

Bile Acids: Fecal or biofluid samples

Service Code: BA

Reference1: William J. Griffiths and Jan Sjövall, Bile acids: analysis in biological fluids and tissues, *Journal of Lipid Research*, 2010, 51, 23.

Reference2 : K. Bentayeb, R. Batlle, C. Sánchez, C. Nerín, C. Domeño, Determination of bile acids in human serum by on-line restricted access material–ultra high-performance liquid chromatography–mass spectrometry;

Reference 3: Metabolic Profiling of Bile Acids in Human and Mouse Blood by LC-MS/MS in combination with phospholipid depletion Solid-Phase extraction, *Analytical Chemistry*, 2015,87,1127-1136.

Summary: Two-step solvent extraction. Supernatants are combined, dried, and re-suspended for LCMS separation by RPLC and measurements by ESI⁻ QQQ MRM methods. Analytes are reported as uM, and CV's are generally <15%.

Container: 2 mL eppendorf-type polypropylene centrifuge tube

Normal Volume:

Minimal Volume: feces or chyme (50 mgs.); plasma or serum (200uL); others (500 uL)

Special Handling: If human or primate, note any known presence of infectious agents

Sample Collection: put feces/chyme into pre-weighed eppendorf tube and provide sample weight on sample submission form.

Analyte	KEGG /CAS number	Normal conc. In human-blood, uM	Normal concentration in human stool	ISTD	MRM (-)	Typical Rt	LOD (uM)
CA (Cholate) *	C00695	0.72 +/- 0.24+ (.040 uM)\$	44+/47 nmol/g feces	CA-d4	407.3->343.2	17.1	0.1
GCA (Glycocholate) *	C01921	0.06 +/- 0.04+	10 +/- 7 nmol/g feces	GCA-d4	464.3->74	12.8	0.005
DCA (Deoxycholate) *	C04483	0.33 +/- 0.06+ or 0.57 +/- 0.35+ (females)	1920 +/- 1390 nmol/mg feces	DCA-d4	391.3->345.3	19.3	0.3
TLCA (Tauroolithocholate) *	C02592	0.614 +/- 0.013+ or 1.8 +/- .002+	.51 +/- .40 nmol/g feces	DCA-d4	482.3->80	18.7	0.005
TCA (Taurocholate) *	C05122	0.38 +/- 0.20+	5.78 +/- 4.32 nmol/g feces	GCA-d4	514.3->80	9.7	0.05
LCA (lithocholate) *	C03990	0.33 +/-	1016 +/-	LCA-d4	375.3-	19.9	0.005

		0.04+ (.00948)\$	647 nmol/g feces		>375.3		
CDCA (chenodeoxycholate) *	C02528 ,	0.98+/- 0.66+		DCA-d4	391.3- >391.3		0.005
<i>a</i> -MCA (<i>alpha</i> -Murocholate) *	C17647	.00047\$		GCA-d4	407.3- >407.3	12.8	
<i>b</i> -MCA (<i>beta</i> -Muricholate) *	C17726	n/d\$		CA-d4	407.3- >407.3	13.6	
<i>g</i> -MCA(<i>gamma</i> -Muricholate) aka <i>a</i> -HCA (<i>alpha</i> -Hyocholate) *	C17649 [547-75- 1]						
<i>w</i> -MCA (<i>omega</i> -Muricholate) *	C17727	.00563\$		GCA-d4	407.3- >407.3	19.3	
GLCA (glycolithocholate) *		0.009 (0.005- 0.015) +		LCA-d4	432.3->74	19.3	
TCDCA (taurochenodeoxycholate) *		0.30 +/- 0.08+		CA-d4	498.3->80	13.8	
TDCA (taurodeoxycholate) *		0.062 (0.001- 0.177) +		CA-d4	498.3->80	14.8	
GHDCa (glycohyodeoxycholate) *	CID114611[1304 2-33-6]	.00163\$		GCA-d4	448.3->74	12.5	
GUDCA (glycoursodeoxycholate) *	CID12310288[64 480-66-6]	0.1-0.7+		GCA-d4	448.3 -> 74.0	12.5	
GCDCA (glycodeoxycholate) *	GCDCA-d4 [32747-08-3]	0.73 uM (355 ng/g)#		GCA-d4	448.3 -> 74.0	18	
GHCA (glychoyocholate) *				GCA-d4	464.3 -> 74.0		
UDCA(urso deoxycholate) *	C07880	0.06 uM (21.3 ng/g)#, 0.1 uM\$		GCDCA-d4	391.3 -> 391.3		
HCA (hyocholic acid)					407.3 -> 407.3	15.4	
HDCA (hyodeoxycholate) *		(only trace amts in humans; except those with choleostatic liver disease (0.00313\$)		GCDCA-d4	391.3 -> 391.3	18.0	
THDCA (taurohyodeoxycholate)/ TUDCA (tauroursodeoxycholate) **		.00489\$		GCA-d4	498.3 -> 80.0	9.2	
GDCA (glycodeoxycholate) *	16409-34-0	0.26 uM(130 ng/g)#		DCA-d4	448.3 -> 74.0	18.5	
MDCA (murideoxycholic acid)	C15515 HMDB00811						
7-oxoDCA(7-oxo-deoxycholate)	[911-40-						

