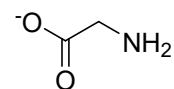
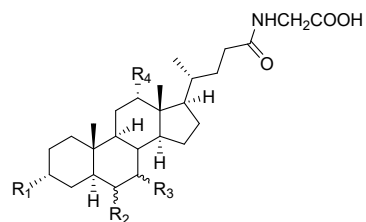


Table S1 Concentration of series working solutions and LQC, MQC, HQC for different BAs

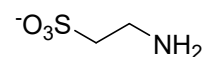
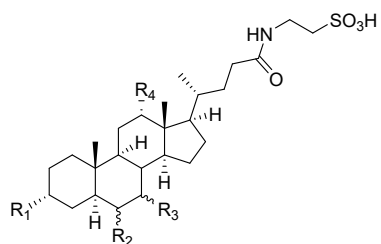
NO.	Analytes	Concentration of STD solutions (nmol/L)	LQC (nmol/L)	MQC (nmol/L)	HQC (nmol/L)
1	ALCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
2	LCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
3	NDCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
4	ACA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
5	IDCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
6	UDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
7	HDCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
8	CDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
9	ω -MA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
10	α -MA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
11	β -MA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
12	γ -MA(HCA)	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
13	CA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
14	DLCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
15	β -DCDCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
16	DCDCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
17	AlloCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
18	12k-LCA	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
19	3,7,12-Trione	50, 100, 200, 500, 1000, 1500, 2000	100	500	1500
20	7,12 dk-LCA	20, 50, 100, 200, 500, 1000, 1500,2000	50	200	1500
21	Methyl Ester	100, 200, 500, 1000, 1500,2000	200	500	1500
22	GLCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
23	GUDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
24	GCDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
25	GDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
26	G β -MA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
27	GCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
28	Glyco-3, 7, 12- Trione	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
29	TLCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
30	TDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
31	TUDCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
32	TCDCDA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
33	T β -MA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
34	THCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500
35	TCA	2, 5, 10, 20, 50, 100, 200, 500, 1000, 1500,2000	5	200	1500

Table S2 Structures and fragmentation patterns of all analytes along with deuterated internal standards.

Type	Structures of analytes		Fragmentation patterns					
	NO.	Compound	R1	R2	R3	R4	R5	Other positions
Free BAs	1	ALCA	OH	H	H	H	H	5 α -H
	2	LCA	OH	H	H	H	H	5 β -H
	3	NDCA	OH	H	H	OH	H	
	4	ACA	OH	H	H	OH	H	8(14)-en
	5	IDCA	H	H	α -OH	OH	H	
	6	UDCA	OH	H	β -OH	H	H	
	7	HDCA	OH	α -OH	H	H	H	
	8	CDCA	OH	H	α -OH	H	H	
	9	ω -MA	OH	α -OH	β -OH	H	H	
	10	α -MA	OH	β -OH	α -OH	H	H	
	11	β -MA	OH	β -OH	β -OH	H	H	
	12	HCA	OH	α -OH	α -OH	H	H	
	13	CA	OH	H	α -OH	OH	H	
	14	DLCA	=O	H	H	H	H	
	15	β -DCDCA	=O	H	=O	H	H	
	16	DCDCA	=O	=O	H	H	H	
	17	AlloCA	OH	=O	H	H	H	
	18	12k-LCA	OH	H	H	=O	H	
	19	3,7,12-Trione	=O	H	=O	=O	H	
	20	7,12 dk-LCA	OH	H	=O	=O	H	
	21	Methyl Ester	OH	H	=O	OH	CH3	
	22	CA-d4	OH	H	α -OH	OH	H	2,2,4,4-D4



Glycine- conjugated BAs	NO.	Compound	R1	R2	R3	R4	Other positions	
	1	GLCA	OH	H	H	H		
	2	GUDCA	OH	H	β -OH	H		
	3	GCDCA	OH	H	α -OH	H		
	4	GDCA	OH	H	H	OH		
	5	G β -MA	OH	β -OH	β -OH	H		
	6	GCA	OH	H	α -OH	OH		
	7	Glyco-3, 7, 12-Trione	=O	H	=O	=O		
	8	GCA-d4	OH	H	α -OH	OH	<input type="checkbox"/>	2,2,4,4-D4



Taurine- conjugated BAs	NO.	Compound	R1	R2	R3	R4	Other positions	
	1	TLCA	OH	H	H	H		
	2	TDCA	OH	H	H	OH		
	3	TUDCA	OH	H	β -OH	H		
	4	TCDCa	OH	H	α -OH	H		
	5	T β -MA	OH	β -OH	β -OH	H		
	6	THCA	OH	α -OH	α -OH	H		
	7	TCA	OH	H	α -OH	OH		
	8	TCA-d4	OH	H	α -OH	OH	<input type="checkbox"/>	2,2,4,4-D4

Table S3 Descriptives
and Tukey analysis
data of all analytes.

		Descriptives							
						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ALCA	MeOH	3	0.0436	0.02204	0.01272	-0.0111	0.0983	0.02	0.06
	ACN	3	0.0494	0.01051	0.00607	0.0233	0.0755	0.04	0.06
	IPA	3	0.2045	0.28177	0.16268	-0.4955	0.9044	0.04	0.53
	MTBE	3	0.0412	0.01590	0.00918	0.0017	0.0807	0.03	0.06
	One-step-1	3	0.0339	0.00196	0.00113	0.0290	0.0387	0.03	0.04
	One-step-2	3	0.0726	0.05152	0.02974	-0.0554	0.2006	0.02	0.11
	One-step-3	3	0.0265	0.02071	0.01196	-0.0249	0.0780	0.01	0.05
	Two-step-1	3	0.0326	0.00635	0.00367	0.0168	0.0483	0.03	0.04
	Two- step-2	3	0.0441	0.02219	0.01281	-0.0110	0.0992	0.02	0.06
	Two-step-3	3	0.0304	0.03892	0.02247	-0.0663	0.1271	0.00	0.08
	Total	30	0.0579	0.09229	0.01685	0.0234	0.0923	0.00	0.53
LCA	MeOH	3	0.0689	0.00661	0.00382	0.0525	0.0853	0.06	0.08
	ACN	3	0.0559	0.03921	0.02264	-0.0415	0.1533	0.02	0.10
	IPA	3	0.2172	0.30257	0.17469	-0.5345	0.9688	0.03	0.57
	MTBE	3	0.1265	0.10897	0.06291	-0.1442	0.3971	0.04	0.25
	One-step-1	3	0.1830	0.18379	0.10611	-0.2736	0.6395	0.02	0.38

NDCa	One-step-2	3	0.1887	0.03328	0.01921	0.1060	0.2713	0.17	0.23
	One-step-3	3	0.0847	0.06412	0.03702	-0.0746	0.2440	0.01	0.14
	Two-step-1	3	0.0804	0.03640	0.02102	-0.0101	0.1708	0.04	0.11
	Two- step-2	3	0.0815	0.02217	0.01280	0.0264	0.1366	0.07	0.11
	Two-step-3	3	0.0616	0.02678	0.01546	-0.0049	0.1281	0.03	0.08
	Total	30	0.1148	0.11597	0.02117	0.0715	0.1581	0.01	0.57
	MeOH	3	2.6811	0.45322	0.26167	1.5553	3.8070	2.33	3.19
	ACN	3	0.0327	0.01403	0.00810	-0.0022	0.0675	0.02	0.05
	IPA	3	0.0792	0.01494	0.00862	0.0421	0.1163	0.06	0.09
	MTBE	3	0.0698	0.04279	0.02470	-0.0364	0.1761	0.03	0.12
	One-step-1	3	3.6446	0.42756	0.24685	2.5825	4.7067	3.29	4.12
	One-step-2	3	3.3522	0.56960	0.32886	1.9373	4.7672	2.69	3.69
	One-step-3	3	3.1812	0.37154	0.21451	2.2582	4.1041	2.89	3.60
ACA	Two-step-1	3	4.3673	0.54305	0.31353	3.0182	5.7163	3.74	4.74
	Two- step-2	3	3.5782	0.20399	0.11777	3.0714	4.0849	3.35	3.73
	Two-step-3	3	3.4605	0.64271	0.37107	1.8639	5.0571	2.85	4.13
	Total	30	2.4447	1.67114	0.30511	1.8207	3.0687	0.02	4.74
	MeOH	3	1.5889	0.24955	0.14408	0.9690	2.2088	1.36	1.85
	ACN	3	0.0468	0.02239	0.01293	-0.0088	0.1024	0.03	0.07
	IPA	3	0.0535	0.02322	0.01341	-0.0042	0.1112	0.03	0.08
	MTBE	3	0.0283	0.02834	0.01636	-0.0421	0.0987	0.01	0.06
	One-step-1	3	2.1787	0.30429	0.17568	1.4228	2.9346	1.96	2.53
	One-step-2	3	2.0278	0.39615	0.22872	1.0437	3.0119	1.61	2.39
	One-step-3	3	1.8822	0.29527	0.17047	1.1488	2.6157	1.64	2.21

