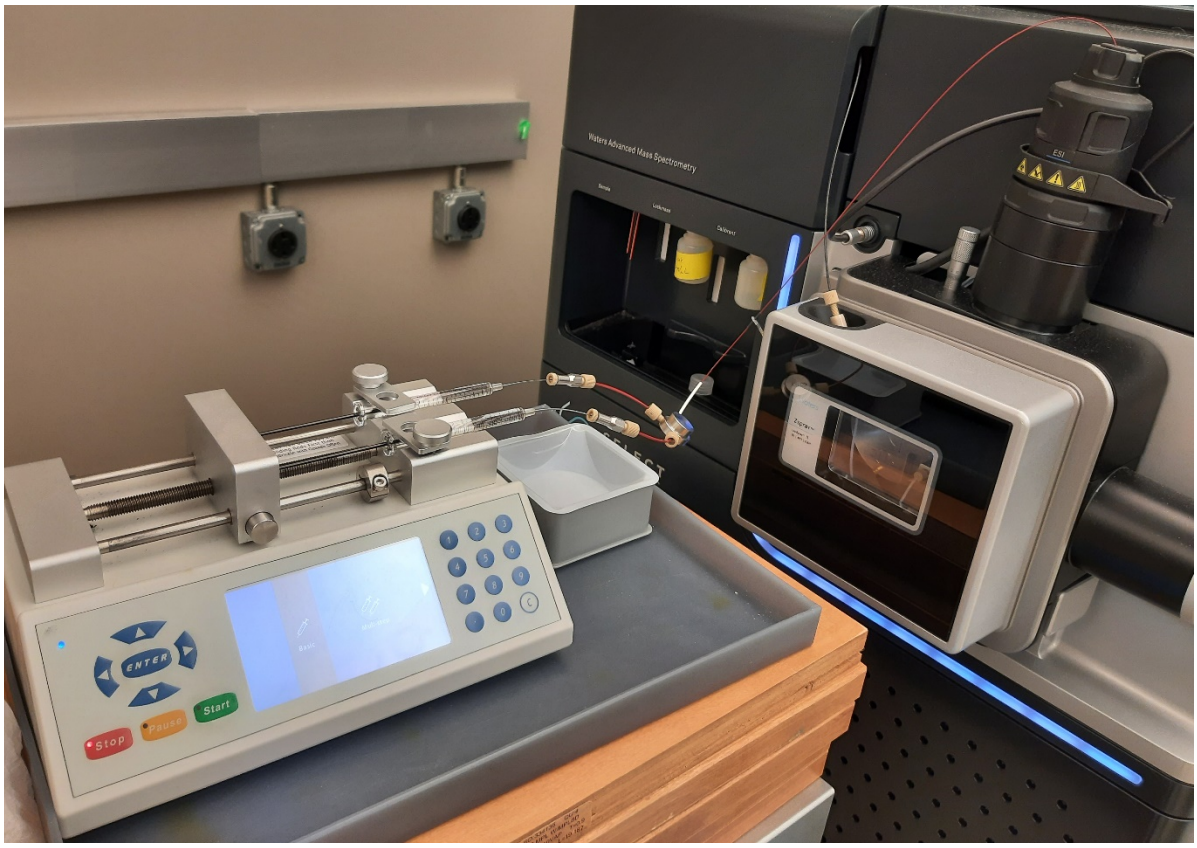

Image S1. The experimental setup of our dual syringe setup.

