

Supplemental information

Characterization of Metabolic Correlations of Ursodeoxycholic Acid with Other Bile Acid Species through In Vitro Sequential Metabolism and Isomer-Focused Identification

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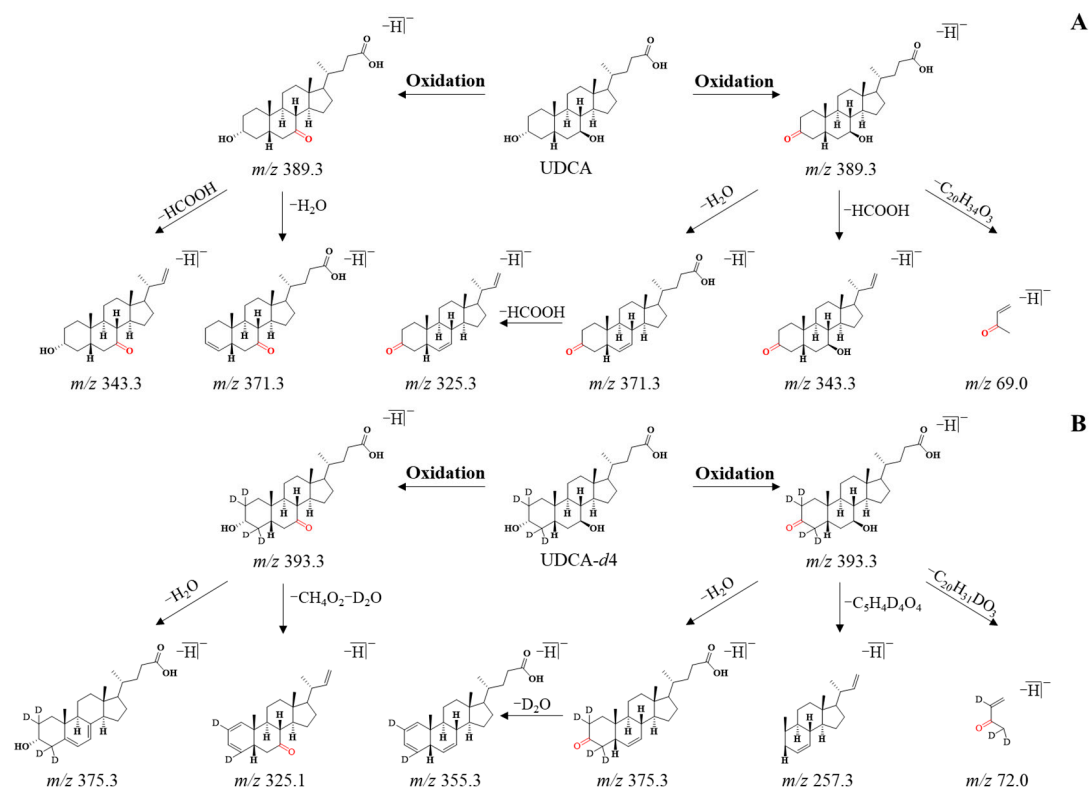


Figure S1. LC–MS chromatogram and proposed fragmentation pathways of oxidative products in the negative ion mode. (A) Proposed fragmentation pathways of 7 β -hydroxy-3-oxo-5 β -cholan-24-oic acid and 7-ketolithocholic acid. (B) Proposed fragmentation pathways of 7 β -hydroxy-3-oxo-5 β -cholan-24-oic acid- and 7-ketolithocholic acid- d_4 .