IDCA	Two-step-1	3	2.5459	0.33893	0.19568	1.7040	3.3879	2.16	2.78
	Two- step-2	3	2.2968	0.16387	0.09461	1.8897	2.7039	2.12	2.44
	Two-step-3	3	2.1287	0.30838	0.17804	1.3627	2.8948	1.83	2.45
	Total	30	1.4778	1.00725	0.18390	1.1017	1.8539	0.01	2.78
	MeOH	3	0.9477	0.20417	0.11788	0.4405	1.4549	0.73	1.13
	ACN	3	0.0200	0.00102	0.00059	0.0175	0.0226	0.02	0.02
	IPA	3	0.0429	0.02403	0.01387	-0.0168	0.1025	0.02	0.07
	MTBE	3	0.0360	0.02520	0.01455	-0.0266	0.0986	0.02	0.07
	One-step-1	3	1.1606	0.12051	0.06958	0.8612	1.4599	1.02	1.25
	One-step-2	3	1.1356	0.25652	0.14810	0.4984	1.7729	0.88	1.39
	One-step-3	3	1.0520	0.17149	0.09901	0.6260	1.4780	0.88	1.22
	Two-step-1	3	1.5828	0.19404	0.11203	1.1008	2.0648	1.41	1.79
	Two- step-2	3	1.2472	0.03662	0.02114	1.1562	1.3382	1.21	1.28
	Two-step-3	3	1.1631	0.18513	0.10689	0.7032	1.6230	1.03	1.37
UDCA	Total	30	0.8388	0.57297	0.10461	0.6248	1.0527	0.02	1.79
	MeOH	3	0.2814	0.04309	0.02488	0.1743	0.3884	0.25	0.33
	ACN	3	0.0138	0.00727	0.00420	-0.0043	0.0318	0.01	0.02
	IPA	3	0.0062	0.00354	0.00205	-0.0026	0.0150	0.00	0.01
	MTBE	3	0.0130	0.01133	0.00654	-0.0152	0.0411	0.00	0.03
	One-step-1	3	0.3854	0.07685	0.04437	0.1945	0.5763	0.32	0.47
	One-step-2	3	0.3727	0.06008	0.03469	0.2234	0.5220	0.30	0.42
	One-step-3	3	0.3139	0.04580	0.02644	0.2002	0.4277	0.28	0.37
	Two-step-1	3	0.4707	0.04205	0.02428	0.3663	0.5752	0.43	0.52
	Two- step-2	3	0.4101	0.01859	0.01073	0.3639	0.4562	0.39	0.42

HDCA	Two-step-3	3	0.3526	0.07400	0.04272	0.1688	0.5364	0.28	0.42
	Total	30	0.2620	0.17835	0.03256	0.1954	0.3286	0.00	0.52
	MeOH	3	0.1182	0.01358	0.00784	0.0845	0.1520	0.11	0.13
	ACN	3	0.0091	0.00463	0.00267	-0.0024	0.0206	0.01	0.01
	IPA	3	0.0056	0.00136	0.00079	0.0022	0.0089	0.00	0.01
	MTBE	3	0.0093	0.00297	0.00171	0.0019	0.0166	0.01	0.01
	One-step-1	3	0.1409	0.01420	0.00820	0.1056	0.1762	0.13	0.16
	One-step-2	3	0.1559	0.02890	0.01669	0.0841	0.2277	0.14	0.19
	One-step-3	3	0.1270	0.04292	0.02478	0.0204	0.2337	0.10	0.18
	Two-step-1	3	0.2451	0.01363	0.00787	0.2112	0.2789	0.23	0.25
CDCA	Two- step-2	3	0.1618	0.01536	0.00887	0.1236	0.1999	0.15	0.18
	Two-step-3	3	0.1372	0.04880	0.02818	0.0160	0.2584	0.11	0.19
	Total	30	0.1110	0.07894	0.01441	0.0815	0.1405	0.00	0.25
	MeOH	3	0.1831	0.06218	0.03590	0.0286	0.3375	0.11	0.22
	ACN	3	0.0064	0.00142	0.00082	0.0029	0.0100	0.01	0.01
	IPA	3	0.0470	0.03429	0.01980	-0.0381	0.1322	0.02	0.09
	MTBE	3	0.0204	0.01955	0.01129	-0.0281	0.0690	0.01	0.04
	One-step-1	3	0.1786	0.04562	0.02634	0.0653	0.2919	0.14	0.23
	One-step-2	3	0.1506	0.03378	0.01950	0.0667	0.2345	0.13	0.19
	One-step-3	3	1.1885	1.83895	1.06172	-3.3797	5.7567	0.11	3.31
Two-step-1	3	0.2374	0.01888	0.01090	0.1905	0.2843	0.22	0.25	
Two- step-2	3	0.1692	0.01314	0.00758	0.1366	0.2018	0.15	0.18	
Two-step-3	3	0.1531	0.03991	0.02304	0.0540	0.2523	0.11	0.19	
Total	30	0.2334	0.58679	0.10713	0.0143	0.4525	0.01	3.31	

ω -MA	MeOH	3	0.2194	0.00879	0.00507	0.1975	0.2412	0.21	0.22
	ACN	3	0.0062	0.00400	0.00231	-0.0038	0.0161	0.00	0.01
	IPA	3	0.0044	0.00138	0.00080	0.0010	0.0078	0.00	0.01
	MTBE	3	0.0037	0.00206	0.00119	-0.0015	0.0088	0.00	0.01
	One-step-1	3	0.2563	0.02889	0.01668	0.1845	0.3280	0.23	0.29
	One-step-2	3	0.2273	0.01348	0.00778	0.1938	0.2608	0.21	0.24
	One-step-3	3	0.2586	0.05341	0.03084	0.1259	0.3913	0.20	0.30
	Two-step-1	3	0.3155	0.01046	0.00604	0.2895	0.3415	0.31	0.33
	Two- step-2	3	0.2497	0.02530	0.01461	0.1869	0.3126	0.23	0.28
	Two-step-3	3	0.2873	0.02922	0.01687	0.2147	0.3599	0.25	0.31
	Total	30	0.1828	0.12302	0.02246	0.1369	0.2288	0.00	0.33
α -MA	MeOH	3	0.1993	0.06441	0.03718	0.0393	0.3593	0.13	0.26
	ACN	3	0.0062	0.00455	0.00263	-0.0051	0.0175	0.00	0.01
	IPA	3	0.0054	0.00243	0.00140	-0.0006	0.0115	0.00	0.01
	MTBE	3	0.0021	0.00117	0.00067	-0.0008	0.0050	0.00	0.00
	One-step-1	3	0.3027	0.06319	0.03648	0.1457	0.4597	0.26	0.37
	One-step-2	3	0.2733	0.04023	0.02323	0.1733	0.3732	0.25	0.32
	One-step-3	3	0.2000	0.01811	0.01046	0.1550	0.2450	0.18	0.21
	Two-step-1	3	0.3305	0.01427	0.00824	0.2950	0.3659	0.32	0.35
	Two- step-2	3	0.2782	0.01652	0.00954	0.2371	0.3192	0.26	0.29
	Two-step-3	3	0.3020	0.02709	0.01564	0.2347	0.3693	0.27	0.32
	Total	30	0.1900	0.13284	0.02425	0.1404	0.2396	0.00	0.37
β -MA	MeOH	3	0.2804	0.06382	0.03684	0.1219	0.4390	0.23	0.35
	ACN	3	0.0113	0.00463	0.00267	-0.0002	0.0228	0.01	0.01

