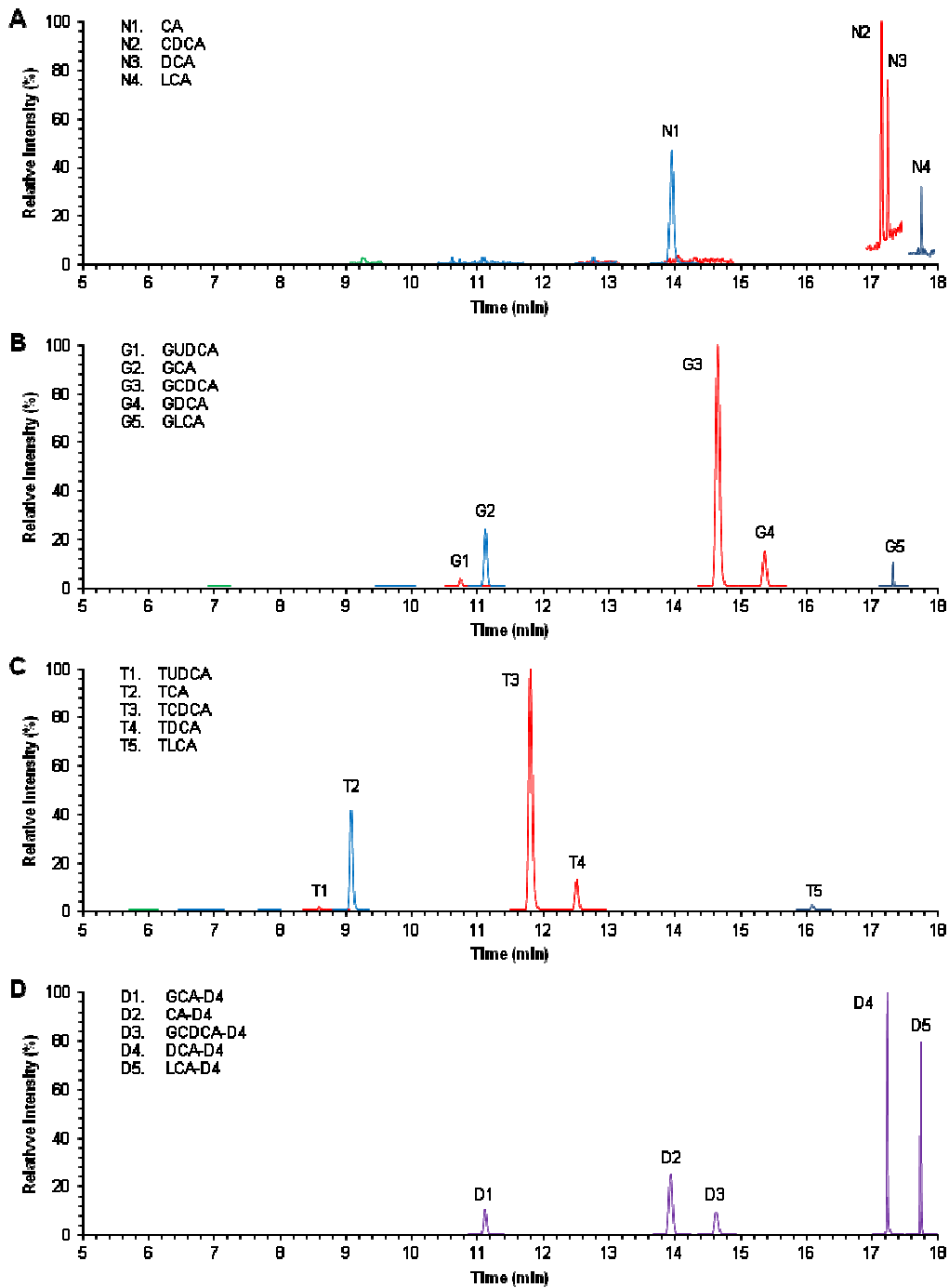
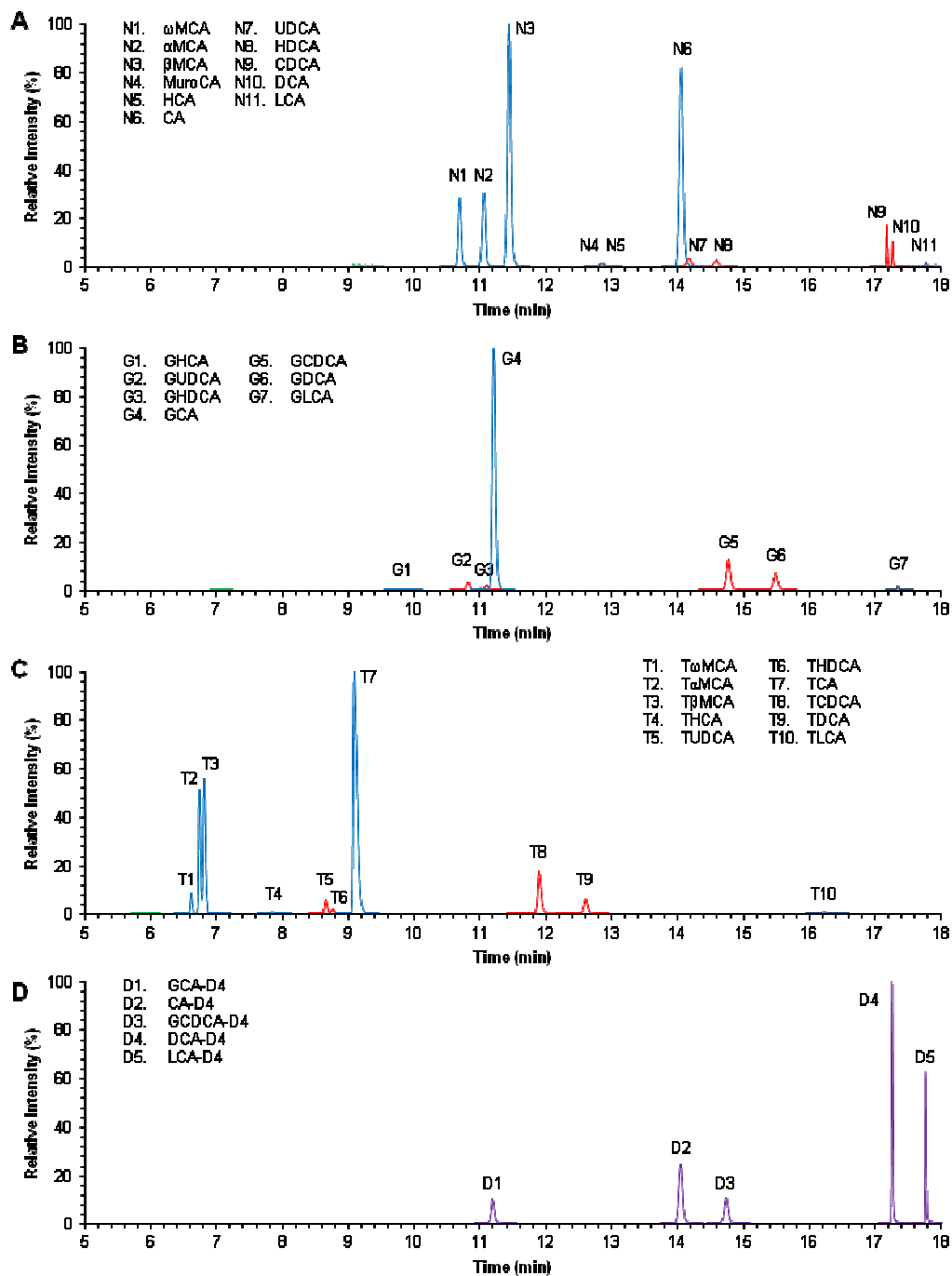


