Supporting Information

Simultaneous Quantitation of Structurally Analogous Bile Acid Isomers via Charge-State-Engineered UPLC-MS

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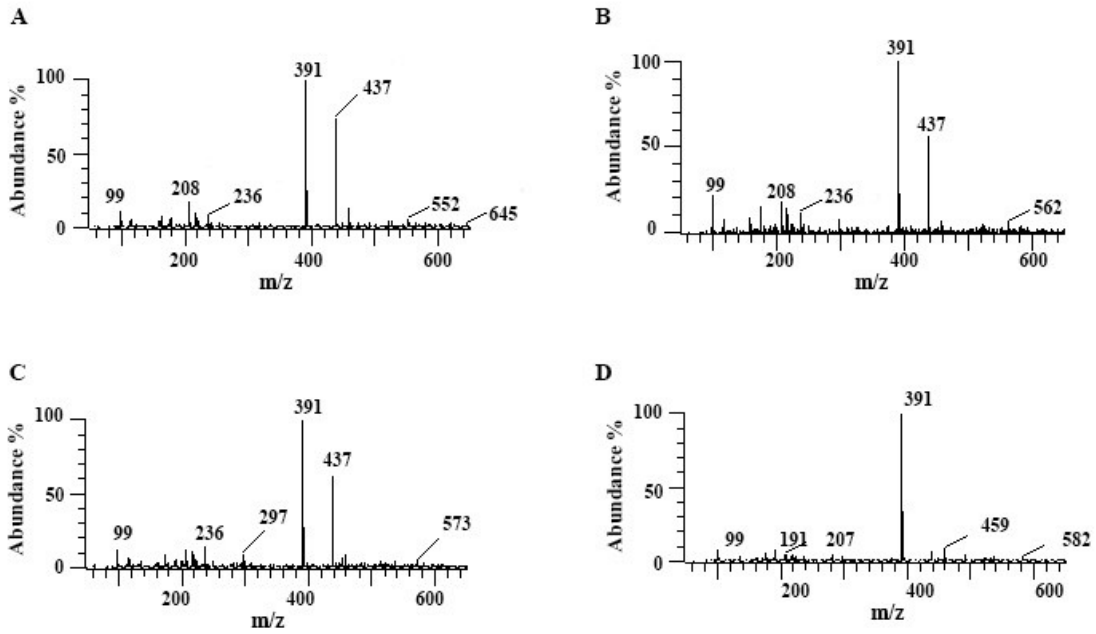

Fig. S1 Electron spray ionization mass spectra of (A) UDCA, (B) CDCA, (C) HDCA and (D) DCA in

negative ion mode. The concentration of standards was 1 μg/mL. Acetonitrile/2 mM aqueous ammonia (50:50, v/v) was used as the mobile phase, and the injection volume was set at 2 μL.