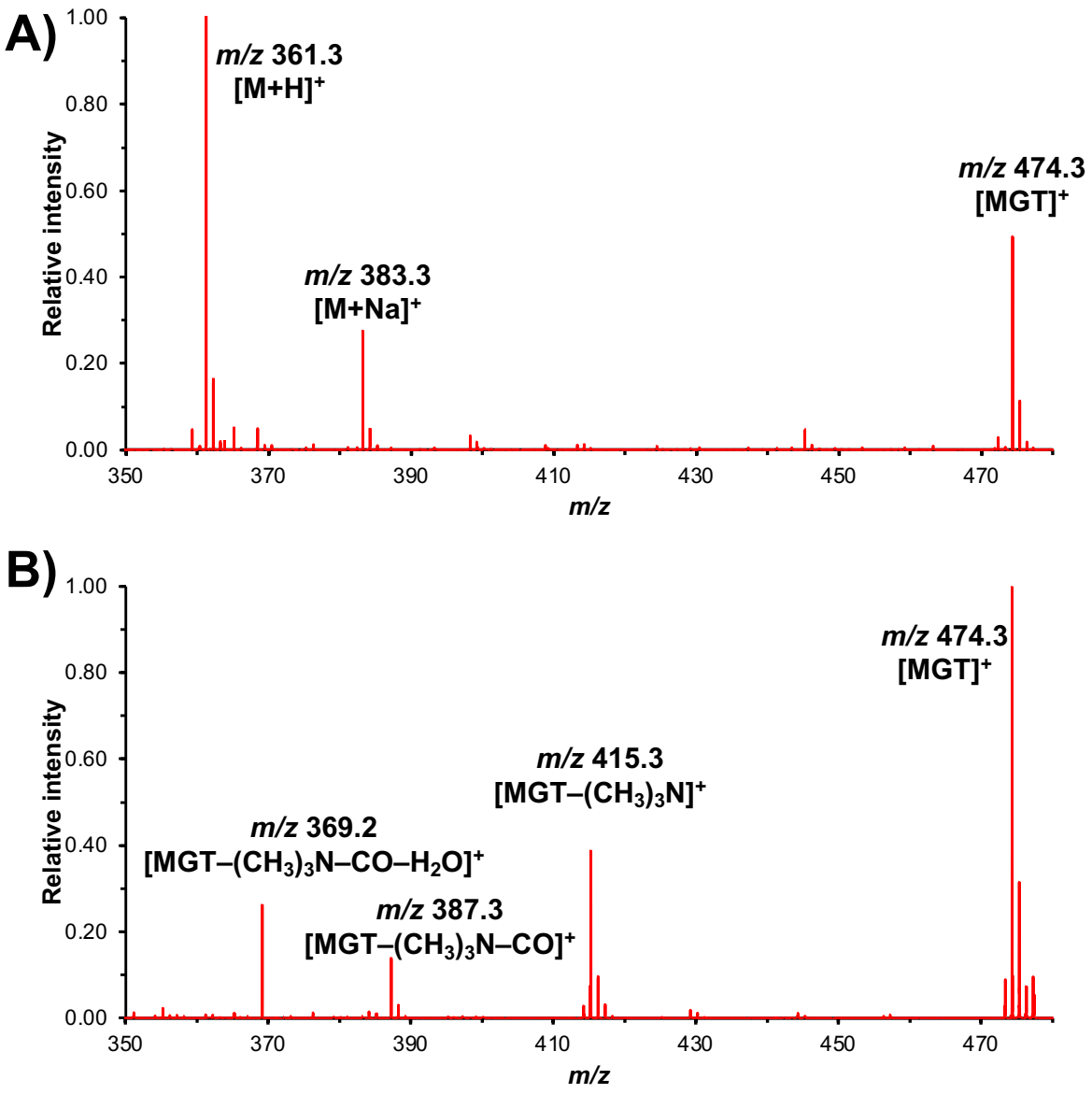

Figure S1. Mass spectra of the product of the reaction of cortisone and GT with signal averaging performed for 2 min (A). Tandem mass spectra highlighting fragment ions for the products of the reaction of cortisone & GT at the CID of 35 V with signal averaging performed for 5 min (B).

Table S1. Comparisons of varying droplet reaction conditions for the reaction of 3-OCA & various hydrazide tags.

#	Bile acid concentration	Derivatizing agent concentration	Solvent composition	Relative intensity to highest intensity conditions
1.	3-OCA (10μM)	GT (10μM)	90/10/0.5 (v/v) MeOH/H_2O/HCOOH	0.7 %
2.	3-OCA (10μM)	GT (10μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	100 %
3.	3-OCA (5μM)	GT (10μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	1.8 %
4.	3-OCA (10μM)	GT (5μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	1.8 %
5.	3-OCA (20μM)	GT (5μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	3.4 %
6.	3-OCA (5μM)	GT (20μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	11.5 %
7.	3-OCA (20μM)	GT (10μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	No product formation observed
8.	3-OCA (10μM)	GT (20μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	11.4 %
9.	3-OCA (10μM)	GT (10μM)	60/40/0.5 (v/v) MeOH/H_2O/HCOOH	7.3 %
10.	3-OCA (10μM)	GT (10μM)	50/50/0.5 (v/v) MeOH/H_2O/HCOOH	No product formation observed
11.	3-OCA (10μM)	GT (10μM)	80/20/2 (v/v) MeOH/H_2O/AcOH	5.2 %
12.	3-OCA (10μM)	GT (10μM)	80/20/0.1 (v/v) MeOH/H_2O/TFA	35.5 %
13.	3-OCA (10μM)	GT (10μM)	80/20/0.5 (v/v) MeCN/H_2O/HCOOH	No product formation observed
14.	3-OCA (10μM)	N-boc-L-histidine hydrazide (10μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	8.9 %
15.	3-OCA (10μM)	N-boc-L-histidine hydrazide (10μM)	90/10/0.5 (v/v) MeOH/H_2O/HCOOH	100 %
16.	3-OCA (10μM)	L-tyrosine hydrazide (10μM)	80/20/0.5 (v/v) MeOH/H_2O/HCOOH	27.7 %
17.	3-OCA (10μM)	L-tyrosine hydrazide (10μM)	90/10/0.5 (v/v) MeOH/H_2O/HCOOH	100 %