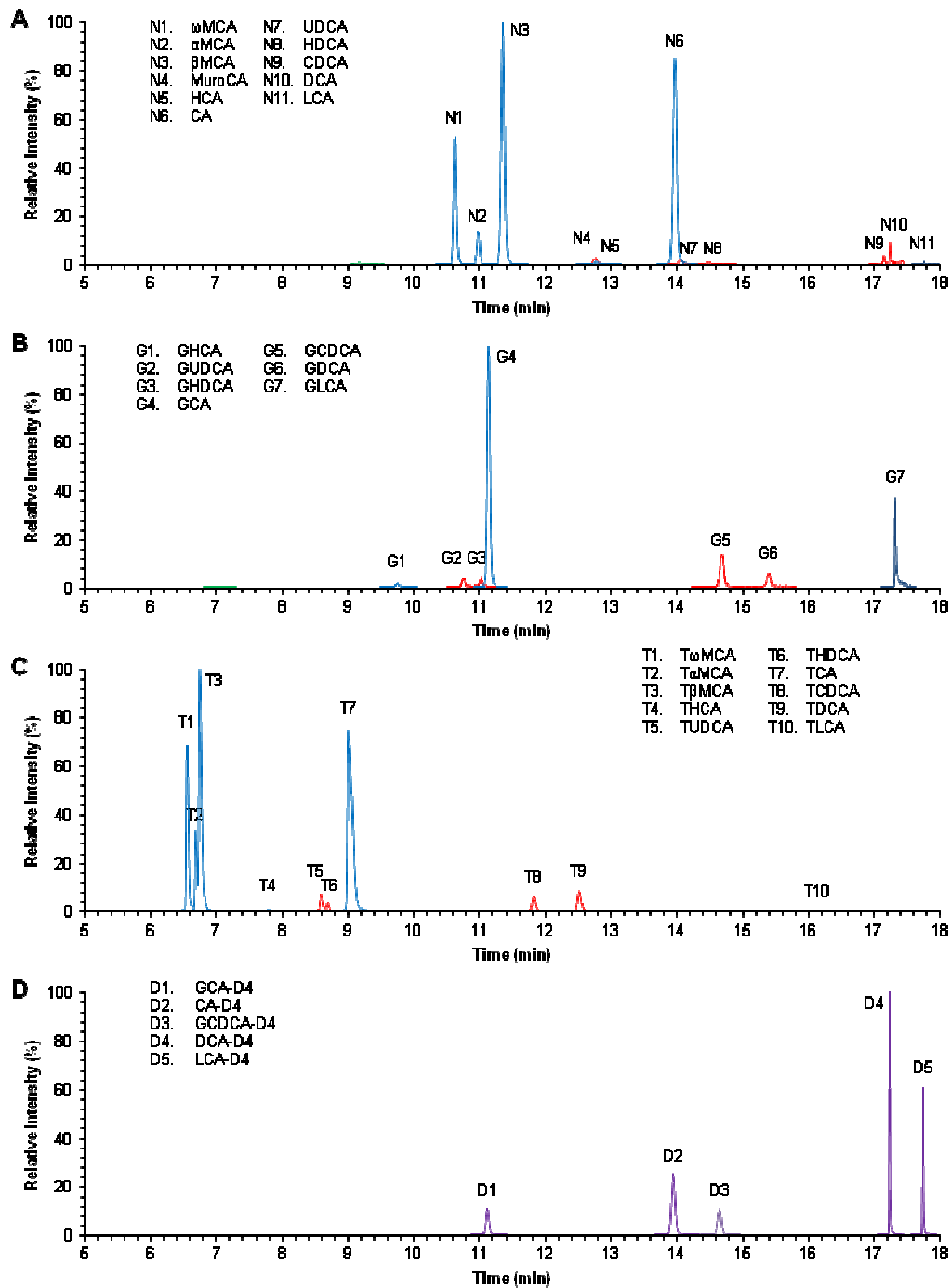
## Supplementary Material



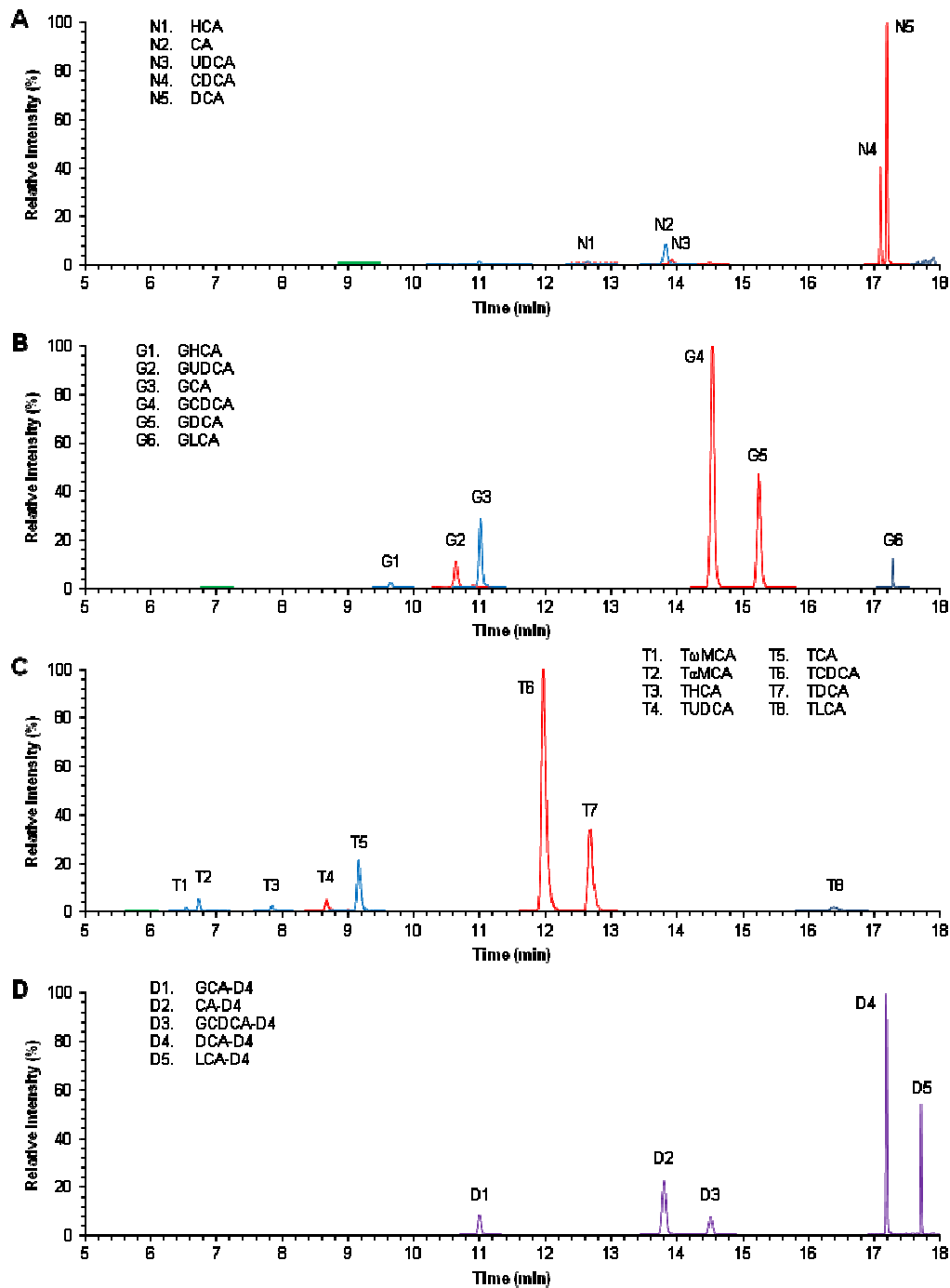
**Fig. S1.** Chromatographic separation of a pooled human liver sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark-blue*: mono-hydroxylated BAs.