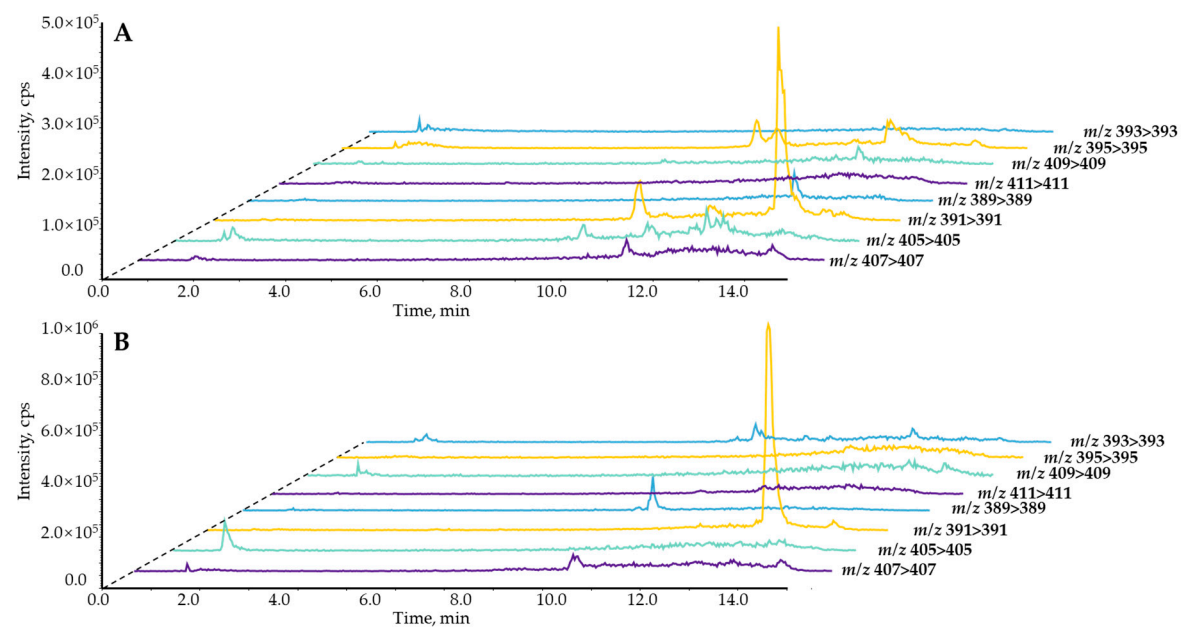


Figure S2. Extraction chromatography of incubation system from the LC-pMRM program (A) without UDCA and with NADPH incubation system involved HLM; (B) without UDCA and NADPH incubation system, with HLM.