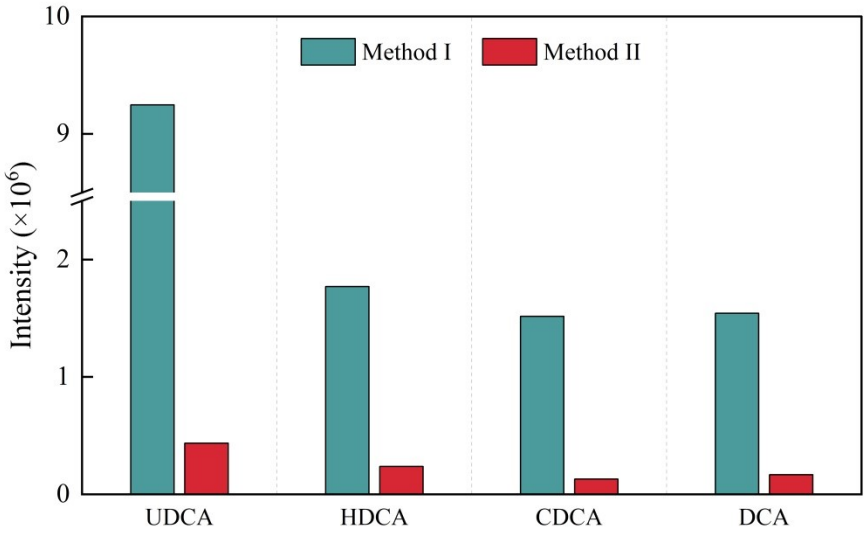

Fig. S2 Sensitivity comparison between SIM and MRM monitoring. Method Ⅰ: SIM monitoring with $[M–H]^-$ at m/z 391.35; the mobile phase consisted of water(A) and acetonitrile(B), both containing 8 mM aqueous ammonia; the gradient elution program was as follows: 0 min, 15% B; 4.0 min, 22% B; 6.0 min, 30%B; 8.0 min, 40% B; 8.1 min, 15% B; 10.0 min, 15% B. Method Ⅱ: MRM monitoring with transitions 357.35>339.40 and 357.35>133.20 for UDCA, 357.35>161.20 and 357.35>221.25 for HDCA, and 357.35>339.40 and 357.35>105.10 for CDCA and DCA; the mobile phase consisted of water(A), acetonitrile (B) and methanol (C), all containing 0.1% formic acid; the gradient elution program was as follows: 0 min, 70% A, 20%B, 10%C; 5.0 min, 50% A, 30%B, 20%C; 10.0 min, 25% A, 55%B, 20%C; 12.0 min, 5% A, 5%B, 90%C; 15.0 min, 70% A, 20%B, 10%C; 20.0 min, 70% A, 20%B, 10%C.

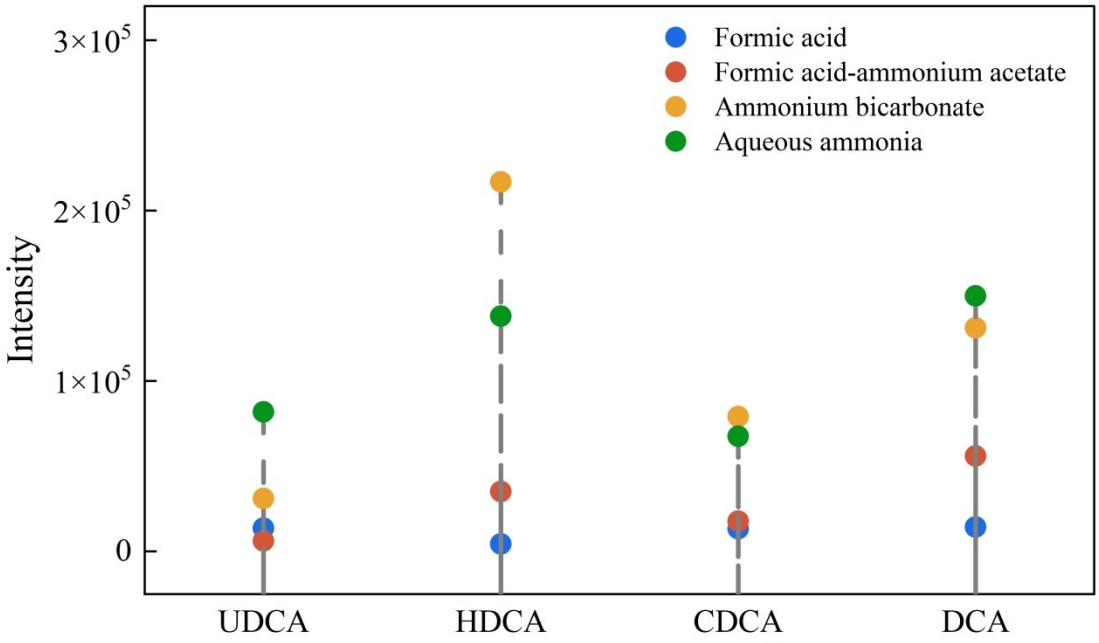

Fig. S3 Effects of mobile phase pH on signal intensity of UDCA, HDCA, CDCA and DCA using MS scan mode. The mobile phase consisted of a mixture of acetonitrile and one of the following aqueous

buffers at a volume ratio of 50:50 (v/v): 0.5% formic acid, 0.01% formic acid-5 mM ammonium acetate, 2 mM ammonium bicarbonate, or 2 mM aqueous ammonia.

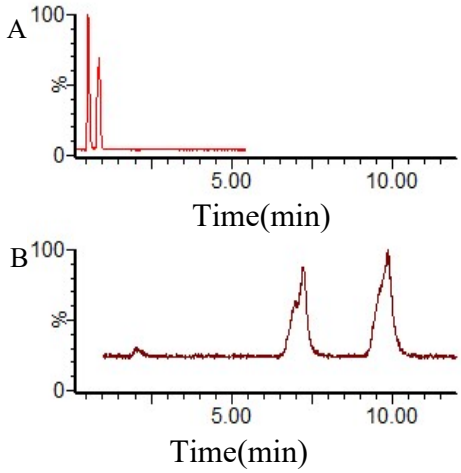

Fig. S4 Mass chromatograms of four bile acids obtained using two mobile phase systems: (A) acetonitrile–8 mM aqueous ammonia (50:50, v/v); (B) methanol–8 mM aqueous ammonia (50:50, v/v). The standard solution of the four bile acids was prepared at a concentration of 1 μg/mL, with an injection volume of 2 μL.