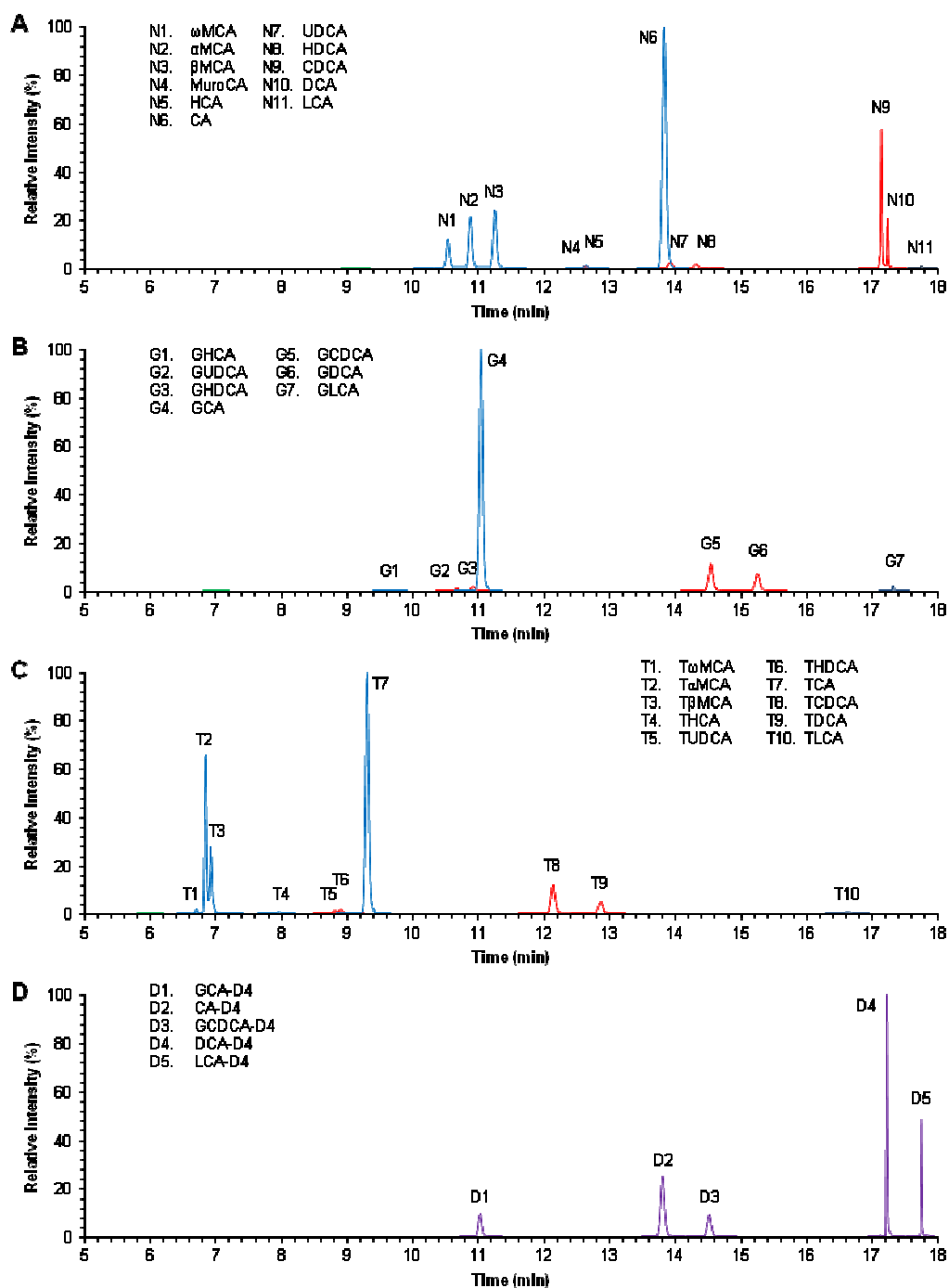
**Fig. S2.** Chromatographic separation of a pooled rat liver sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark blue*: mono-hydroxylated BAs.