	0]1426091-04-4						
T-12-epiDCA(tauro-12-epi deoxycholate)							
TaMCA(tauro-alpha-muricholate) * (TaMCA)	C1893-000			GCA-d4	514.3 -> 80.0	6.25	
TbMCA(tauro-beta-muricholate) * (TbMCA)	C1899-000			GCA-d4	514.3 -> 80.0	6.4	
TwMCA(tauro-omega-muricholate)	C1889-000						
TMDCA(tauro-murideoxycholate)	AlanHofmannUC SD						
isoDCA(iso-deoxycholic acid)							
isoLCA(iso-lithocholate) *	1534-35-6 C1475-000	.00959\$		LCA-d4	375.3 -> 375.3		
TUCA(tauroursocolanate)	C0835-000	.01uM (1.9 ng/g)#					
Glyco-gamma-muricholate aka glycohyocholate	C1860-000						
THCA Tauro-gamma-muricholate aka taurohyocholate *	C1887-000			GCA-d4	514.3 -> 80.0	7.7	
Rodent species in blue font							
+ = HMDB; #=Reference 2 \$=Reference3							

Materials

1. Agilent 6410 QQQ with 1260 LC unit, chilled autosampler, with standard 54-well autosampler plate
2. Vortexer
3. Refrigerated centrifuge, capable of 13,000g with eppendorf tube compatible rotor
4. Eppendorf Vacufuge
5. ice bucket, ice
6. Prepared 10 mM stock solutions of each authentic standard and 100uM of isotope-labelled internal standard (cholic acid-d₄) in methanol.
7. eppendorf tubes (polypropylene)
8. LCMS grade water, acetonitrile (ACN), and methanol (MeOH)
9. ACS Reagent Grade chloroform, ammonium acetate, ammonium hydroxide, and acetic acid
10. Water-bath sonicator

PROCEDURE

Sample Preparation – Fecal, Jejunum, Cecal

1. Prepare standard curve solutions (0.2, 0.6, 2, 6, 20 uM) using 10 mM aqueous stocks of authentic standards

2. prepare extraction solvent A: 100% ethanol with 0.2 uM IS, B: chloroform/Methanol, 1:1 with 0.2uM IS, chill on ice
3. Add 1500 uL of extraction solvent A to each tube, pipette 750 ul to another 1.5 ml tube
4. Sonicate using water-bath sonicator for 30min
5. Centrifuge 10 minutes at 13,000g and 4°C. Combine supernatant from step 4 and transfer 750ul to a labeled autosampler vial for drying
6. Add 750ul of extraction solvent B) to the pellet from step 1 (2 tubes, so 750x2 =1500ul). Vortex briefly. Sonicate using water-bath sonicator for 30min.
7. Centrifuge 10 minutes at 15,000g at 4°C. Combine supernatant from step 6 and transfer 750ul of supernatant to a labeled autosampler vial.
8. Dry extracts from step 5 and 7 by vacuum centrifuge to dryness at 45°C.
9. Resuspend sample extracts in 50 uL of 75:25 Methanol/H₂O and transfer to autosampler insert tube
10. Keep samples at 4°C until analysis.

Sample Preparation Notes – Plasma

1. ?