We would like to note that comparisons with methanol/formic acid 100/0.5 (v/v) solvent mixture were performed with bile acid substrates other than 3-OCA and yielded decrease in signal intensity compared to 90/10/0.5 (v/v) MeOH/H_2O/HCOOH solvent mixture.

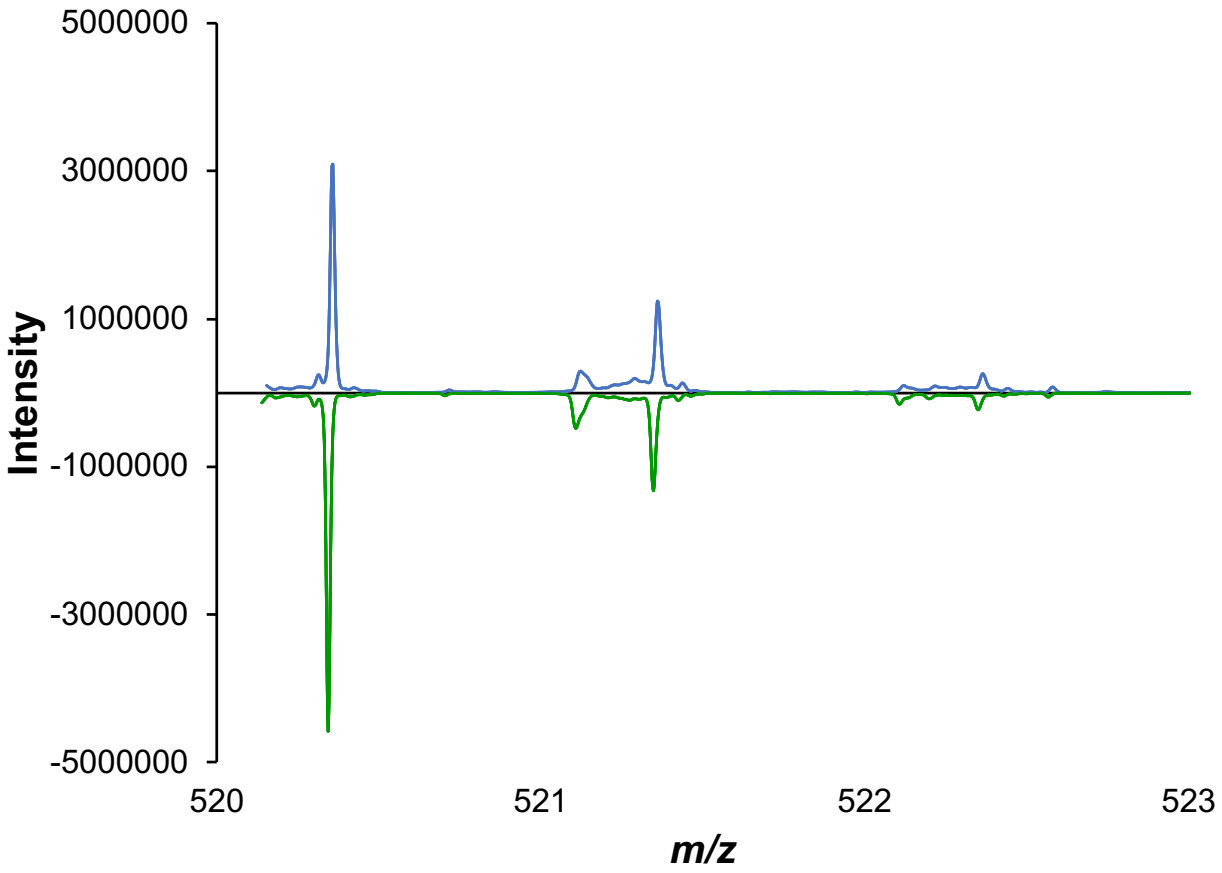

Figure S2. Inverted mass spectra of the product of the reaction of 3-OCA and GT with microflow of 2 µL/min preceding direct infusion (blue trace) and direct infused sample (green trace). Signal averaging performed for three minutes for each. PEEK tube used: “TRAJAN” 1302004006-10F, 1/16’’OD X 0.004’’ ID. Length used ~70 cm. The length of PEEK tubing used was identical to that used in the direct infusion droplet chemistry experiments.