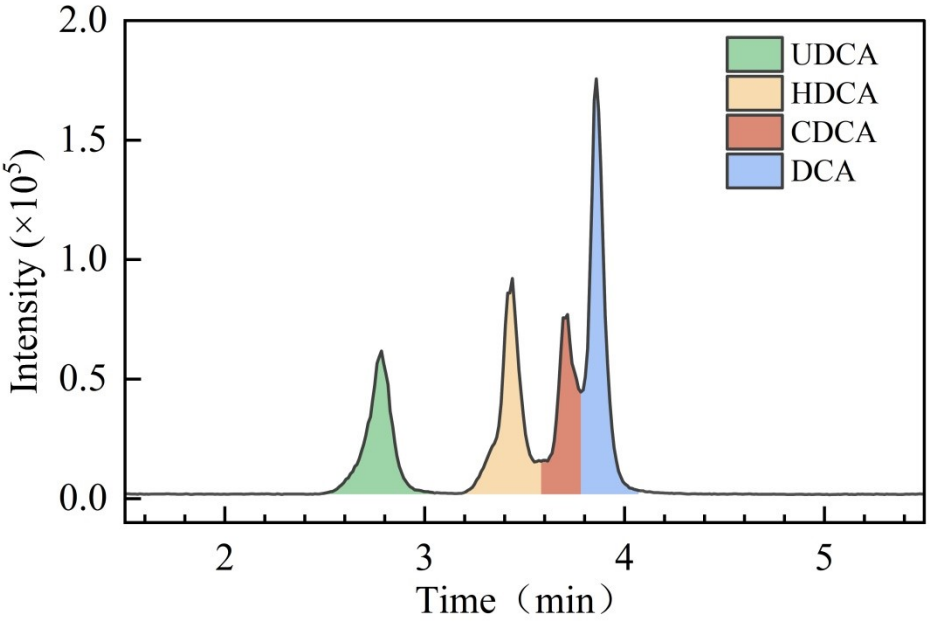

Fig. S5 Chromatograms of UDCA, CDCA, HDCA and DCA analysed with Fluoro-Phenyl chromatography column. The mobile phase was composed of 8 mM aqueous ammonia (A) and acetonitrile (B). The flow rate was 0.3 mL/min. The gradient elution program was as follows: 0 min, 15% B; 4.0 min, 22% B; 6.0 min, 30%B; 8.0 min, 40% B; 8.1 min, 15% B; 10.0 min, 15% B.

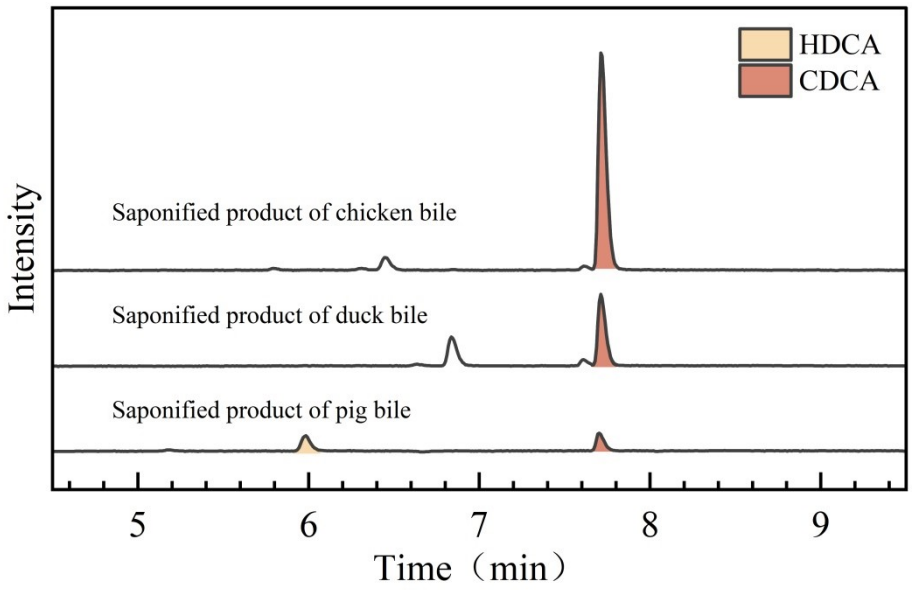

Fig. S6 Chromatograms of bile samples from poultry and pig. The mobile phase was composed of 8 mM aqueous ammonia (A) and acetonitrile (B). The flow rate was 0.3 mL/min. The gradient elution program was as follows: 0 min, 15% B; 4.0 min, 22% B; 6.0 min, 30%B; 8.0 min, 40% B; 8.1 min, 15% B; 10.0 min, 15% B.