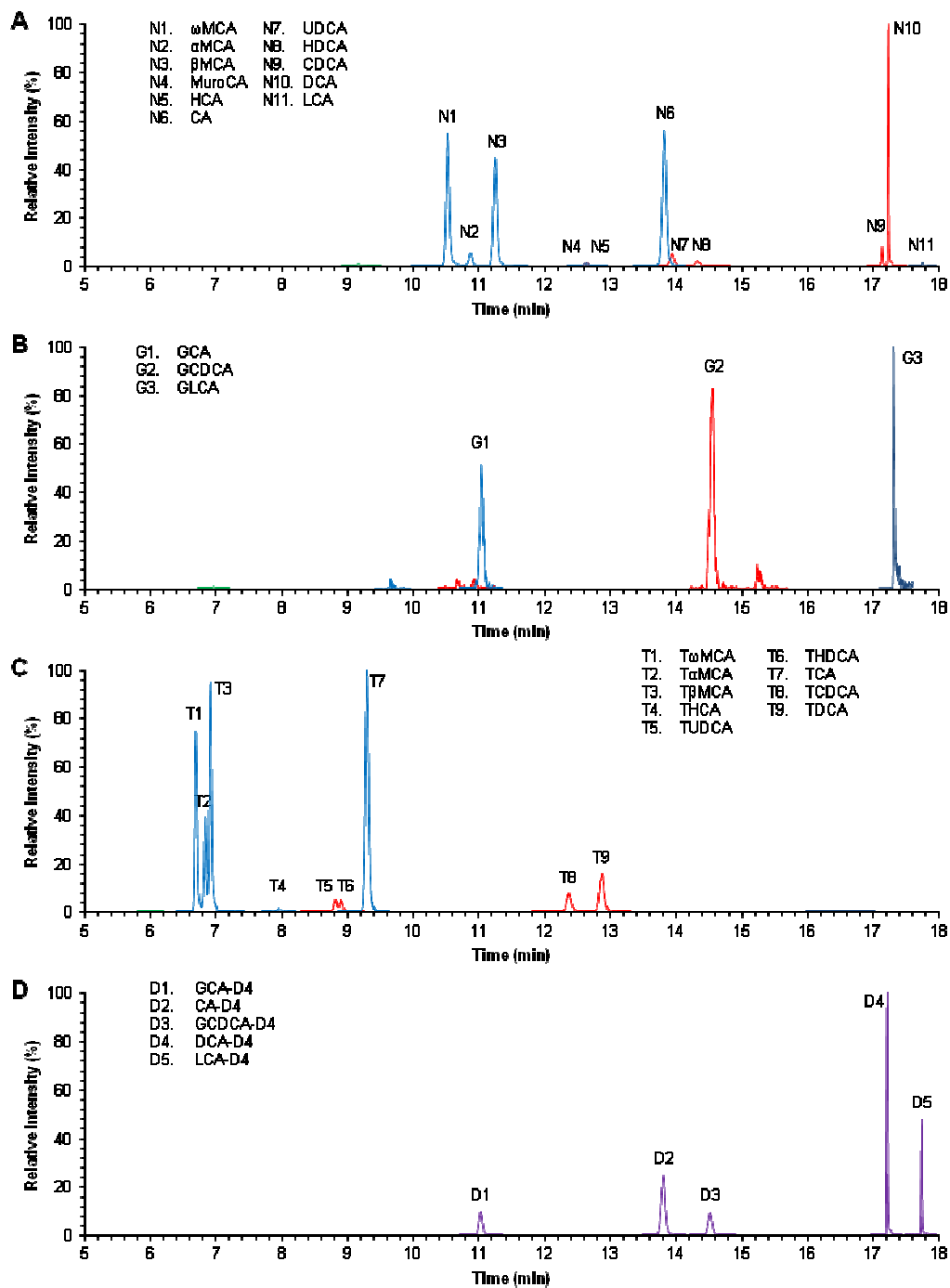
**Fig. S3.** Chromatographic separation of a pooled mouse liver sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark blue*: mono-hydroxylated BAs.



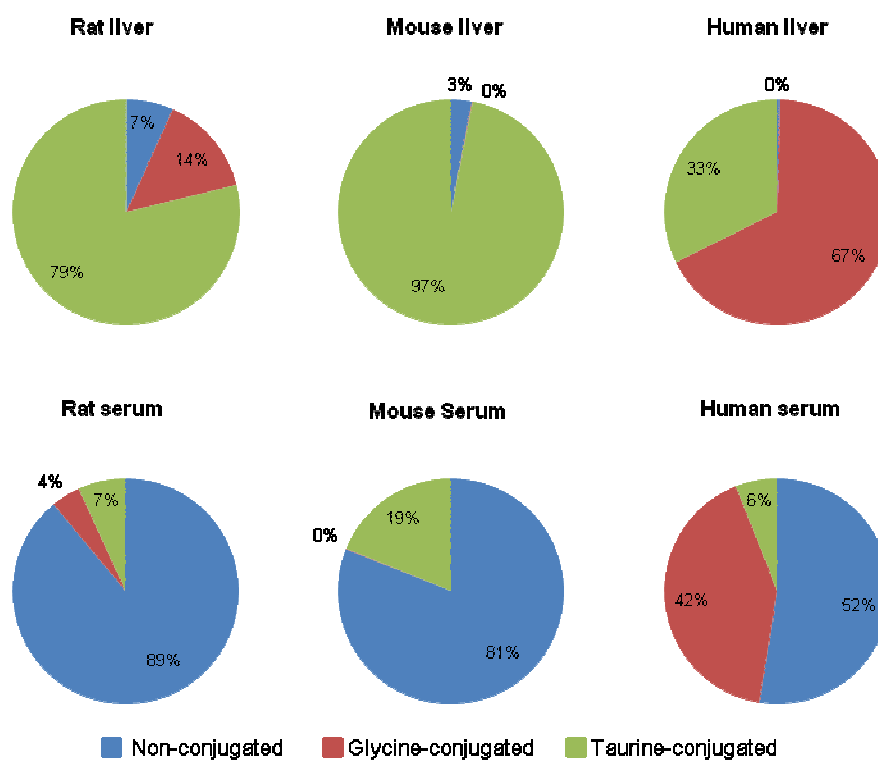
**Fig. S4.** Chromatographic separation of a pooled human serum sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark blue*: mono-hydroxylated BAs.