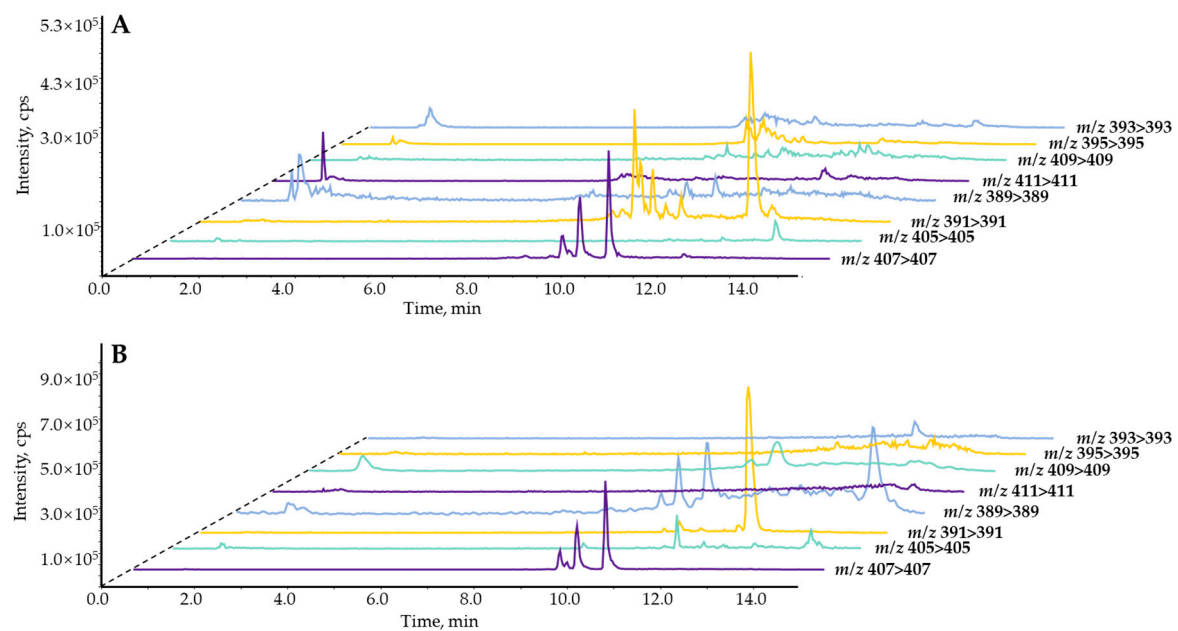


Figure S3. Extraction chromatography of incubation system from the LC-pMRM program (A) without UDCA and with NADPH incubation system involved MLM; (B) without UDCA and NADPH incubation system, with MLM.

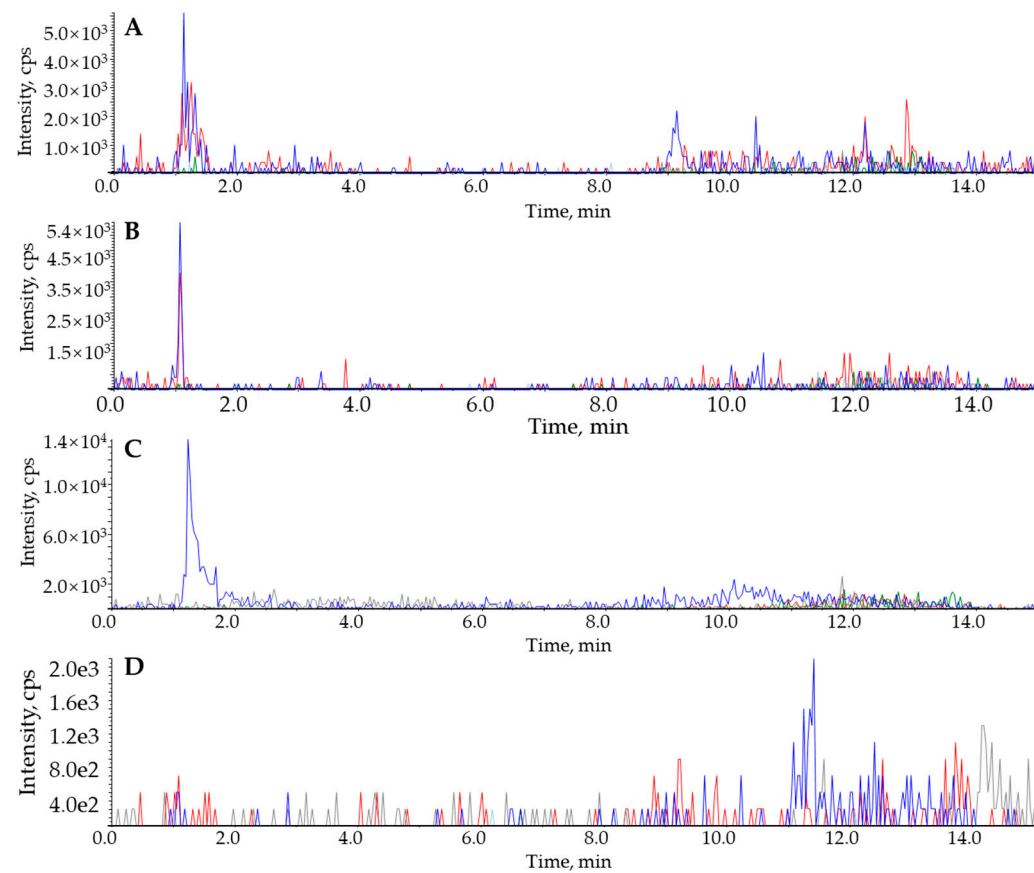


Figure S4. Extraction chromatography of incubation system from the LC-*p*MRM program (A) without UDCA and with UDPGA incubation system involved HLM or MLM; (B) without UDCA and UDPGA incubation system, with HLM or MLM; (C) without UDCA and with PAPS incubation system involved HLM; (D) without UDCA and PAPS incubation system, with HLM or MLM.