	IPA	3	0.0148	0.01668	0.00963	-0.0267	0.0562	0.00	0.03
	MTBE	3	0.0041	0.00084	0.00048	0.0020	0.0062	0.00	0.00
	One-step-1	3	0.4093	0.05021	0.02899	0.2846	0.5341	0.37	0.47
	One-step-2	3	0.3723	0.06204	0.03582	0.2182	0.5265	0.34	0.44
	One-step-3	3	0.3728	0.05032	0.02905	0.2478	0.4979	0.31	0.40
	Two-step-1	3	0.4749	0.00541	0.00312	0.4615	0.4884	0.47	0.48
	Two- step-2	3	0.3943	0.03891	0.02246	0.2976	0.4909	0.36	0.44
	Two-step-3	3	0.4268	0.03828	0.02210	0.3317	0.5219	0.38	0.45
	Total	30	0.2761	0.18640	0.03403	0.2065	0.3457	0.00	0.48
HCA	MeOH	3	1.5966	0.18934	0.10931	1.1263	2.0670	1.40	1.78
	ACN	3	0.0124	0.00185	0.00107	0.0078	0.0170	0.01	0.01
	IPA	3	0.0344	0.01073	0.00619	0.0078	0.0611	0.03	0.05
	MTBE	3	0.0108	0.00409	0.00236	0.0006	0.0209	0.01	0.02
	One-step-1	3	2.0990	0.25752	0.14868	1.4593	2.7387	1.86	2.37
	One-step-2	3	2.1396	0.35473	0.20481	1.2584	3.0208	1.76	2.46
	One-step-3	3	1.8956	0.26051	0.15040	1.2484	2.5427	1.71	2.19
	Two-step-1	3	2.6328	0.08105	0.04679	2.4315	2.8342	2.55	2.71
	Two- step-2	3	2.1325	0.03954	0.02283	2.0342	2.2307	2.10	2.18
	Two-step-3	3	2.1832	0.08888	0.05132	1.9624	2.4040	2.08	2.25
	Total	30	1.4737	1.01014	0.18443	1.0965	1.8509	0.01	2.71
CA	MeOH	3	2.4531	0.35742	0.20635	1.5652	3.3409	2.20	2.86
	ACN	3	0.0327	0.01348	0.00778	-0.0008	0.0661	0.02	0.05
	IPA	3	0.0530	0.00957	0.00553	0.0292	0.0767	0.04	0.06
	MTBE	3	0.0102	0.00428	0.00247	-0.0004	0.0209	0.01	0.02

	One-step-1	3	3.3915	0.60485	0.34921	1.8890	4.8940	3.03	4.09
	One-step-2	3	3.0515	0.48112	0.27778	1.8563	4.2467	2.50	3.37
	One-step-3	3	2.9270	0.38650	0.22314	1.9669	3.8871	2.57	3.34
	Two-step-1	3	4.1404	0.38369	0.22152	3.1872	5.0935	3.91	4.58
	Two- step-2	3	3.3590	0.28955	0.16717	2.6397	4.0783	3.10	3.67
	Two-step-3	3	3.5961	0.62411	0.36033	2.0457	5.1465	2.97	4.22
	Total	30	2.3014	1.60143	0.29238	1.7035	2.8994	0.01	4.58
DLCA	MeOH	3	0.0697	0.02581	0.01490	0.0056	0.1339	0.04	0.10
	ACN	3	0.0441	0.02806	0.01620	-0.0257	0.1138	0.01	0.07
	IPA	3	0.0560	0.04234	0.02444	-0.0492	0.1611	0.01	0.10
	MTBE	3	0.0936	0.09789	0.05652	-0.1495	0.3368	0.04	0.21
	One-step-1	3	0.0995	0.06634	0.03830	-0.0653	0.2643	0.06	0.18
	One-step-2	3	0.0885	0.04475	0.02584	-0.0227	0.1996	0.05	0.13
	One-step-3	3	0.0475	0.03112	0.01797	-0.0298	0.1248	0.02	0.08
	Two-step-1	3	0.1049	0.02295	0.01325	0.0479	0.1619	0.08	0.13
	Two- step-2	3	0.0525	0.02526	0.01458	-0.0102	0.1153	0.03	0.08
	Two-step-3	3	0.0457	0.06156	0.03554	-0.1072	0.1986	0.01	0.12
	Total	30	0.0702	0.04774	0.00872	0.0524	0.0880	0.01	0.21
β-DCDCA	MeOH	3	0.7403	0.06418	0.03706	0.5809	0.8997	0.69	0.81
	ACN	3	0.0302	0.03568	0.02060	-0.0584	0.1189	0.01	0.07
	IPA	3	0.0098	0.00557	0.00322	-0.0041	0.0236	0.01	0.02
	MTBE	3	0.0284	0.01428	0.00824	-0.0071	0.0638	0.01	0.04
	One-step-1	3	0.8717	0.15417	0.08901	0.4887	1.2547	0.77	1.05
	One-step-2	3	0.8009	0.14912	0.08609	0.4305	1.1714	0.66	0.96

DCDCA	One-step-3	3	0.2714	0.38067	0.21978	-0.6743	1.2170	0.04	0.71
	Two-step-1	3	1.2304	0.10376	0.05991	0.9726	1.4881	1.12	1.32
	Two- step-2	3	1.0827	0.03236	0.01868	1.0024	1.1631	1.06	1.12
	Two-step-3	3	0.5634	0.45957	0.26533	-0.5782	1.7051	0.04	0.90
	Total	30	0.5629	0.47044	0.08589	0.3873	0.7386	0.01	1.32
	MeOH	3	0.6265	0.13454	0.07767	0.2923	0.9607	0.47	0.71
	ACN	3	0.0415	0.03520	0.02033	-0.0460	0.1289	0.01	0.08
	IPA	3	0.0199	0.00905	0.00523	-0.0026	0.0424	0.01	0.03
	MTBE	3	0.0454	0.02003	0.01156	-0.0044	0.0951	0.03	0.07
	One-step-1	3	0.8803	0.14579	0.08417	0.5181	1.2424	0.79	1.05
One-step-2	3	0.7835	0.17437	0.10067	0.3504	1.2167	0.61	0.96	
One-step-3	3	0.7268	0.04396	0.02538	0.6176	0.8360	0.69	0.78	
Two-step-1	3	1.2651	0.05023	0.02900	1.1403	1.3899	1.23	1.32	
Two- step-2	3	1.0645	0.02356	0.01360	1.0060	1.1230	1.05	1.09	
Two-step-3	3	0.8366	0.08727	0.05039	0.6198	1.0534	0.75	0.93	
Total	30	0.6290	0.43710	0.07980	0.4658	0.7922	0.01	1.32	
AlloCA	MeOH	3	0.2798	0.05220	0.03014	0.1501	0.4095	0.23	0.33
	ACN	3	0.0502	0.01637	0.00945	0.0095	0.0908	0.03	0.07
	IPA	3	0.0476	0.01954	0.01128	-0.0010	0.0961	0.03	0.07
	MTBE	3	0.0677	0.01740	0.01005	0.0245	0.1110	0.06	0.09
	One-step-1	3	0.3400	0.06614	0.03819	0.1757	0.5043	0.30	0.42
	One-step-2	3	0.2985	0.03218	0.01858	0.2186	0.3784	0.28	0.34
	One-step-3	3	0.3721	0.06884	0.03975	0.2011	0.5431	0.32	0.45
	Two-step-1	3	0.4395	0.04528	0.02614	0.3270	0.5520	0.39	0.48

12k-LCA	Two- step-2	3	0.3716	0.04104	0.02370	0.2697	0.4736	0.33	0.40
	Two-step-3	3	0.3557	0.01907	0.01101	0.3083	0.4031	0.33	0.37
	Total	30	0.2623	0.14833	0.02708	0.2069	0.3177	0.03	0.48
	MeOH	3	0.0205	0.00431	0.00249	0.0097	0.0312	0.02	0.02
	ACN	3	0.0074	0.00321	0.00185	-0.0005	0.0154	0.00	0.01
	IPA	3	0.0032	0.00133	0.00077	-0.0001	0.0065	0.00	0.00
	MTBE	3	0.0110	0.01063	0.00613	-0.0154	0.0374	0.00	0.02
	One-step-1	3	0.0103	0.00541	0.00312	-0.0032	0.0237	0.01	0.02
	One-step-2	3	0.0091	0.00118	0.00068	0.0061	0.0120	0.01	0.01
	One-step-3	3	0.0235	0.00868	0.00501	0.0019	0.0450	0.02	0.03
3,7,12-Trione	Two-step-1	3	0.0207	0.00449	0.00259	0.0095	0.0318	0.02	0.02
	Two- step-2	3	0.0125	0.00528	0.00305	-0.0006	0.0256	0.01	0.02
	Two-step-3	3	0.0147	0.00437	0.00252	0.0038	0.0255	0.01	0.02
	Total	30	0.0133	0.00783	0.00143	0.0104	0.0162	0.00	0.03
	MeOH	3	0.0216	0.01471	0.00849	-0.0149	0.0582	0.01	0.04
	ACN	3	0.0121	0.00673	0.00389	-0.0046	0.0288	0.01	0.02
	IPA	3	0.0051	0.00465	0.00268	-0.0065	0.0166	0.00	0.01
	MTBE	3	0.0142	0.00463	0.00267	0.0028	0.0257	0.01	0.02
	One-step-1	3	0.0436	0.02851	0.01646	-0.0272	0.1145	0.03	0.08
	One-step-2	3	0.0222	0.01075	0.00621	-0.0046	0.0489	0.01	0.03
One-step-3	3	0.0424	0.02560	0.01478	-0.0212	0.1060	0.02	0.07	
Two-step-1	3	0.0437	0.00885	0.00511	0.0217	0.0657	0.03	0.05	
Two- step-2	3	0.0326	0.02552	0.01474	-0.0308	0.0960	0.01	0.06	
Two-step-3	3	0.0288	0.00569	0.00328	0.0147	0.0430	0.02	0.03	