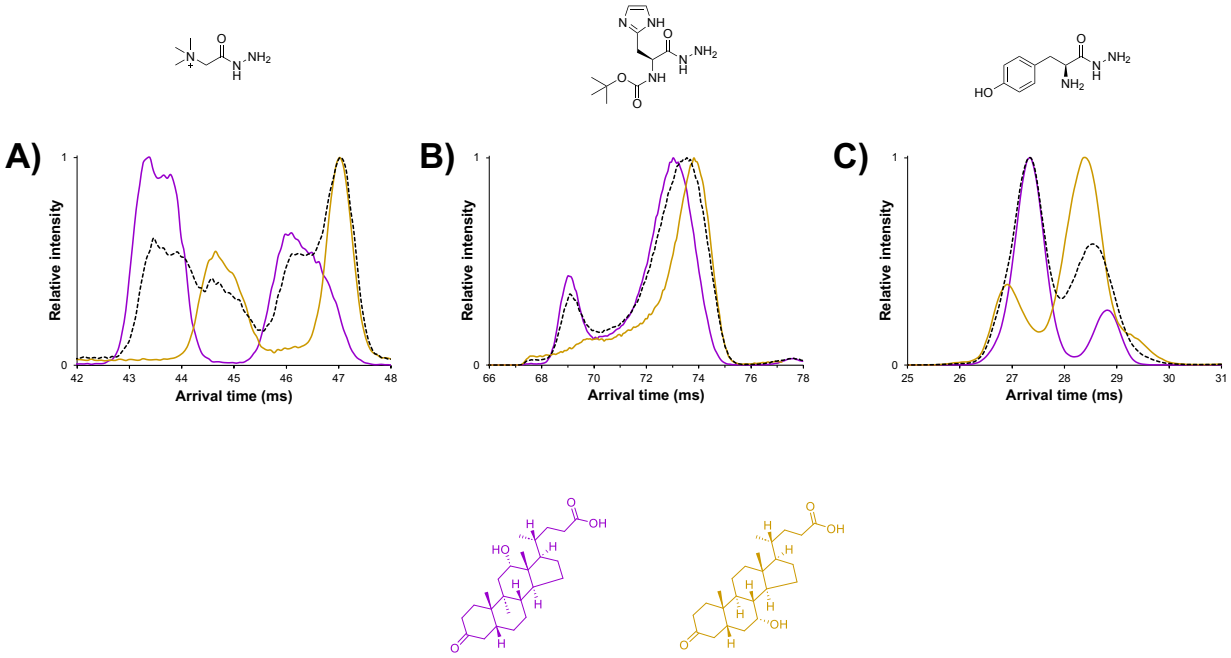

Figure S3. cIMS-MS separation (5 m) of the products of the 3-ODCA and 3-OCDOCA isomers with GT reaction as their $[M]^+$ ions at TW conditions of 480 m/s and 28 V with signal averaging performed for 5 min (A). cIMS-MS separation (2 m) of the products of the 3-ODCA and 3-OCDOCA isomers with L-tyrosine hydrazide reaction as its $[M + H]^+$ adducts at TW conditions of 480 m/s and 28 V with signal averaging performed for 5 min (B). cIMS-MS separation (5 m) of the products of the 3-ODCA and 3-OCDOCA isomers with N-boc-L-histidine hydrazide as its $[M + H]^+$ adducts under TW conditions of 550 m/s and 30 V with signal averaging performed for 3 min (C). The dotted black trace represents the isomeric mixture.