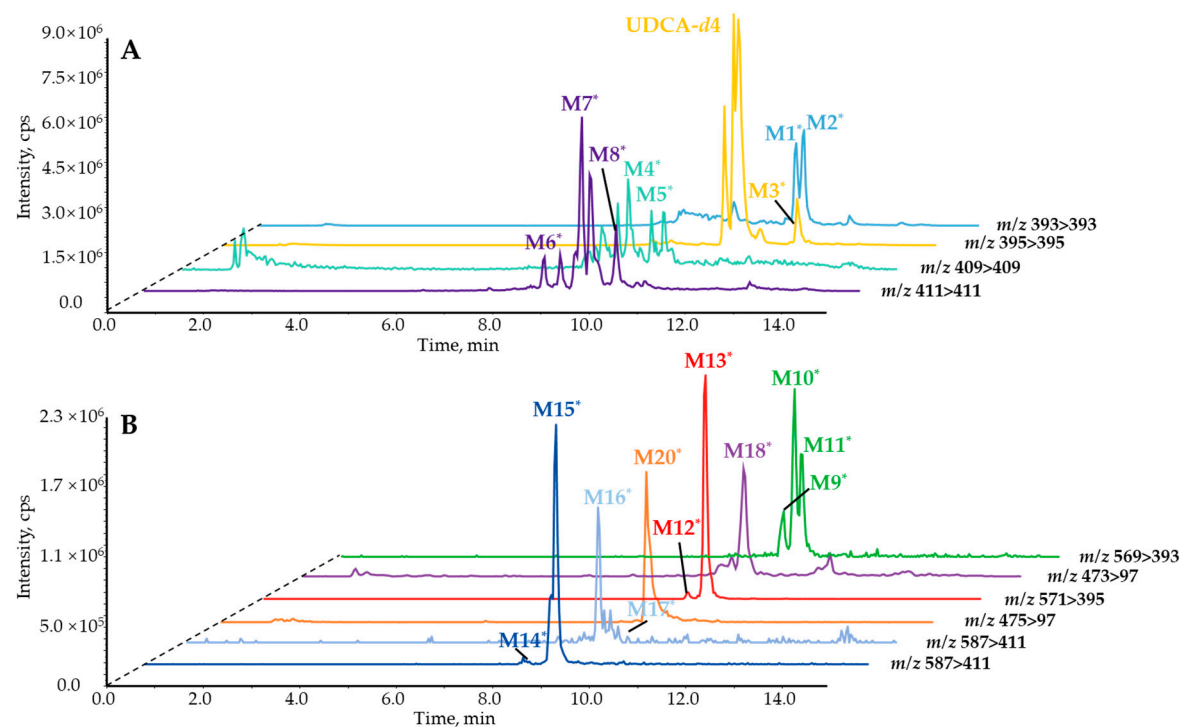


Figure S5. (A) Extracted ion current chromatogram (phase I metabolites of UDCA-*d*₄) from the LC-*p*MRM program; (B) Extracted ion current chromatogram (phase II glucuronidated and sulfated metabolites of UDCA-*d*₄) from the LC-*p*MRM program.

Table S1 The chromatographic, MS/MS information, optimized ion transitions and collision energy information of UDCA *in vitro* metabolites generated from incubation of liver microsomes (human and mouse) by *p*MRM program.