7,12 dk-LCA	Total	30	0.0266	0.01906	0.00348	0.0195	0.0338	0.00	0.08
	MeOH	3	0.0220	0.01508	0.00871	-0.0155	0.0594	0.01	0.04
	ACN	3	0.0101	0.00103	0.00060	0.0076	0.0127	0.01	0.01
	IPA	3	0.0101	0.00335	0.00193	0.0018	0.0184	0.01	0.01
	MTBE	3	0.0169	0.00338	0.00195	0.0086	0.0253	0.01	0.02
	One-step-1	3	0.0466	0.05327	0.03076	-0.0858	0.1789	0.01	0.11
	One-step-2	3	0.0107	0.00503	0.00291	-0.0018	0.0232	0.01	0.02
	One-step-3	3	0.0124	0.00512	0.00296	-0.0003	0.0251	0.01	0.02
	Two-step-1	3	0.0496	0.00741	0.00428	0.0312	0.0680	0.04	0.05
	Two- step-2	3	0.0165	0.00882	0.00509	-0.0054	0.0384	0.01	0.02
	Two-step-3	3	0.0105	0.00479	0.00277	-0.0014	0.0224	0.01	0.02
	Methyl Ester	Total	30	0.0205	0.02093	0.00382	0.0127	0.0284	0.01
MeOH		3	0.0290	0.01480	0.00855	-0.0078	0.0658	0.01	0.04
ACN		3	0.0407	0.04252	0.02455	-0.0649	0.1464	0.01	0.09
IPA		3	0.0490	0.02470	0.01426	-0.0124	0.1103	0.02	0.06
MTBE		3	0.0302	0.01729	0.00998	-0.0127	0.0732	0.01	0.05
One-step-1		3	0.0655	0.06518	0.03763	-0.0964	0.2274	0.03	0.14
One-step-2		3	0.0716	0.05268	0.03042	-0.0592	0.2025	0.01	0.11
One-step-3		3	0.0607	0.04947	0.02856	-0.0622	0.1836	0.02	0.12
Two-step-1		3	0.1131	0.02834	0.01636	0.0427	0.1835	0.09	0.14
Two- step-2		3	0.0763	0.03661	0.02114	-0.0146	0.1673	0.05	0.12
Two-step-3		3	0.0819	0.07507	0.04334	-0.1046	0.2684	0.01	0.16
GLCA		Total	30	0.0618	0.04491	0.00820	0.0450	0.0786	0.01
	MeOH	3	1.3432	0.22122	0.12772	0.7936	1.8927	1.20	1.60

	ACN	3	0.0039	0.00018	0.00010	0.0034	0.0043	0.00	0.00
	IPA	3	0.0232	0.01001	0.00578	-0.0017	0.0481	0.02	0.03
	MTBE	3	0.0030	0.00133	0.00077	-0.0003	0.0063	0.00	0.00
	One-step-1	3	2.1150	0.21788	0.12579	1.5738	2.6563	1.90	2.34
	One-step-2	3	1.7169	0.37504	0.21653	0.7853	2.6486	1.29	1.98
	One-step-3	3	0.7294	0.03536	0.02042	0.6415	0.8172	0.70	0.77
	Two-step-1	3	2.6078	0.31098	0.17954	1.8353	3.3804	2.25	2.83
	Two- step-2	3	2.0179	0.01534	0.00886	1.9797	2.0560	2.00	2.03
	Two-step-3	3	0.8004	0.19205	0.11088	0.3233	1.2774	0.64	1.01
	Total	30	1.1361	0.94391	0.17233	0.7836	1.4885	0.00	2.83
GUDCA	MeOH	3	1.5771	0.12742	0.07356	1.2606	1.8937	1.50	1.72
	ACN	3	0.0012	0.00030	0.00017	0.0005	0.0020	0.00	0.00
	IPA	3	0.0188	0.00366	0.00212	0.0097	0.0279	0.02	0.02
	MTBE	3	0.0004	0.00023	0.00013	-0.0002	0.0010	0.00	0.00
	One-step-1	3	2.5598	0.37693	0.21762	1.6234	3.4961	2.24	2.98
	One-step-2	3	2.3080	0.34921	0.20162	1.4405	3.1754	1.92	2.59
	One-step-3	3	1.9648	0.25880	0.14942	1.3219	2.6077	1.80	2.26
	Two-step-1	3	2.8669	0.08568	0.04947	2.6541	3.0798	2.78	2.95
	Two- step-2	3	2.4235	0.12590	0.07269	2.1108	2.7363	2.33	2.57
	Two-step-3	3	2.3352	0.35124	0.20279	1.4626	3.2077	1.97	2.67
	Total	30	1.6056	1.12909	0.20614	1.1840	2.0272	0.00	2.98
GCDCA	MeOH	3	0.9570	0.13164	0.07600	0.6300	1.2840	0.86	1.11
	ACN	3	0.0014	0.00033	0.00019	0.0006	0.0022	0.00	0.00
	IPA	3	0.0108	0.00372	0.00215	0.0016	0.0201	0.01	0.02

	MTBE	3	0.0004	0.00025	0.00014	-0.0002	0.0010	0.00	0.00
	One-step-1	3	1.4271	0.22922	0.13234	0.8577	1.9965	1.29	1.69
	One-step-2	3	1.2606	0.26247	0.15154	0.6086	1.9126	0.97	1.49
	One-step-3	3	0.9955	0.09127	0.05269	0.7688	1.2222	0.93	1.10
	Two-step-1	3	1.6703	0.11408	0.06586	1.3869	1.9537	1.55	1.77
	Two- step-2	3	1.4312	0.11110	0.06415	1.1552	1.7072	1.31	1.54
	Two-step-3	3	1.1492	0.22545	0.13016	0.5891	1.7092	0.92	1.37
	Total	30	0.8904	0.63626	0.11616	0.6528	1.1279	0.00	1.77
GDCA	MeOH	3	1.2578	0.04826	0.02787	1.1379	1.3777	1.22	1.31
	ACN	3	0.0038	0.00487	0.00281	-0.0083	0.0159	0.00	0.01
	IPA	3	0.0152	0.00439	0.00253	0.0043	0.0261	0.01	0.02
	MTBE	3	0.0006	0.00024	0.00014	0.0000	0.0012	0.00	0.00
	One-step-1	3	1.7923	0.12550	0.07246	1.4806	2.1041	1.69	1.93
	One-step-2	3	1.6864	0.21800	0.12586	1.1449	2.2280	1.46	1.89
	One-step-3	3	1.5492	0.20058	0.11581	1.0509	2.0474	1.38	1.77
	Two-step-1	3	2.1952	0.22234	0.12837	1.6429	2.7475	1.94	2.37
	Two- step-2	3	1.8673	0.04201	0.02426	1.7630	1.9717	1.82	1.90
	Two-step-3	3	1.6071	0.24728	0.14277	0.9929	2.2214	1.34	1.82
	Total	30	1.1975	0.83457	0.15237	0.8859	1.5091	0.00	2.37
Gβ-MA	MeOH	3	0.7450	0.05960	0.03441	0.5969	0.8930	0.68	0.79
	ACN	3	0.0006	0.00019	0.00011	0.0001	0.0010	0.00	0.00
	IPA	3	0.0077	0.00238	0.00138	0.0018	0.0136	0.01	0.01
	MTBE	3	0.0002	0.00016	0.00009	-0.0002	0.0006	0.00	0.00
	One-step-1	3	1.3315	0.24687	0.14253	0.7182	1.9447	1.16	1.61

	One-step-2	3	1.1969	0.21931	0.12662	0.6521	1.7417	0.94	1.34
	One-step-3	3	1.1131	0.10816	0.06245	0.8444	1.3818	1.02	1.23
	Two-step-1	3	1.4435	0.04873	0.02813	1.3224	1.5645	1.39	1.48
	Two- step-2	3	1.3380	0.00678	0.00392	1.3212	1.3549	1.33	1.34
	Two-step-3	3	1.1833	0.13988	0.08076	0.8358	1.5307	1.03	1.30
	Total	30	0.8360	0.59153	0.10800	0.6151	1.0568	0.00	1.61
GCA	MeOH	3	0.9346	0.04455	0.02572	0.8239	1.0453	0.88	0.97
	ACN	3	0.0008	0.00038	0.00022	-0.0002	0.0017	0.00	0.00
	IPA	3	0.0085	0.00101	0.00058	0.0060	0.0110	0.01	0.01
	MTBE	3	0.0003	0.00005	0.00003	0.0002	0.0004	0.00	0.00
	One-step-1	3	1.5236	0.20252	0.11692	1.0205	2.0266	1.40	1.76
	One-step-2	3	1.4910	0.21314	0.12306	0.9616	2.0205	1.26	1.68
	One-step-3	3	1.3721	0.17952	0.10365	0.9262	1.8181	1.26	1.58
	Two-step-1	3	1.8351	0.08190	0.04728	1.6317	2.0386	1.76	1.92
	Two- step-2	3	1.5655	0.09885	0.05707	1.3200	1.8111	1.45	1.63
	Two-step-3	3	1.4501	0.12328	0.07118	1.1438	1.7563	1.33	1.58
	Total	30	1.0182	0.71609	0.13074	0.7508	1.2856	0.00	1.92
Glyco-3, 7, 12-Trione	MeOH	3	0.3715	0.05620	0.03244	0.2319	0.5111	0.31	0.42
	ACN	3	0.0013	0.00066	0.00038	-0.0003	0.0029	0.00	0.00
	IPA	3	0.0048	0.00372	0.00215	-0.0045	0.0140	0.00	0.01
	MTBE	3	0.0003	0.00031	0.00018	-0.0005	0.0010	0.00	0.00
	One-step-1	3	0.7592	0.13229	0.07638	0.4306	1.0879	0.65	0.90
	One-step-2	3	0.7116	0.05274	0.03045	0.5806	0.8426	0.65	0.75
	One-step-3	3	0.6344	0.04669	0.02696	0.5184	0.7504	0.60	0.69

