

Fig. S1. The product ion spectra of the $[M-H]^-$ ions of analytes and ISs

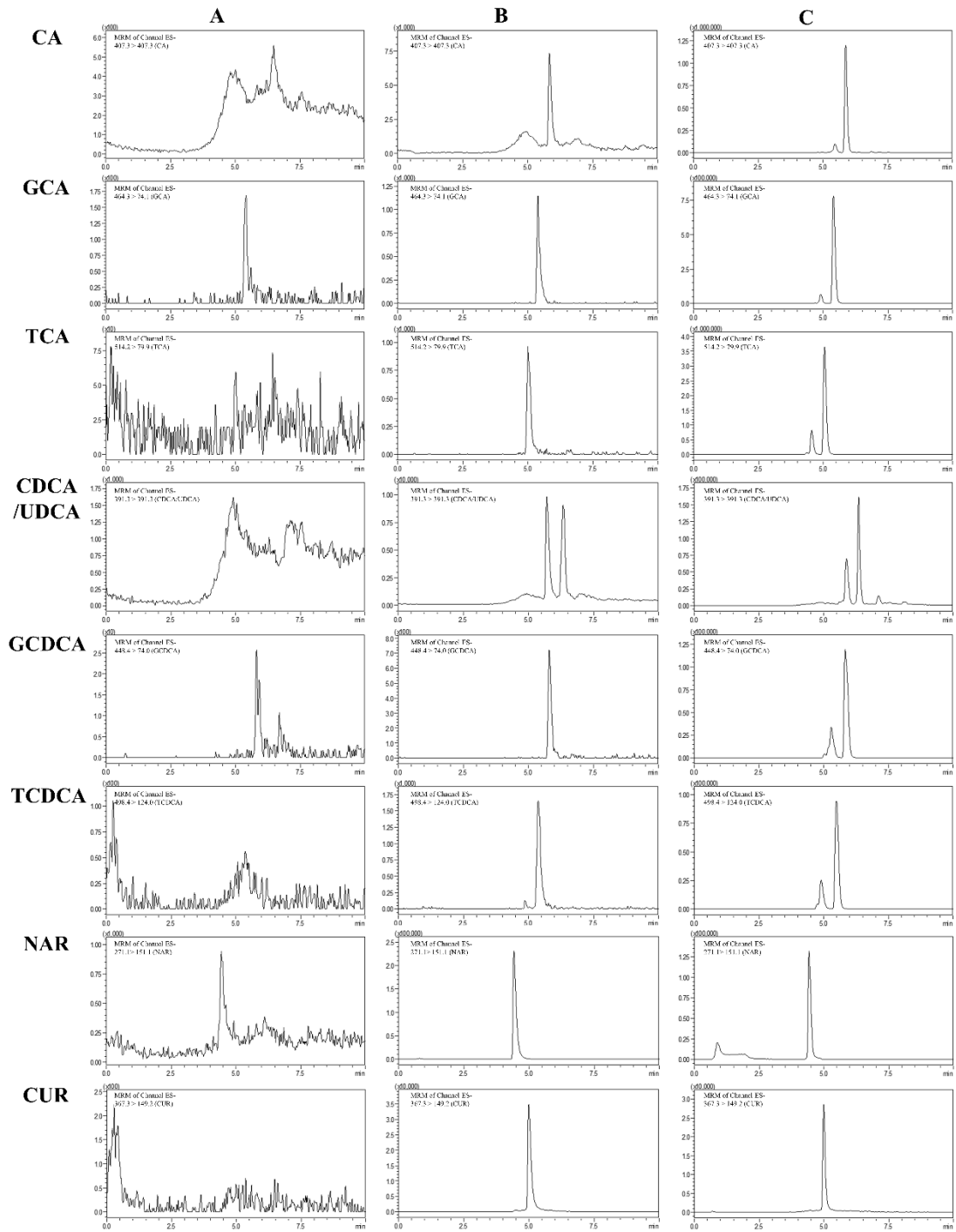


Fig. S2. MRM chromatograms for analytes and ISs from (A) a BAS-free liver sample, (B) a BAS-free liver sample spiked with LLOQ samples and IS solution (C) an authentic liver sample.

Table S1 Linearity and LLOQ of analytes in liver samples.