LC-MS/MS Procedure

1. LC column: waters XBridge C18 (150mm x 2.1mm, 3.5um) at ?? °C
2. Solvent A: 10 mM ammonium acetate in ACN/MeOH (3:1, v/v)
3. Solvent B: 10 mM ammonium acetate in H₂O, adjust pH to 8.0 with NH₄OH
4. Gradient: 0min, 70%B; 6min, 35%B; 14min, 28%B; 15min, 10%B, 20min, 70%B; 30min,70%B; flow rate: 200ul/min
5. Autosampler: 4°C, 10 uL injection
6. Agilent 6410 MS/MS: ESI⁻, 350 °C, 800 ms cycle time, N₂ collision gas, see MRM table
7. Agilent method: xxxx.m or equivalent
8. Collect standard curve data first, then sample data if system is suitable.
9. Bile acids are measured by

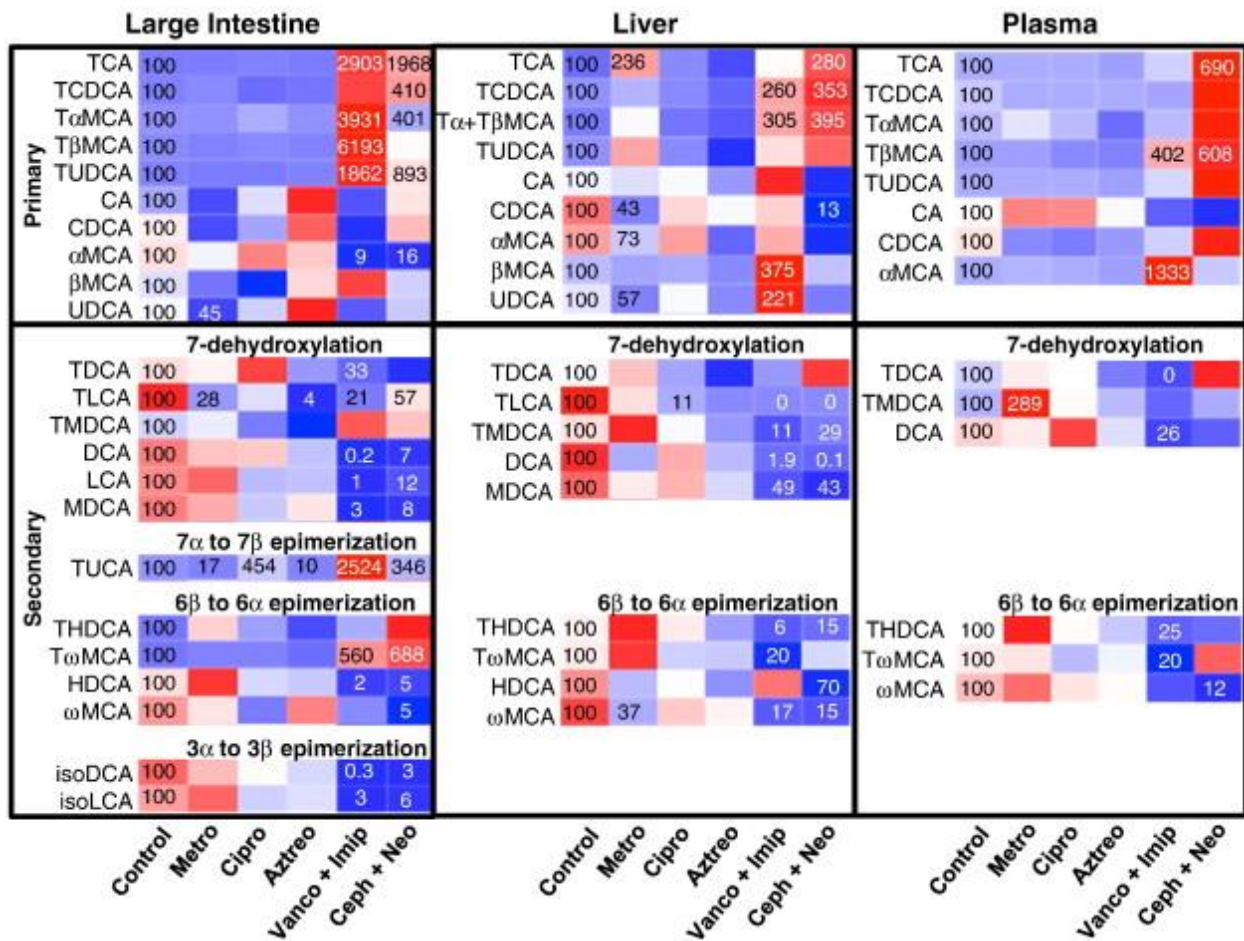
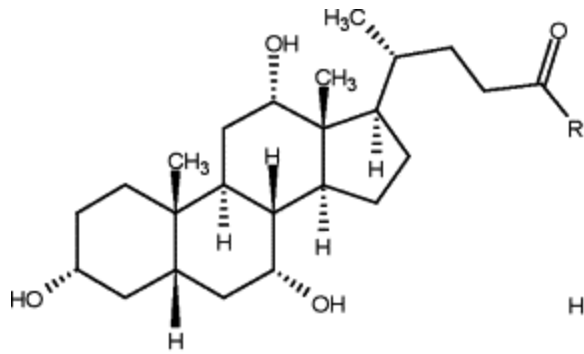
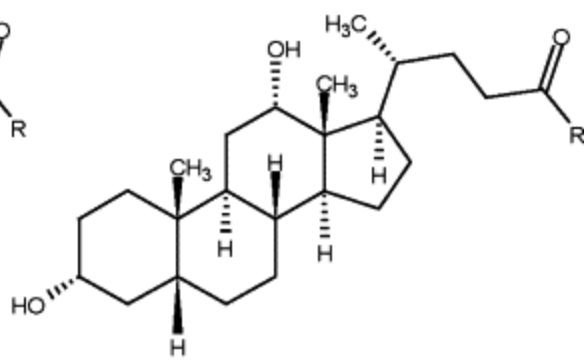