TLCA	Two-step-1	3	0.8532	0.03490	0.02015	0.7665	0.9399	0.83	0.89
	Two- step-2	3	0.7821	0.04470	0.02581	0.6711	0.8931	0.73	0.82
	Two-step-3	3	0.7637	0.11973	0.06913	0.4662	1.0611	0.66	0.89
	Total	30	0.4882	0.35108	0.06410	0.3571	0.6193	0.00	0.90
	MeOH	3	0.7482	0.12265	0.07081	0.4435	1.0528	0.61	0.85
	ACN	3	0.0050	0.00071	0.00041	0.0032	0.0067	0.00	0.01
	IPA	3	0.0152	0.00656	0.00379	-0.0011	0.0315	0.01	0.02
	MTBE	3	0.0001	0.00005	0.00003	-0.0001	0.0002	0.00	0.00
	One-step-1	3	0.9677	0.10607	0.06124	0.7042	1.2312	0.90	1.09
	One-step-2	3	0.8515	0.12565	0.07254	0.5394	1.1636	0.71	0.96
	One-step-3	3	0.3761	0.02679	0.01547	0.3095	0.4426	0.35	0.40
	Two-step-1	3	1.2031	0.04296	0.02480	1.0963	1.3098	1.17	1.25
	Two- step-2	3	0.8886	0.02234	0.01290	0.8331	0.9441	0.87	0.91
	Two-step-3	3	0.3909	0.08718	0.05034	0.1743	0.6075	0.32	0.49
TDCA	Total	30	0.5446	0.43411	0.07926	0.3825	0.7067	0.00	1.25
	MeOH	3	1.3780	0.08257	0.04767	1.1728	1.5831	1.32	1.47
	ACN	3	0.0063	0.00087	0.00050	0.0041	0.0084	0.01	0.01
	IPA	3	0.0214	0.00529	0.00305	0.0082	0.0345	0.02	0.03
	MTBE	3	0.0002	0.00005	0.00003	0.0001	0.0003	0.00	0.00
	One-step-1	3	2.3384	0.35258	0.20356	1.4625	3.2143	2.12	2.74
	One-step-2	3	2.1958	0.25876	0.14939	1.5530	2.8386	1.91	2.40
	One-step-3	3	1.5445	0.32527	0.18779	0.7365	2.3525	1.25	1.90
	Two-step-1	3	2.5700	0.16338	0.09432	2.1642	2.9759	2.43	2.75
	Two- step-2	3	2.2490	0.10407	0.06009	1.9905	2.5076	2.15	2.35

TUDCA	Two-step-3	3	1.7506	0.26964	0.15568	1.0808	2.4205	1.53	2.05
	Total	30	1.4054	1.00823	0.18408	1.0289	1.7819	0.00	2.75
	MeOH	3	1.1800	0.18183	0.10498	0.7283	1.6317	1.03	1.38
	ACN	3	0.0047	0.00018	0.00011	0.0042	0.0051	0.00	0.00
	IPA	3	0.0185	0.00361	0.00209	0.0095	0.0275	0.02	0.02
	MTBE	3	0.0003	0.00032	0.00019	-0.0005	0.0011	0.00	0.00
	One-step-1	3	1.9188	0.12500	0.07217	1.6082	2.2293	1.83	2.06
	One-step-2	3	1.8989	0.23970	0.13839	1.3035	2.4944	1.63	2.08
	One-step-3	3	1.5130	0.21040	0.12148	0.9904	2.0357	1.37	1.75
	Two-step-1	3	2.1873	0.06565	0.03790	2.0242	2.3504	2.14	2.26
TCDCA	Two- step-2	3	1.9377	0.03002	0.01733	1.8631	2.0122	1.91	1.97
	Two-step-3	3	1.6842	0.30208	0.17440	0.9338	2.4346	1.41	2.01
	Total	30	1.2343	0.86743	0.15837	0.9104	1.5582	0.00	2.26
	MeOH	3	1.3747	0.10700	0.06178	1.1089	1.6405	1.30	1.50
	ACN	3	0.0049	0.00049	0.00028	0.0037	0.0062	0.00	0.01
	IPA	3	0.0193	0.00405	0.00234	0.0092	0.0293	0.02	0.02
	MTBE	3	0.0005	0.00035	0.00020	-0.0004	0.0013	0.00	0.00
	One-step-1	3	1.8300	0.24075	0.13900	1.2320	2.4281	1.67	2.11
	One-step-2	3	1.8222	0.28259	0.16315	1.1202	2.5242	1.52	2.08
	One-step-3	3	1.4644	0.15531	0.08967	1.0786	1.8503	1.32	1.63
Two-step-1	3	2.2020	0.13735	0.07930	1.8608	2.5432	2.06	2.34	
Two- step-2	3	1.8841	0.06394	0.03692	1.7252	2.0429	1.81	1.93	
Two-step-3	3	1.5850	0.31290	0.18065	0.8077	2.3623	1.33	1.94	
Total	30	1.2187	0.84844	0.15490	0.9019	1.5355	0.00	2.34	

Tβ-MA	MeOH	3	0.3798	0.06974	0.04026	0.2066	0.5531	0.33	0.46
	ACN	3	0.0011	0.00066	0.00038	-0.0006	0.0027	0.00	0.00
	IPA	3	0.0062	0.00065	0.00038	0.0046	0.0078	0.01	0.01
	MTBE	3	0.0001	0.00005	0.00003	0.0000	0.0002	0.00	0.00
	One-step-1	3	0.6013	0.00295	0.00170	0.5939	0.6086	0.60	0.60
	One-step-2	3	0.5926	0.11113	0.06416	0.3165	0.8686	0.47	0.68
	One-step-3	3	0.5050	0.05865	0.03386	0.3594	0.6507	0.45	0.57
	Two-step-1	3	0.7416	0.02142	0.01236	0.6884	0.7948	0.73	0.77
	Two- step-2	3	0.6582	0.01458	0.00842	0.6220	0.6945	0.65	0.67
	Two-step-3	3	0.6043	0.10530	0.06080	0.3428	0.8659	0.53	0.73
	Total	30	0.4090	0.28931	0.05282	0.3010	0.5171	0.00	0.77
THCA	MeOH	3	0.3173	0.03722	0.02149	0.2249	0.4098	0.28	0.35
	ACN	3	0.0014	0.00115	0.00067	-0.0015	0.0042	0.00	0.00
	IPA	3	0.0053	0.00053	0.00031	0.0040	0.0067	0.00	0.01
	MTBE	3	0.0014	0.00133	0.00077	-0.0019	0.0047	0.00	0.00
	One-step-1	3	0.6181	0.11062	0.06386	0.3434	0.8929	0.53	0.74
	One-step-2	3	0.5778	0.06128	0.03538	0.4255	0.7300	0.51	0.63
	One-step-3	3	0.4605	0.07681	0.04435	0.2697	0.6514	0.42	0.55
	Two-step-1	3	0.6601	0.00264	0.00153	0.6535	0.6667	0.66	0.66
	Two- step-2	3	0.5826	0.05743	0.03316	0.4399	0.7253	0.54	0.65
	Two-step-3	3	0.5398	0.05263	0.03039	0.4091	0.6705	0.48	0.58
	Total	30	0.3764	0.26868	0.04905	0.2761	0.4768	0.00	0.74
TCA	MeOH	3	0.1396	0.01672	0.00965	0.0981	0.1811	0.13	0.16
	ACN	3	0.0004	0.00027	0.00015	-0.0003	0.0010	0.00	0.00