Analytes	Linear range (ng/mL)	Calibration curve	<i>r</i>	LLOQ (ng/mL)
CA	5-10000	$Y = 2.131 \times 10^{-2}X + 3.979 \times 10^{-2}$	0.9977	5
GCA	5-20000	$Y = 3.714 \times 10^{-3}X + 5.490 \times 10^{-3}$	0.9961	5
TCA	5-40000	$Y = 1.166 \times 10^{-3}X + 2.161 \times 10^{-3}$	0.9970	5
CDCA	10-2000	$Y = 2.508 \times 10^{-3}X + 7.607 \times 10^{-3}$	0.9981	10
GCDCA	1-2000	$Y = 2.159 \times 10^{-2}X + 9.421 \times 10^{-3}$	0.9974	1
TCDC	5-20000	$Y = 2.423 \times 10^{-3}X + 3.674 \times 10^{-3}$	0.9984	5
UDCA	10-5000	$Y = 3.407 \times 10^{-2}X + 6.393 \times 10^{-2}$	0.9944	10

Table S2 Intra-day and inter-day precision and accuracy for assay of analytes in rat liver (mean \pm SD, n = 6, three consecutive days).

Analytes	Added (ng/mL)	Found (ng/mL) Mean \pm SD	Accuracy	Precision	
			RE (%)	Intra-day RSD (%)	Inter-day RSD (%)
CA	5	5.069 \pm 0.254	1.4	4.9	5.8
	10	10.18 \pm 0.59	1.9	5.6	7.7
	250	248.1 \pm 13.1	-0.8	4.5	9.5
	8000	7520 \pm 255	-6.0	3.6	2.1
GCA	5	5.039 \pm 0.343	0.8	6.7	8.1
	10	10.29 \pm 0.69	2.9	6.7	7.7
	500	494.5 \pm 38.0	-1.1	7.7	7.9
	16000	15046 \pm 891	-6.0	5.3	9.7
TCA	5	5.066 \pm 0.311	1.3	5.5	9.8
	10	10.16 \pm 0.72	1.6	7.5	2.3
	500	506.8 \pm 36.4	1.4	7.2	7.1
	32000	32562 \pm 2101	1.8	6.1	8.8
CDCA	10	10.04 \pm 0.71	0.4	7.3	5.7
	20	20.42 \pm 1.47	2.1	7.0	9.2
	200	200.9 \pm 13.1	0.4	6.1	9.4
	1600	1742 \pm 92	8.8	4.5	9.3
GCDCA	1	1.011 \pm 0.075	1.1	7.7	6.2
	2	2.032 \pm 0.112	1.7	4.7	9.8
	50	48.31 \pm 2.50	-3.4	4.9	7.0
	1600	1603 \pm 99	0.2	5.2	11
TCDCA	5	5.047 \pm 0.295	0.9	5.9	5.6
	10	10.04 \pm 0.70	0.4	6.9	8.3
	500	493.6 \pm 31.8	-1.3	6.2	8.4
	16000	15599 \pm 983	-2.5	5.9	8.9
UDCA	10	9.98 \pm 0.66	-0.2	6.8	5.5
	20	20.88 \pm 1.09	4.5	5.4	4.4
	200	208.0 \pm 11.3	4.0	4.6	9.6
	4000	4380 \pm 184	9.5	4.0	5.6

Table S3 Matrix effect and extraction recovery for analytes in rat liver (n = 6).

Analytes	Analyte concentration (ng/mL)	Extraction recovery (%)	Matrix effect (%)
		Mean \pm SD	Mean \pm SD
CA	10	85.6 \pm 3.4	111.8 \pm 6.2
	250	90.4 \pm 6.8	101.5 \pm 5.4
	8000	89.1 \pm 3.0	95.3 \pm 1.7
GCA	10	93.0 \pm 9.8	106.3 \pm 3.8
	500	88.0 \pm 7.6	103.7 \pm 5.7
	16000	92.8 \pm 7.5	108.3 \pm 4.8
TCA	10	83.4 \pm 3.9	110.3 \pm 7.1
	500	88.7 \pm 9.6	98.6 \pm 5.0
	32000	89.3 \pm 6.8	100.6 \pm 5.5
CDCA	20	88.1 \pm 3.8	107.0 \pm 7.5
	200	88.6 \pm 6.3	100.2 \pm 4.8
	1600	87.2 \pm 7.5	110.1 \pm 6.4
GCDCA	2	88.5 \pm 4.7	99.1 \pm 5.4
	50	92.6 \pm 2.1	100.3 \pm 5.5
	1600	87.8 \pm 6.6	104.0 \pm 7.2
TCDCA	10	84.4 \pm 5.0	104.5 \pm 4.1
	500	89.7 \pm 4.1	100.0 \pm 5.2
	16000	87.9 \pm 6.8	111.1 \pm 7.9
UDCA	20	83.1 \pm 3.4	109.8 \pm 5.7
	200	90.2 \pm 7.5	99.0 \pm 5.2
	4000	85.0 \pm 4.8	97.9 \pm 5.4

Table S4 Stability for assay of analytes in rat liver under different storage conditions (mean \pm SD, n = 3).