Fig. 3 from ToxicolAppliedPharmacol_277_138

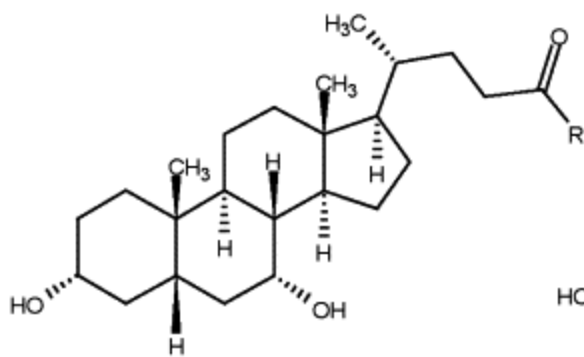
Methods: PLoSone DOI: 10.1371/journal.pone.0034522 J Lipid Res_51_13



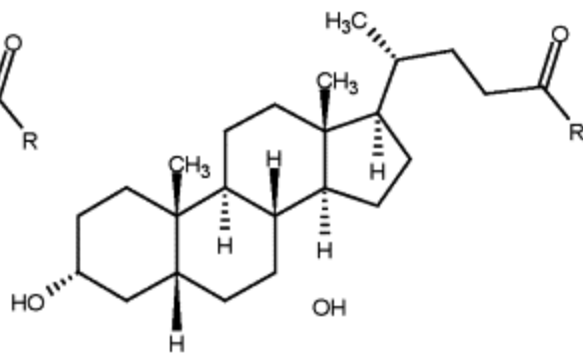
R = OH cholic acid
 R = glycine glycocholic acid
 R = taurine taurocholic acid



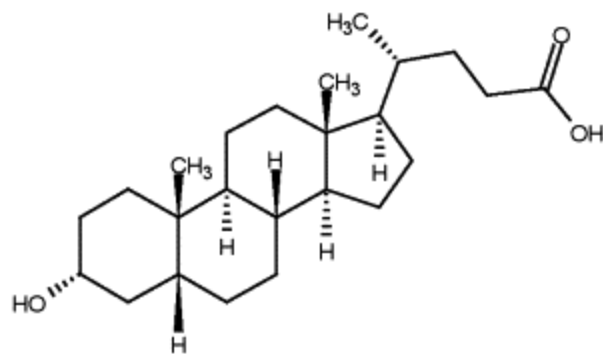
R = OH deoxycholic acid
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R = OH chenodeoxycholic acid
 R = glycine glycochenodeoxycholic acid
 R = taurine taurochenodeoxycholic acid



R = OH ursodeoxycholic acid
 R = glycine glycoursoxycholic acid
 R = taurine tauroursodeoxycholic acid



lithocholic acid