	IPA	3	0.0017	0.00045	0.00026	0.0006	0.0028	0.00	0.00
	MTBE	3	0.0001	0.00004	0.00002	0.0000	0.0002	0.00	0.00
	One-step-1	3	0.2448	0.01922	0.01110	0.1971	0.2926	0.23	0.26
	One-step-2	3	0.2561	0.03804	0.02196	0.1617	0.3506	0.21	0.28
	One-step-3	3	0.2217	0.04065	0.02347	0.1207	0.3227	0.18	0.27
	Two-step-1	3	0.3116	0.00939	0.00542	0.2882	0.3349	0.30	0.32
	Two- step-2	3	0.2470	0.03624	0.02092	0.1570	0.3370	0.22	0.29
	Two-step-3	3	0.2226	0.02160	0.01247	0.1690	0.2763	0.21	0.25
	Total	30	0.1646	0.11808	0.02156	0.1205	0.2087	0.00	0.32
Two-step-3	3		0.8004	0.8004					
MeOH	3			1.3432	1.3432				
One-step-2	3				1.7169	1.7169			
Two- step-2	3					2.0179			
One-step-1	3					2.1150	2.1150		
Two-step-1	3						2.6078		
Sig.		1.000	1.000	0.061	0.390	0.312	0.112		

Table S4 Summary of method validation results

NO.	Analytes	linearity		Accuracy (%)						Precision (RSD %)						Recovery (%)			Matrix effect (%)						Repeatability (%)			
		Range (nmol/L)	R ²	LLOQ (nmol/L)	Within-run (n=6×3)			Between-runs (n=18×3)			Within-run (n=6×3)			Between-runs (n=18×3)			LQC (n=6)	MQC (n=6)	HQC (n=6)	LQC (n=6)		MQC (n=6)		HQC (n=6)		LQC (n=6)	MQC (n=6)	HQC (n=6)
					LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC				Mean	RSD	Mean	RSD	Mean	RSD			
					(n=6)	(n=6)	(n=6)	(n=18)	(n=18)	(n=18)	(n=6)	(n=6)	(n=6)	(n=18)	(n=18)	(n=18)												
1	ALCA	50-2000	0.991	50	83.0- 119	92.7- 110	99.3- 101	81.2- 119	80.0- 118	94.2- 118	11	5.8	0.5	11	7.7	8.6	34.4- 40.6	39.6- 47.7	47.6- 52.9	103	7.5	105	7.1	101	8.4	4.2	5.5	3.2
2	LCA	50-2000	0.995	50	88.9- 116	88.9- 116	89.5- 104	83.6- 116	80.5- 118	80.9- 117	8.5	8.5	5.0	11	10	12	30.6- 36.3	39.4- 41.6	38.2- 45.8	96.2	5.4	98.4	6.5	103	3.0	15	4.1	11
3	NDCA	50-2000	0.996	50	87.2- 106	91.4- 119	101- 113	87.2- 109	82.5- 119	87.1- 115	8.0	9.2	3.6	7.1	9.9	7.7	67.0- 72.4	65.8- 72.4	69.4- 76.3	109	4.6	95.1	4.3	102	5.4	7.4	8.5	3.4
4	ACA	50-2000	0.994	50	98.9- 119	98.9- 114	100- 117	90.9- 119	81.3- 117	89.0- 117	6.1	5.3	6.3	6.8	10	4.5	75.7- 82.0	73.7- 83.2	76.8- 80.1	104	3.7	105	4	108	5.5	4.6	4.5	5.3
5	IDCA	50-2000	0.994	50	93.1- 107	89.1- 115	93.1- 107	81.6- 118	82.1- 115	93.1- 118	4.9	10	5.0	7.5	9.6	5.0	63.1- 71.9	63.4- 70.6	68.3- 74.7	104	4.4	91.0	2.7	97.9	8.1	7.5	6.4	4.4
6	UDCA	2-2000	0.995	2	86.8- 102	95.8- 108	92.2- 112	82.6- 113	87.4- 109	85.5- 112	6.3	4.7	6.6	9.5	9.1	7.8	70.3- 77.1	74.5- 82.4	75.5- 81.9	104	3.9	94.8	3.3	104	4.0	5.9	5.2	6.1
7	HDCA	50-2000	0.997	50	83.3- 106	89.1- 115	93.1- 107	83.3- 112	81.1- 118	82.3- 110	8.5	10	4.9	9.4	13	8.8	70.1- 78.4	64.9- 69.4	68.3- 76.1	101	7.6	91.1	5.1	104	6.2	4.7	5.9	2.5
8	CDCA	2-2000	0.994	2	80.2- 105	89.3- 106	94.5- 102	88.6- 110	81.2- 109	81.4- 119	8.6	6.2	2.4	7.6	10	8.9	72.3- 79.9	75.1- 83.8	70.8- 78.6	98.4	8.7	93.6	6.5	97.4	5.2	5.6	4.3	2.9
9	ω-MA	2-2000	0.993	2	94.1- 105	90.2- 108	93.1- 102	87.1- 115	81.2- 119	84.4- 111	3.9	6.3	3.7	7.5	13	8.9	74.4- 81.1	76.8- 87.5	77.9- 85.9	103	1.7	93.5	5.9	97.2	8.2	5.7	2.0	6.0
10	α-MA	2-2000	0.994	2	84.1- 112	84.8- 105	86.3- 102	82.4- 116	84.8- 116	86.0- 111	9.4	7.6	7.6	14	11	8.8	80.9- 87.5	76.7- 84.8	75.4- 80.4	101	4.2	93.4	5.8	104	6.0	6.7	7.1	5.6

11	β -MA	2-2000	0.995	2	83.7-105	81.4-112	84.2-94.6	83.7-120	81.4-118	84.2-112	8.0	9.4	3.6	7.9	4.7	7.9	72.0-77.9	73.0-78.6	76.4-79.8	96.7	7.7	100	4.7	104	5.2	5.7	5.3	4.4
12	γ -MA	2-2000	0.991	2	90.3-111	85.0-111	82.5-97.7	84.1-116	81.3-115	82.5-110	7.4	9.3	6.2	10	7.8	6.4	73.9-79.0	78.8-81.2	74.3-78.0	103	6.1	98.1	7.1	105	3.2	4.6	3.4	5.8
13	CA	2-2000	0.999	2	90.5-108	88.3-109	92.4-105	86.2-110	88.3-112	92.4-112	7.4	7.6	4.7	9.0	4.9	2.4	71.4-77.8	73.0-78.1	78.0-83.0	98.6	3.7	104	7.8	102	5.9	6.2	4.7	4.9
14	DLCA	50-2000	0.992	50	90.3-110	103-116	99.4-111	86.6-115	80.2-116	86.5-111	7.8	4.4	3.5	8.7	10	8.3	70.9-79.3	70.1-75.2	72.9-77.6	104	7.5	102	4.5	97.6	6.0	3.2	3.2	2.9
15	β -DCDCA	50-2000	0.991	50	96.3-115	101-119	88.7-106	80.4-115	81.9-119	87.1-116	6.8	5.5	6.2	12	6.4	10	61.3-72.6	60.4-67.2	62.8-69.3	98.1	7.2	96.1	3.2	106	4.6	2.1	5.8	5.7
16	DCDCA	50-2000	0.995	50	86.0-106	80.4-92.3	91.0-108	86.0-110	80.4-108	82.8-117	7.4	4.6	5.6	6.2	9.0	9.6	69.7-76.6	65.8-72.0	67.9-72.2	107	3.6	95.0	3.6	106	4.1	7.1	7.8	6.3
17	AlloCA	50-2000	0.995	50	83.8-112	82.1-110	87.2-110	83.8-118	82.1-110	87.2-113	10	11	8.4	8.4	7.6	8.0	75.3-81.1	76.4-82.4	76.6-80.7	102	1.7	102	8.3	91.7	4.0	2.3	2.2	4.0
18	12k-LCA	50-2000	0.994	50	88.5-109	84.4-109	85.9-95.3	84.9-109	83.5-110	82.6-106	6.7	7.6	3.4	4.9	8.6	6.5	69.5-78.1	68.9-76.5	69.7-76.0	105	5.0	99.8	8.0	97.6	6.1	8.2	4.1	5.2
19	3,7,12-Trione	50-2000	0.993	50	84.4-120	83.0-132	96.8-107	84.4-120	80.9-117	84.9-111	12	17	3.4	11	12	9.4	29.9-35.2	35.1-39.4	33.9-37.1	102	7.6	87.8	2.0	105	4.3	11	4.8	9.6
20	7,12 dk-LCA	20-2000	0.994	20	88.5-112	84.4-109	85.9-99.9	88.5-115	84.4-109	83.6-115	7.6	7.6	4.8	6.9	4.7	8.5	69.9-75.9	66.9-73.6	63.1-71.0	105	7.4	105	4.0	102	6.2	14	8.1	4.4
21	Methyl Ester	100-2000	0.992	100	81.5-118	83.1-103	94.3-101	81.5-118	83.1-115	83.6-108	12	6.6	2.4	6.6	7.9	7.6	69.6-76.1	71.4-77.5	72.5-80.2	102	9.9	101	8.1	102	7.1	11	8.0	7.1
22	GLCA	2-2000	0.997	2	81.7-109	86.6-110	98.3-107	81.6-115	85.4-114	85.7-113	11	8.6	2.9	7.0	6.1	8.0	75.0-83.8	78.4-85.3	79.5-86.3	105	4.6	102	7.8	107	3.0	8.9	6.1	4.3
23	GUDCA	2-2000	0.991	2	97.1-112	83.5-97.4	82.3-103	93.0-114	83.5-112	82.3-119	4.7	5.9	7.1	7.0	5.1	6.6	73.4-79.3	72.8-80.7	77.5-83.6	105	6.1	102	5.7	112	3.3	6.9	6.5	4.9