**Fig. S5.** Chromatographic separation of a pooled rat serum sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark blue*: mono-hydroxylated BAs.



**Fig. S6.** Chromatographic separation of a pooled mouse serum sample. A: Non conjugated BAs. B: Glycine-conjugated BAs. C: Taurine-conjugated BAs. D: Deuterium-labeled internal standards. All the BAs were separated and detected in a single analytical run. *Green*: DHCA; *blue*: tri-hydroxylated BAs; *red*: di-hydroxylated BAs; *dark blue*: mono-hydroxylated BAs.



**Fig. S7.** Percentage of non conjugated (blue), glycine-conjugated (red) and taurine-conjugated (green) bile acids in rat, mouse and human serum and in liver tissue. The results are expressed as a percentage of the total amount of BAs.

## Supplemental Material

TABLE S1. Validation of the serum bile acid extraction protocol

Bile Acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Mean	CV (Injection)	CV (Preparation)
CDCA	238.6	237.8	238.6	242.6	239.5	233.5	238.4	2.3	1.2
DCA	297.2	295.2	296.3	299.9	297.6	293.2	296.5	3.0	0.8
UDCA	19.6	19.6	19.0	20.4	19.9	19.8	19.7	3.4	2.4
CA	133.6	128.7	127.2	135.0	135.2	134.7	132.4	1.8	2.7
HCA	6.9	7.7	7.6	7.7	7.2	7.4	7.4	6.8	4.1
GLCA	41.7	39.6	46.4	44.4	37.8	38.8	41.4	11.6	8.1
GCDCA	1210.5	1206.4	1195.1	1197.7	1219.4	1196.1	1204.2	1.1	0.8
GDCA	507.2	492.9	484.9	490.6	495.4	478.4	491.6	2.0	2.0
GUDCA	94.7	94.6	92.5	93.1	95.2	92.4	93.8	2.2	1.3
GCA	268.7	271.5	260.8	262.4	269.0	262.0	265.7	1.8	1.7
GHCA	19.5	18.9	18.9	19.0	19.3	18.8	19.1	2.5	1.5
TCDCa	148.2	149.7	143.7	145.9	147.2	143.6	146.4	1.6	1.7
TDCA	43.7	43.5	43.4	44.5	43.7	42.8	43.6	2.6	1.2
TCA	30.8	30.4	30.6	30.6	30.6	29.5	30.4	3.7	1.5
Non conjugated	696.0	689.0	688.6	705.6	699.3	688.6	694.5	2.3	1.0
Glycine-conjugated	2142.3	2124.0	2098.6	2107.3	2136.1	2086.5	2115.8	0.9	1.0
Taurine-conjugated	222.7	223.7	217.7	220.9	221.6	215.9	220.4	1.5	1.4
Total	3060.9	3036.7	3004.8	3033.8	3057.0	2991.1	3030.7	0.9	0.9

BAs concentrations are expressed in nM as the mean value of four replicates. CV: coefficient of variation expressed as a percentage (%). Injection variability is expressed as the mean of the CV obtained for each sample injection replicate. Preparation variability is expressed as the CV obtained for the concentration mean values of each sample.



TABLE S2. Validation of the liver bile acid extraction protocol

Bile Acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Mean (1-6)	CV (1-6)	Accuracy
LCA	29.0	19.8	39.7	63.5	29.4	36.8	20.4	36.4	41.2	-44.0
MuroCA	114.2	93.7	111.1	102.9	155.3	136.0	130.4	118.9	19.2	9.7
CDCA	275.2	167.5	169.2	141.2	324.0	237.4	234.5	219.1	32.7	7.0
DCA	83.2	56.7	69.1	125.3	94.6	96.5	73.3	87.6	27.3	-16.3
UDCA	105.2	82.0	89.6	90.2	139.7	111.5	114.8	103.0	20.4	11.5
HDCA	275.4	240.3	275.7	310.3	361.0	334.0	272.3	299.4	14.7	-9.1
CA	5587.1	3435.4	4604.4	2547.7	7070.4	6123.6	4697.8	4894.7	34.7	-4.0
$\omega$ MCA	1859.4	1586.0	1765.5	2022.7	2353.0	2154.1	1957.8	1956.8	14.2	0.1
$\alpha$ MCA	2360.6	1701.8	2215.0	1551.3	3299.4	2897.1	2286.9	2337.5	28.9	-2.2
$\beta$ MCA	4834.5	3952.2	4797.2	4424.9	6686.6	5943.6	5158.8	5106.5	19.9	1.0
HCA	24.9	18.7	22.9	22.3	32.2	29.5	25.0	25.1	19.8	-0.4
GLCA	122.6	90.1	184.6	139.0	168.7	165.0	85.6	145.0	24.1	-41.0
GCDCA	2463.5	2048.4	2069.6	1809.5	2423.5	2309.5	2671.3	2187.4	11.6	22.1
GDCA	1439.9	1177.7	1388.8	1279.6	1548.5	1644.1	1441.6	1413.1	12.1	2.0
GUDCA	401.0	332.1	374.9	316.2	474.5	433.5	477.5	388.7	15.5	22.8
GHDCa	225.4	205.6	214.5	214.2	266.9	234.7	284.4	226.9	9.7	25.3
GCA	20502.6	15708.7	18168.7	15663.4	24057.0	23658.6	22477.9	19626.5	19.0	14.5
TLCA	525.6	614.8	609.6	938.4	958.3	1000.6	381.8	774.6	27.5	-50.7
TCDCa	6526.4	6165.4	6540.3	6180.7	6619.7	6362.9	7123.5	6399.2	3.0	11.3
TDCA	2376.6	2199.0	2682.0	3076.3	2597.1	3072.1	2382.3	2667.2	13.4	-10.7
TUDCA	1418.7	1301.7	1552.3	1466.3	1734.5	1637.2	1709.2	1518.4	10.3	12.6
THDCa	975.1	959.1	1041.3	1159.1	1222.7	1152.6	1222.7	1085.0	10.0	12.7
TCA	48649.5	41144.2	48989.4	45990.1	57916.0	57734.1	49317.9	50070.6	13.2	-1.5
T $\omega$ MCA	4914.4	4555.9	5099.2	6067.2	5743.4	5371.0	6007.3	5291.9	10.5	13.5
T $\alpha$ MCA	18684.8	16231.0	19370.8	18691.8	22566.8	22030.4	20453.9	19595.9	12.0	4.4
T $\beta$ MCA	12539.0	11399.2	13321.1	13694.6	15806.0	14747.6	14730.1	13584.6	11.5	8.4
THCA	206.7	189.0	205.4	221.8	226.8	214.6	196.8	210.7	6.4	-6.6
Non conjugated	15548.7	11354.1	14159.3	11402.3	20545.6	18100.1	14972.1	15185.0	24.2	-1.4
Glycine-conjugated	25155.0	19562.6	22401.1	19422.0	28939.2	28445.4	27438.3	23987.6	17.6	14.4
Taurine-conjugated	96816.7	84759.3	99411.2	97486.4	115391.4	113323.2	103525.5	101198.1	11.3	2.3
Total	137520.5	115676.0	135971.6	128310.7	164876.2	159868.7	145935.9	140370.6	13.4	4.0