Metabolite	<i>t_R</i> (min)	Q1 (Da)	Q3 (Da)	OCE (eV)	DP (V)	MS ¹ (Da)	MS ² (Da)	AF2 level corresponding to 50% relative intensity	Compound name	Incubation system
M1	10.99	389.3	389.3	-22.8	-100	–	–	<i>m/z</i> 389.3>389.3>389.3(0.01802V)	7β-hydroxy-3-oxo-5β- cholan-24-oic acid	HLM & MLM
	8.66	–	–	–	–	389.2705	371.2637,343.2656,325.2553,69.03 46			MLM
M1-<i>d</i>₄	8.63	393.3	393.3	-22.8	-100	393.2952	375.2863,355.2267,257.1544,72.05 35	–	7β-hydroxy-3-oxo-5β- cholan-24-oic acid- <i>d</i> ₄	HLM & MLM
M2	11.25	389.3	389.3	-22.3	-100	–	–	<i>m/z</i> 389.3>389.3>389.3(0.02379V)	7-ketolithocholic acid*	HLM & MLM
	8.78	–	–	–	–	389.2705	371.2610,343.2649			MLM
M2-<i>d</i>₄	8.79	393.3	393.3	-22.3	-100	393.2954	375.2910,325.1848	–	7-ketolithocholic acid- <i>d</i> ₄	HLM & MLM
M3	12.00	391.3	391.3	-20.1	-100	–	–	<i>m/z</i> 391.3>391.3>391.3(0.02077V)	Chenodeoxycholic acid*	HLM & MLM
	8.34	–	–	–	–	391.286	373.2764,345.7297			
M3-<i>d</i>₄	9.53	395.3	395.3	-20.1	-100	395.3117	377.3023	–	Chenodeoxycholic acid- <i>d</i> ₄	HLM & MLM
	9.40	405.3	405.3	-16.79	-100	–	–		3α,7β-dihydroxy-6-oxo-5β- cholan-24-oic acid or 3α,6β- Dihydroxy-7-oxo-5β- cholan-24-oic acid	HLM & MLM HLM
M4	7.69	–	–	–	–	405.2659	387.2552,375.2551,369.2458,357.2 482	–		
M5	10.12	405.3	405.3	-20.67	-100	–	–	–	3α,7β-dihydroxy-12-oxo- 5β-cholan-24-oic acid	HLM & MLM MLM
	7.98	–	–	–	–	405.2659	387.2559,353.2128,69.0345			
M5-<i>d</i>₄	7.94	409.3	409.3	-20.67	-100	409.2907	391.2817	–	3α,7α-dihydroxy-12-oxo- 5β-cholan-24-oic acid - <i>d</i> ₄	MLM
M6	8.30	407.3	407.3	-21.02	-100	–	–	<i>m/z</i> 407.3>407.3>407.3(0.02594V)	3β,7β,12α-trihydroxy-5β-	HLM