24	GCDCa	2-2000	0.994	2	103- 109	84.7- 95.3	89.0- 102	99.1- 109	82.0- 115	89.0- 118	1.6	4.0	5.1	8.0	10	4.2	76.5- 83.2	76.4- 83.4	74.3- 80.9	94.1	5.2	96.8	5.2	96.3	2.4	6.1	5.1	4.0
25	GDCA	2-2000	0.997	2	102- 111	80.4- 94.9	81.3- 93.6	85.4- 115	80.4- 116	81.3- 119	3.2	5.9	5.1	5.9	10	2.8	67.5- 74.0	72.3- 76.7	67.5- 70.4	95.8	8.1	101	4.8	98.1	6.6	5.3	4.8	7.7
26	Gβ-MA	2-2000	0.995	2	84.8- 105	84.4- 113	81.2- 107	82.6- 117	81.8- 118	81.2- 107	6.9	10	11	8.0	8.1	6.5	70.9- 78.6	73.2- 79.8	75.2- 83.3	106	3.8	100	4.5	104	4.0	7.3	8.3	7.6
27	GCA	2-2000	0.997	2	94.2- 107	82.4- 105	91.6- 108	94.2- 111	80.2- 113	88.6- 110	4.0	8.1	6.4	4.6	9.7	7.1	73.2- 83.5	78.1- 85.6	76.0- 84.9	106	5.9	99.5	4.3	106.8	4.6	7.0	3.3	4.0
28	Gly-3, 7, 12- Trione	2-2000	0.99	2	80.8- 98.9	87.9- 104	104- 107	80.8- 117	83.2- 117	88.6- 110	7.6	7.1	1.1	9.9	12	5.0	75.6- 83.5	78.0- 83.1	71.6- 75.0	97.3	5.2	98.3	5.3	93.1	4.6	6.8	7.1	6.0
29	TLCA	2-2000	0.998	2	93.4- 112	81.9- 98.0	93.8- 113	89.0- 114	81.9- 109	86.4- 113	6.4	6.7	6.4	1.8	5.8	6.1	80.2- 86.8	77.7- 84.7	78.7- 84.5	101	10	106	4.1	105	4.6	6.9	1.6	4.3
30	TDCA	2-2000	0.994	2	93.1- 105	81.8- 93.6	82.4- 98.5	93.1- 107	81.8- 120	82.4- 113	4.1	11	5.2	7.7	9.9	7.1	72.1- 77.2	70.3- 74.6	72.5- 79.2	108	6.5	93.8	3.4	103	6.8	8.9	5.2	5.2
31	TUDCA	2-2000	0.993	2	101- 113	80.2- 115	98.6- 116	88.8- 113	80.2- 115	87.9- 116	4.1	11	5.2	8.5	9.2	7.9	80.2- 84.6	79.0- 84.2	81.2- 87.4	94.0	4.7	96.8	4.1	96.4	5.9	11	4.2	5.0
32	TCDCa	2-2000	0.996	2	96.5- 111	83.6- 107	87.9- 116	90.5- 112	83.6- 114	86.9- 116	4.1	8.4	9.2	3.4	5.2	6.4	77.1- 84.8	80.0- 89.8	83.7- 89.5	94.1	6.1	102	5.7	112	3.3	6.8	4.3	4.3
33	Tβ-MA	2-2000	0.999	2	91.5- 113	83.2- 105	99.9- 107	82.6- 118	83.2- 120	93.0- 113	4.1	8.4	9.2	13	9.1	7.1	80.2- 84.6	84.7- 87.1	82.0- 87.9	93.5	6.6	108	5.5	98.9	4.3	6.1	4.5	8.2
34	THCA	2-2000	0.995	2	94.3- 112	84.7- 109	100- 112	92.5- 112	83.0- 119	86.1- 115	4.3	5.5	6.7	6.8	12	10	78.5- 85.4	81.0- 85.6	82.3- 86.8	104	5.7	93.9	5.5	88.1	3.3	4.7	3.6	3.5
35	TCA	2-2000	0.998	2	93.3- 107	81.3- 99.3	91.8- 106	88.6- 107	81.3- 104	86.9- 117	5.2	5.9	5.9	7.8	5.3	10	82.2- 86.8	83.3- 86.6	86.8- 93.5	103	8.2	94.6	8.5	89.1	3.8	6.1	5.3	3.4

Table S5 Summary of stability results.

NO.	Analyte	Standard solution stability of MQC (%)	Autosampler stability (%)		Freeze–thaw stability (%)						Room-temperature Stability (%)		Long-term Stability of LQC and HQC (%)			
			LQC	HQC	1 cycle		2 cycles		3 cycles		LQC	HQC	LQC		HQC	
					LQC	HQC	LQC	HQC	LQC	HQC			14d	30d	14d	30d
1	ALCA	102	86.9-	96.3-	91.9-	85.4-	92.6-	89.6-	85.9-	90.2-108	89.4-97.3	95.8-103	86.8-104	87.7-100	99.2-	96.6-
			103	104	114	113	109	104	99.1						106	102
2	LCA	103	85.2-	87.4-	97.3-	88.6-	95.0-	97.4-	87.8-	94.9-97.6	97.4-105	85.7-95.2	90.8-102	93.3-	95.3-	
			108	105	109	103	102-114	107						104	99.6	103
3	NDCA	91.7	103-115	85.9-	89.0-	90.7-	88.2-	86.8-	97.3-	89.6-108	92.0-105	88.6-105	89.6-92.1	87.0-99.2	92.1-	91.9-
			115	112	108	98.1	94.8	108	98.2						103	
4	ACA	104	87.2-	98.7-	87.9-	91.5-	93.3-	88.6-	94.3-	85.4-100	92.4-99.4	90.9-	86.4-102	89.4-106	93.9-	90.1-
			113	108	109	107	101	105	114			99.4			101	97.7
5	IDCA	100	91.7-	92.0-	89.5-	91.5-	93.5-	89.5-	90.6-	87.7-	88.7-97.3	93.7-103	90.4-106	84.3-105	101-107	90.9-
			111	109	111	107	111	97.1	109	99.5					98.6	
6	UDCA	91.0	90.9-	90.3-	96.8-	91.2-	94.9-	93.9-	91.1-	86.3-	91.4-99.8	89.4-	92.8-111	102-111	102-108	104-107
			111	104	115	110	108	100	106	98.1		99.7			97.1	
7	HDCA	96.5	90.1-	92.1-	89.4-	93.6-	91.2-	90.6-	85.5-	90.8-104	92.4-104	98.1-104	96.1-107	84.5-104	100-109	93.0-
			102	106	103	111	114	103	96.2						97.1	
8	CDCA	98.8	86.9-	92.8-	92.5-	92.1-	87.8-	88.3-	97.3-	90.2-105	97.5-104	95.4-106	97.1-103	96.3-100	90.2-	102-109
			95.7	105	105	111	113	106	102						98.4	
9	ω-MA	94.4	86.8-	86.0-	87.5-	86.9-	96.0-	89.3-	96.6-	87.1-103	89.0-90.3	95.1-101	86.8-114	86.4-113	95.8-	101-108
			98.2	99.7	91.8	104	103	96.4	113						103	