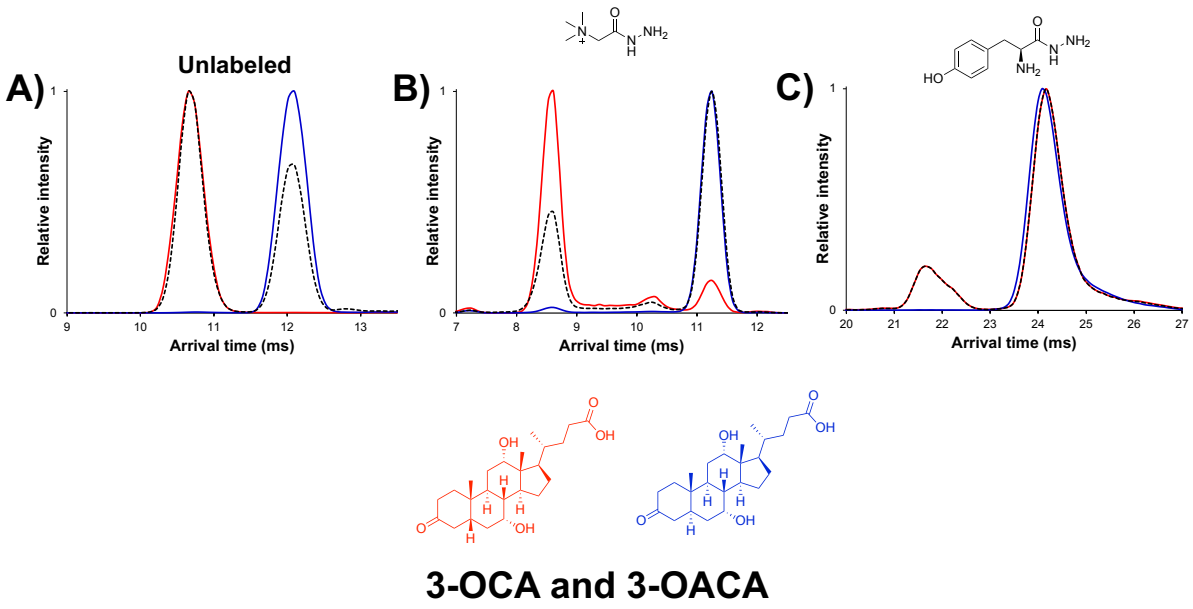

Figure S4. cIMS-MS separation of 3-OCA and 3-OACA as their $[M + Na]^+$ adduct at 1 m at TW conditions of 525 m/s and 25 V with signal averaging performed for 2 min (A). cIMS-MS separation (1 m) of the products of the 3-OCA and 3-OACA isomers with GT as their $[M]^+$ ions TW conditions of 500 m/s and 32 V with signal averaging performed for 4 min (B). cIMS-MS separation (2 m) of the products of the 3-OCA and 3-OACA isomers with L-tyrosine hydrazide as its $[M + H]^+$ adducts under TW conditions of 480 m/s and 28 V with signal averaging performed for 4 min (C). The dotted black trace represents the isomeric mixture.

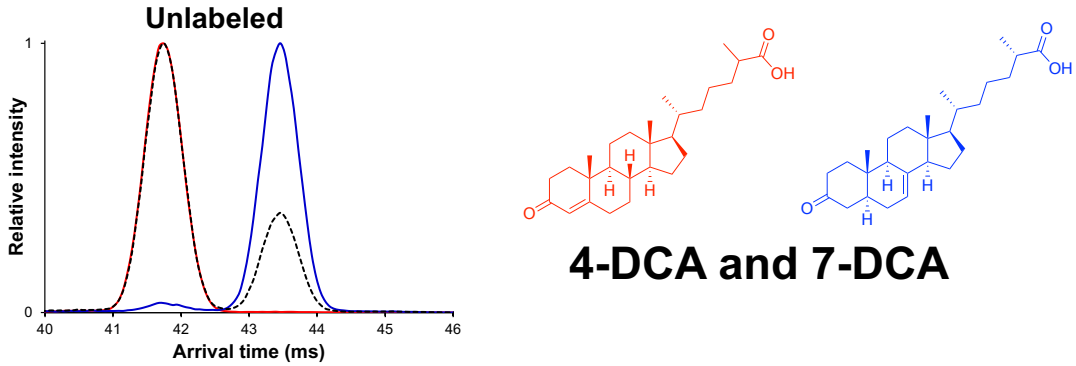

Figure S5. cIMS-MS separations of 4-DCA & 7-DCA isomers as their $[M + Na]^+$ adducts at 5 m at TW conditions of 450 m/s and 27 V with signal averaging performed for 2 min. The dotted black trace represents the isomeric mixture.