	6.60	–	–	–	–	407.2815	389.2744,371.2604		cholan-24-oic acid	
M6-<i>d</i>₄	6.58	411.3	411.3	–21.02	–100	411.3063	375.2857,365.3013,69.0346	–	3β,7β,12α-trihydroxy-5β-cholan-24-oic acid- <i>d</i> ⁴	HLM
M7	9.11	407.3	407.3	–18.1	–100	–	–	<i>m/z</i> 407.3>407.3>407.3(0.03270V)	Ursocholic acid *	HLM & MLM
	6.70	–	–	–	–	407.2813	391.2476,345.2444,271.2069			HLM
M7-<i>d</i>₄	6.70	411.3	411.3	–18.1	–100	411.3065	395.2760,349.2695	–	Ursocholic acid- <i>d</i> ₄	MLM
M8	9.86	407.3	407.3	–22.3	–100	–	–	<i>m/z</i> 407.3>407.3>407.3(0.03836V)	β-muricholic acid *	HLM & MLM
	7.40	–	–	–	–	407.2812	371.2602,331.2287,69.0347			MLM
M8-<i>d</i>₄	7.37	411.3	411.3	–22.3	–100	411.3063	393.2951,365.1661,349.2690	–	β-Muricholic acid- <i>d</i> ₄	MLM
M9	9.16	565.3	389.3	–47.68	–100	–	–	<i>m/z</i> 565.3>389.3>389.3(0.02630V)	7β-hydroxy-3-oxo-5β-cholan-24-oic acid-7- <i>O</i> -glucuronide	HLM & MLM
M10	9.37	565.3	389.3	–48.38	–100	–	–	<i>m/z</i> 565.3>389.3>389.3(0.02491V)	7β-hydroxy-3-oxo-5β-cholan-24-oic acid-24- <i>O</i> -glucuronide	HLM & MLM
M11	9.52	565.3	389.3	–49.74	–100	–	–	<i>m/z</i> 565.3>389.3>389.3(0.01962V)	7-ketolithocholic acid-24- <i>O</i> -glucuronide	HLM & MLM
M12	8.83	567.3	391.3	–49.96	–100	–	–	<i>m/z</i> 567.3>391.3>391.3(0.02576V)	Ursodeoxycholic acid-7- <i>O</i> -glucuronide	HLM & MLM
M13	9.16	567.3	391.3	–51.5	–100	–	–		Ursodeoxycholic acid-3- <i>O</i> -glucuronide	HLM & MLM
	6.65	–	–	–	–	567.3202	391.2863,175.0256,129.0200,113.0250	<i>m/z</i> 567.3>391.3>391.3(0.03289V)		MLM
M13-<i>d</i>₄	6.62	571.3	395.3	–51.5	–100	571.345	395.3119,175.0255,133.0149,113.0250	–	Ursodeoxycholic acid-3- <i>O</i> -glucuronide- <i>d</i> ₄	MLM
M14	7.78	583.3	407.3	–50.57	–100	–	–	<i>m/z</i> 583.3>407.3>407.3(0.03090V)	Ursocholic acid-7- <i>O</i> -glucuronide	MLM

									glucuronide		
M15	8.44	583.3	407.3	−51.33	−100	−	−	<i>m/z</i> 583.3>407.3>407.3(0.03688V)	β-Muricholic	acid-3- <i>O</i> -	MLM
	6.00	583.3	407.3	−51.33	−100	583.3144	407.2813,175.0255,129.0201,113.0250		glucuronide		
M16	8.62	583.3	407.3	−53.74	−100	−	−	<i>m/z</i> 583.3>407.3>407.3(0.03289V)	Ursocholic	acid-3- <i>O</i> -	HLM
M17	9.2	583.3	407.3	−54.11	−100	−	−	<i>m/z</i> 583.3>407.3>407.3(0.02803V)	3β,7β,12α-trihydroxy-5β-	acid-3- <i>O</i> -	HLM
									cholan-24-oic		
M18	9.22	469.3	97	−99.87	−100	−	−	<i>m/z</i> 469.3>389.3>389.3(0.02525V)	7β-hydroxy-3-oxo-5β-	acid-7-sulfate	HLM
									cholan-24-oic		
M19	8.69	471.3	97	−102.5	−100	−	−	<i>m/z</i> 471.3>391.3>391.3(0.02812V)	Ursodeoxycholic	acid-3-	HLM & MLM
	6.64	−	−	−	−	471.2427	391.2880,96.9605,79.9576		sulfate*		HLM
M19- <i>d</i> ₄	6.62	475.3	97	−102.5	−100	475.2677	395.3124	−	Ursodeoxycholic	acid- <i>d</i> ₄ -3-	HLM & MLM
M20	8.92	471.3	97	−101.1	−100	−	−	<i>m/z</i> 471.3>389.3>389.3(0.02516V)	Ursodeoxycholic	acid-7-	HLM & MLM
									sulfate		
									sulfate		

Note: “*” identified by authentic reference. HLM: human liver microsomes; MLM: mouse liver microsomes.