10	α -MA	94.0	85.2-106	93.1-105	96.1-108	88.2-109	87.5-95.3	94.9-98.5	94.1-99.9	89.0-104	92.3-103	94.1-98.3	92.6-109	86.0-113	93.2-104	101-104
11	β -MA	103	87.4-102	93.2-102	91.4-105	88.1-104	85.9-109	86.4-99.3	92.0-105	90.7-102	89.8-101	95.1-98.0	97.1-104	85.6-105	95.7-102	97.9-106
12	γ -MA	91.8	85.9-94.2	95.7-107	88.2-108	85.5-101	91.8-111	89.7-95.8	93.5-107	93.1-108	100-101	100-102	87.2-106	95.4-113	95.0-104	93.2-95.5
13	CA	92.7	87.6-109	90.2-109	87.9-106	89.9-106	91.7-103	92.4-105	90.0-108	91.0-99.7	85.6-109	92.5-97.4	88.9-106	86.9-106	96.5-105	90.6-100
14	DLCA	95.3	97.0-109	86.8-97.9	91.6-103	85.1-104	88.9-97.6	90.5-107	87.0-94.9	98.3-109	98.1-102	100-101	106-116	87.0-88.9	88.0-104	95.9-97.9
15	β -DCDCA	99.9	94.7-106	95.7-109	95.7-102	98.5-112	97.3-103	103-113	94.6-98.9	89.1-106	98.0-103	93.0-105	98.3-103	97.4-104	88.9-106	96.5-105
16	DCDCA	98.3	85.5-97.8	90.9-113	94.2-109	91.6-105	90.5-100	96.6-109	93.2-105	87.3-105	98.8-104	93.2-105	87.8-99.2	85.7-96.6	88.9-102	94.4-98.0
17	AlloCA	97.7	93.1-108	86.5-106	85.3-93.5	86.7-93.2	96.1-103	87.5-104	87.6-102	95.2-109	96.5-100	93.3-102	85.6-109	90.0-102	86.9-104	92.1-94.0
18	12k-LCA	96.4	87.5-93.4	91.5-102	96.7-106	92.9-104	99.4-114	87.8-97.6	90.6-113	95.2-114	102-107	92.6-105	104-111	96.0-99.3	98.9-105	95.1-101
19	3,7,12-Trione	101	90.2-113	89.9-100	93.9-103	87.5-98.0	88.0-95.9	86.3-94.7	97.0-105	86.3-111	96.5-100	94.4-103	90.1-103	88.8-99.3	95.6-108	87.3-92.1
20	7,12 dk-LCA	101	89.9-105	91.9-108	94.6-107	93.2-112	90.8-115	86.8-98.3	92.7-108	94.7-104	96.5-101	93.3-101	100-113	91.4-95.5	90.4-106	90.8-105
21	Methyl Ester	102	87.9-97.4	94.7-106	95.7-103	87.0-103	90.0-99.1	90.9-114	87.2-106	89.2-101	92.3-95.3	91.0-96.6	84.4-105	87.9-102	97.3-101	93.4-101
22	GLCA	100	92.3-107	94.5-102	90.2-112	93.3-109	85.5-105	89.0-98.5	88.4-111	91.4-102	96.5-111	94.5-102	84.2-95.8	96.3-114	88.7-98.3	102-103

23	GUDCA	99.4	88.6- 98.9	95.3- 111	86.6- 110	88.8- 104	93.1- 98.8	86.9- 91.8	91.6- 109	88.9- 98.6	91.2-91.6	90.3- 94.1	88.8-99.3	87.8-101	87.3- 92.1	94.0- 98.3
24	GCDCA	112	103-110	88.0- 105	89.2- 110	94.2- 112	86.3- 95.4	89.3- 106	96.2- 105	93.0-100	89.8-110	96.4- 99.0	85.7-96.6	86.4-94.5	94.4- 98.0	96.0- 105
25	GDCA	102	93.7- 108	90.0- 109	89.6- 114	91.4- 109	102-114	90.6- 98.6	86.2- 102	88.2-103	85.3-93.3	93.2- 96.3	96.1-102	94.8-106	89.9- 101	91.2- 103
26	Gβ-MA	96.0	87.0- 109	95.9- 105	95.5- 105	92.8- 114	98.8- 110	91.7- 103	96.0- 105	90.7-111	85.4-91.5	90.7- 98.8	85.6-99.2	86.8-109	92.1- 94.5	94.4- 104
27	GCA	101	98.5- 112	88.1- 104	86.7- 112	89.1- 107	92.9- 96.7	97.3- 106	94.1- 108	95.3-102	88.8-94.2	91.0- 95.1	84.3-105	89.5-107	90.9- 98.6	91.9- 94.5
28	Gly-3, 7, 12- Trione	96.8	86.1- 102	95.6- 101	88.3- 115	86.8- 102	85.5- 113	90.7- 99.0	96.3- 105	94.7-104	88.9-93.4	94.2- 98.3	101-105	88.7-98.3	93.6- 106	91.6- 95.0
29	TLCA	97.9	106-118	90.4- 102	86.9- 113	94.2- 104	86.5- 99.9	88.1- 105	95.6- 112	94.3-109	87.1-93.6	89.5- 93.3	86.9-104	85.2-102	92.1- 94.0	91.8- 99.8
30	TDCA	94.1	106-115	87.0- 105	85.6- 100	91.4- 110	88.7- 105	95.5- 106	105-110	87.5-104	95.1-114	90.6- 94.6	84.5-104	92.0-98.6	93.0- 97.1	98.0- 99.9
31	TUDCA	103.1	85.6- 99.1	95.3- 102	93.0- 97.4	94.3- 109	94.4- 110	93.5- 103	89.3- 104	86.9-101	92.9-110	91.2- 95.7	90.8-102	91.8-103	95.3- 101	96.9- 101
32	TCDCa	103.2	106-109	92.1- 102	87.2- 99.4	89.7- 105	100-109	86.5- 101	87.7- 107	87.1-101	92.1-99.8	90.5- 96.6	89.4-106	89.0-106	90.1- 97.7	89.2- 97.6
33	Tβ-MA	106.3	92.9- 102	87.3- 105	86.2- 99.2	91.2- 104	88.5- 108	92.2- 107	91.9- 102	87.1- 95.9	89.4-94.2	89.1- 95.7	89.8-97.6	87.3-108	88.3- 101	103-106
34	THCA	99.0	110-115	93.7- 98.0	98.5- 105	86.1- 112	97.1- 105	87.8- 94.8	95..8- 108	85.8-108	94.6-103	93.2-102	87.7-100	98.2-111	96.6- 102	95.1- 101
35	TCA	102.2	93.6- 109	96.4- 106	87.3- 105	89.5- 98.7	88.6- 96.7	86.0- 101	97.0- 111	86.2- 92.9	89.0-93.7	96.1- 98.2	87.0-99.2	92.4-102	91.9- 103	91.1- 98.2

Table S6 Summary of BAs quantitation results

NO.	Analytes	Con1	Con2	Con3	Con4	Con5	Con6	Average of Con group	ANIT1	ANIT2	ANIT3	ANIT4	ANIT5	ANIT6	Average of ANIT group	T-test Value
1	CA	1368	1328	1364	1104	1291	1523	1330	19826	5555	14064	9131	10792	36422	15965.07	9.14E-03
2	α -MA	1247	984	871	1069	1293	947	1069	4804	2403	5920	3947	4120	2185	3896.33	6.90E-04
3	β -MA	859	441	493	851	900	1225	795	4928	3261	4430	3656	3799	4002	4012.59	3.00E-07
4	ω -MA	518	497	92	572	384	818	480	433	663	565	818	618	529	604.18	2.90E-01
5	CDCA	517	624	802	581	770	674	661	114	114	112	48	122	311	136.81	3.88E-06
6	UDCA	161	229	288	270	260	227	239	737	360	722	362	630	541	558.77	1.17E-03
7	HDCA	517	624	82	581	370	674	475	110	114	74	88	211	143	123.33	3.32E-03
8	GCA	1675	1640	1456	1639	1846	1294	1592	12440	11355	11884	11121	12488	12149	11906.28	1.32E-12
9	GCDCA	258	88	245	198	87	137	169	2694	2268	2872	2766	2899	2409	2651.18	6.23E-10
10	GDCA	29	39	17	25	29	11	25	576	195	100	56	125	218	211.72	3.58E-02
11	GUDCA	3	2	3	3	2	4	3	17	23	16	31	39	25	25.36	7.32E-05

12	TCA	1368	162	1364	1104	1000	1523	1087	29826 6	30755 5	40406 4	21093 1	27999 2	36412 2	310821.7 3	5.23E- 07
13	Tα-MA	518	42	354	43	524	412	315	25822	32383	40735	34022	23495	27156	30602.28	3.88E- 07
14	Tβ-MA	74	43	84	30	143	47	70	35608	36175	35668	36039	34120	37234	35807.33	1.06E- 15
15	TCDCa	72	57	92	36	179	143	96	4756	4533	4537	4117	4529	4145	4436.06	1.66E- 12
16	TDCA	74	6	92	2	92	137	67	6568	4535	5539	3567	5413	4246	4977.84	5.59E- 07
17	TUDCA	6	3	8	7	7	4	6	73	56	56	75	29	63	58.58	1.62E- 05
18	Nonconjugate d	5189	4727	3993	5029	5269	6087	5049	30952	12469	25887	18049	20293	44132	25297.09	1.31E- 03
19	Glycine- conjugated	1965	1768	1721	1865	1965	1446	1788	15728	13841	14872	13975	15552	14801	14794.54	2.47E- 12
20	Taurine- conjugated	2112	313	1994	1222	1945	2266	1642	37109 3	38523 8	49059 9	28875 1	34757 8	43696 5	386703.8 2	1.01E- 07
21	Total	9266	6808	7707	8116	9179	9799	8479	41777 3	41154 8	53135 8	32077 5	38342 2	49589 8	426795.4 6	1.01E- 07