Bile acids concentrations are expressed in fmol/mg of tissue. CV: coefficient of variation expressed as a percentage (%). Mean and CV were obtained using those values obtained for Samples 1 to 6. Accuracy is expressed as a percentage and is calculated using the following formula: Accuracy (%) = 100 x [Sample 7 concentration – mean (1-6) concentration]/ mean (1-6) concentration. Only those bile acids with values above the lower limit of quantification are shown.

TABLE S3. Accuracy in biological matrices

Bile Acid	Blank matrix			Rat Liver			Rat Serum			Mouse Liver			Mouse Serum			Human Liver			Human Serum		
	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H
LCA	-18.6	-7.6	-4.0	13.3	12.4	4.9	29.0	13.6	3.7	13.1	5.7	2.3	16.3	6.8	2.2	18.2	8.5	2.4	21.1	11.2	4.3
MuroCA	-2.2	-19.1	-7.3	-2.7	-2.6	3.3	4.3	-6.4	4.9	-0.4	-4.1	2.0	-4.6	-7.8	3.1	-0.3	-7.8	3.1	-3.0	-10.7	2.9
CDCA	12.3	-18.5	-9.9	1.2	-3.2	-1.7	11.9	-2.4	-1.8	2.5	-3.2	-4.5	0.9	-3.7	-2.8	2.8	-2.8	-2.7	16.0	-4.3	-2.0
DCA	-9.5	-15.0	-11.4	9.0	4.6	0.8	20.4	6.3	0.4	10.5	4.0	-1.4	7.1	3.3	-0.1	8.1	4.2	0.0	19.9	3.6	0.3
UDCA	7.0	-11.4	-0.8	-5.7	-1.7	1.7	4.5	-7.3	2.5	-1.2	-2.7	-0.4	-1.7	-6.9	1.7	-0.2	-6.8	1.7	-0.1	-10.4	1.2
HDCA	15.9	-11.4	-3.2	-11.0	-9.1	-1.7	-0.1	-11.9	-0.9	-11.2	-11.9	-3.8	-12.1	-13.0	-2.0	-10.1	-13.0	-2.0	3.4	-14.4	-1.3
DHCA	-9.7	-9.4	-3.7	-2.6	0.7	4.0	-6.5	5.7	2.7	-3.6	9.1	1.4	-1.5	7.0	4.7	-9.6	7.0	4.7	-5.1	4.0	1.7
CA	-16.8	-7.5	-5.4	10.4	7.7	1.6	13.5	6.1	0.3	11.8	6.6	-0.7	13.0	7.1	0.9	11.4	7.3	1.0	17.4	7.3	-0.7
ωMCA	-17.9	-5.0	-4.6	2.1	6.8	4.6	9.5	5.2	1.4	3.3	5.8	0.5	1.7	5.4	2.3	4.9	5.4	2.3	-1.4	3.4	0.7
αMCA	-10.4	-3.8	-1.9	2.6	7.0	5.3	12.2	5.7	1.4	2.6	6.2	1.0	-1.0	5.7	3.0	3.4	5.7	3.0	13.3	4.9	1.1
βMCA	-19.1	-6.6	-5.6	4.9	4.1	4.9	10.9	4.4	3.6	3.5	3.4	1.8	1.2	6.3	3.9	3.2	6.4	3.9	9.3	2.5	2.5
HCA	-12.5	-7.6	-5.8	8.7	6.6	6.8	16.1	7.4	5.1	4.4	6.5	3.6	5.2	5.5	5.1	6.2	5.5	5.1	4.7	7.3	2.9
GLCA	-16.6	7.1	20.9	-14.9	-11.1	-10.2	-12.7	-10.4	-4.4	-16.1	-13.2	-10.3	-11.6	-7.6	-6.7	-17.2	-13.2	-10.3	10.9	6.9	5.0
GCDCA	-5.8	-10.5	-7.1	8.3	3.2	-1.5	17.6	3.7	-1.2	7.7	3.2	-3.1	8.5	3.8	-2.6	7.0	4.1	-2.5	13.6	2.7	-0.7