Analytes	Added (ng/mL)	Room temperature for 12 h		Pretreatment for 8 h		Freeze–thaw stability		-80°C frozen storage for 30 days	
		Found (ng/mL)	RSD	Found (ng/mL)	RSD	Found (ng/mL)	RSD	Found (ng/mL)	RSD
		Mean \pm SD	(%)	Mean \pm SD	(%)	Mean \pm SD	(%)	Mean \pm SD	(%)
CA	10	10.20 \pm 0.42	4.1	9.85 \pm 0.70	7.1	9.10 \pm 0.18	2.0	9.63 \pm 0.60	6.3
	250	264.2 \pm 25.4	9.7	278.2 \pm 2.9	1.1	272.3 \pm 15.6	5.8	265.5 \pm 25.5	9.7
	8000	7372 \pm 403	5.5	7738 \pm 435	5.7	7571 \pm 668	8.9	7449 \pm 294	4.0
GCA	10	10.45 \pm 0.12	1.1	10.56 \pm 0.67	6.4	10.71 \pm 0.36	3.3	10.71 \pm 0.54	5.1
	500	538.6 \pm 48.5	9.0	560.9 \pm 1.4	0.3	524.8 \pm 11.9	2.3	546.5 \pm 41.3	7.6
	16000	14310 \pm 299	2.1	14917 \pm 1374	9.3	14777 \pm 658	4.5	14029 \pm 331	2.4
TCA	10	11.04 \pm 0.34	3.1	10.50 \pm 1.04	9.9	10.38 \pm 0.62	6.0	10.39 \pm 0.30	2.9
	500	520.1 \pm 52.9	11	558.5 \pm 9.6	1.8	559.4 \pm 6.8	1.3	496.1 \pm 52.2	11
	32000	32890 \pm 996	3.1	31491 \pm 1662	5.3	34840 \pm 2659	7.7	28432 \pm 650	2.3
CDCA	20	18.94 \pm 1.19	6.3	19.00 \pm 1.69	8.9	18.57 \pm 0.90	4.9	18.49 \pm 0.71	3.9
	200	182.0 \pm 8.7	4.8	179.6 \pm 6.8	3.8	178.5 \pm 6.2	3.5	182.6 \pm 14.1	7.8
	1600	1383 \pm 21	1.5	1437 \pm 30	2.1	1444 \pm 67	4.7	1441 \pm 55	3.8
GCDCA	2	2.063 \pm 0.191	9.3	2.096 \pm 0.066	3.2	1.966 \pm 0.204	11	2.079 \pm 0.165	8.0
	50	46.11 \pm 2.39	5.2	52.35 \pm 4.59	8.8	54.19 \pm 3.07	5.7	45.15 \pm 2.85	6.4
	1600	1690 \pm 57	3.4	1628 \pm 78	4.9	1730 \pm 118	6.8	1435 \pm 58	4.1
TCDCA	10	10.94 \pm 0.13	1.3	10.97 \pm 0.32	2.9	8.93 \pm 0.16	1.8	9.98 \pm 0.96	9.7
	500	498.2 \pm 59.7	12	515.8 \pm 33.4	6.5	512.2 \pm 50.8	10	490.0 \pm 34.9	7.2
	16000	16562 \pm 646	3.9	16797 \pm 1109	6.6	14821 \pm 354	2.4	15310 \pm 1818	12
UDCA	20	21.07 \pm 0.90	4.3	21.23 \pm 1.43	6.8	20.28 \pm 0.18	0.9	19.07 \pm 0.63	3.3
	200	201.0 \pm 11.7	5.9	209.8 \pm 3.4	1.7	187.1 \pm 5.2	2.8	184.3 \pm 9.5	5.2
	4000	4459 \pm 131	3.0	3890 \pm 305	7.9	3917 \pm 277	7.1	4501 \pm 107	2.4

Table S5 Dilution integrity for assay of analytes in rat liver (mean \pm SD, n = 6).

Analytes	Dilution factors	Added (ng/mL)	Found (ng/mL) Mean \pm SD	Accuracy	Precision
				RE (%)	RSD (%)
CA	4	30000	7927 \pm 294	5.7	3.7
GCA	4	60000	14712 \pm 328	-1.9	4.2
TCA	8	240000	29895 \pm 880	-0.4	12
CDCA	4	6000	1457 \pm 146	-2.9	2.0
GCDCA	4	6000	1503 \pm 124	0.2	1.6
TCDC	4	60000	14122 \pm 792	-5.9	10
UDCA	4	16000	4283 \pm 158	7.1	2.0