GDCA	-11.5	-0.9	-6.2	8.2	3.6	-3.3	17.6	7.0	-3.5	10.0	5.0	-6.3	10.1	0.5	-5.2	6.6	0.5	-5.2	19.2	1.6	-4.0
GUDCA	-3.0	-8.0	-5.9	0.3	-0.3	-4.9	3.3	-4.0	-7.4	-2.5	1.3	-8.4	-2.2	-1.8	-6.9	-0.9	-1.8	-6.9	-3.5	-5.6	-8.1
GHDCA	-2.6	-8.0	-5.7	1.8	3.6	-1.8	9.8	1.2	-3.1	1.1	2.6	-4.8	-0.6	2.1	-3.6	1.4	2.1	-3.6	1.3	-1.3	-3.6
GDHCA	-10.1	-4.9	-3.6	-1.9	1.3	-4.8	-2.7	-0.8	-7.7	-9.4	2.6	-5.6	-11.9	0.3	-5.9	-8.5	0.3	-5.9	-7.5	-1.3	-6.2
GCA	-4.0	-8.9	-6.4	5.6	3.3	-1.4	12.4	1.4	-3.8	1.1	3.5	-2.4	4.2	1.8	-2.7	5.7	1.9	-2.7	11.8	1.0	-2.6
GHCA	-14.6	-2.1	-8.2	10.8	7.8	-1.5	12.1	6.2	-2.3	0.8	5.9	-2.2	0.3	6.5	-4.7	2.2	6.5	-4.7	3.2	6.8	-1.4
TLCA	7.6	12.0	17.3	23.4	1.9	-0.5	15.3	2.3	5.3	10.1	-4.5	-9.7	-7.3	-6.1	-1.3	16.0	9.1	-9.8	-1.2	0.7	7.0
TCDCa	-7.9	-6.3	-9.1	17.8	3.8	-2.7	15.5	5.9	2.6	19.3	3.9	-4.8	18.3	2.0	-3.6	9.8	2.0	-3.6	17.5	4.4	-2.3
TDCA	-5.9	-5.5	-4.1	10.2	5.4	1.5	18.2	7.3	6.7	10.7	4.4	-0.3	15.1	5.1	0.2	8.0	5.3	0.3	19.6	7.9	3.5
TUDCA	-1.7	-11.0	-10.3	1.8	2.6	-6.0	12.8	2.2	-3.9	2.4	3.4	-7.6	3.5	0.5	-7.9	3.1	0.5	-7.9	-0.4	-1.3	-7.5
THDCA	-2.7	-15.8	-12.1	-1.6	-9.5	-14.5	14.8	-3.3	-7.9	9.4	-2.4	-8.5	11.0	-5.4	-10.5	12.6	-5.4	-10.5	14.2	-5.3	-7.6
TDHCA	-7.8	-5.0	-7.3	-5.4	5.8	-4.5	1.0	3.8	-4.0	-10.0	8.0	-2.3	-7.3	4.2	-4.0	-7.0	4.2	-4.0	-6.2	3.2	-3.6
TCA	-2.7	-10.4	-8.0	8.4	5.2	1.6	17.1	7.6	3.9	7.2	6.3	1.8	11.5	3.5	0.6	6.9	3.5	0.7	13.6	5.1	2.1
TωMCA	-7.8	-6.2	-10.5	6.7	8.0	-3.2	17.0	9.1	0.2	7.9	9.1	-1.1	8.2	8.0	-2.9	1.8	8.0	-2.9	7.5	8.9	-1.4
TαMCA	-7.8	-4.3	-9.3	8.9	5.1	-3.1	20.4	5.9	0.4	6.7	6.5	-1.0	14.0	3.7	-3.7	15.9	3.7	-3.7	12.0	3.9	-1.5
TβMCA	-13.0	-5.9	-8.5	12.2	0.4	-2.7	20.8	10.1	-2.8	9.8	0.5	4.4	13.1	8.5	0.9	7.4	8.5	0.9	15.6	5.8	3.5
THCA	-2.7	-11.0	-11.5	14.2	2.8	-0.2	17.8	4.7	3.5	13.0	2.1	1.1	14.3	2.9	-0.7	10.7	2.9	-0.7	14.4	2.5	0.9

Accuracy is expressed as the relative measurement error (RME) and is calculated using the following formula:  $RME (\%) = 100 \times (\text{calculated concentration} - \text{nominal concentration}) / \text{nominal concentration}$ . L: low concentration (40nM. each BA); M: medium concentration (312nM. each BA); H: high concentration (2500nM. each BA)

