High-Precision Metabolomics since 2003

BEVITAL

Method for the analysis of bile acids and related metabolites

Chemicals/metabolites

Compound/IS	Abbr.	Source	Purity
7α-hydroxy-cholesten-3-one	C4	MedChemExpress (https://www.medchemexpress.com)	99.60
Cholic acid	CA	Merck(https://www.sigmaaldrich.com)	>98
	d4CA	Merck(https://www.sigmaaldrich.com)	>98
Glycocholic acid	GCA	MedChemExpress (https://www.medchemexpress.com)	99.90
Taurocholic acid	TCA	MedChemExpress (https://www.medchemexpress.com)	99.64
Chenodeoxycholic acid	CDCA	Merck(https://www.sigmaaldrich.com)	>98
Glycochenodeoxycholic acid	GCDCA	avantiresearch(https://avantiresearch.com/)	>98
Taurochenodeoxycholic acid	TCDCA	avantiresearch(https://avantiresearch.com/)	>98
	d4TCDCA	Avantiresearch (https://avantiresearch.com)	>98
Hyocholic acid	HCA	MedChemExpress (https://www.medchemexpress.com)	99.46
Glycohyocholic acid	GHCA	MedChemExpress (https://www.medchemexpress.com)	99.68
Taurohyocholic acid	THCA	Merck (https//www.sigmaaldrich.com)	>98
Tauro-alfa-muricholic acid	TaMCA	Merck (https//www.sigmaaldrich.com)	>98
Deoxycholic acid	DCA	Merck (https//www.sigmaaldrich.com)	>98
Glycodeoxycholic acid	GDCA	MedChemExpress (https://www.medchemexpress.com)	99.08
Taurodeoxycholic acid	TDCA	MedChemExpress (https://www.medchemexpress.com)	99.80
Lithocholic acid	LCA	British Pharmacopoeia (https://www.pharmacopoeia.com)	>98
	d4LCA	Avantiresearch (https://avantiresearch.com)	>98
Glycolithocholic acid	GLCA	MedChemExpress (https://www.medchemexpress.com)	99.90
Taurolithocholic acid	TLCA	MedChemExpress (https://www.medchemexpress.com)	99.81
Lithocholic acid sulfate	LCAsulf	Avantiresearch (https://avantiresearch.com)	>98
Iso-litocholic acid	isoLCA	Merck(https://www.sigmaaldrich.com)	>98
Ursodeoxycholic acid	UDCA	Merck(https://www.sigmaaldrich.com)	>98
Glycoursodeoxycholic acid	GUDCA	MedChemExpress (https://www.medchemexpress.com)	≥98.0
Tauroursodeoxycholic acid	TUDCA	MedChemExpress (https://www.medchemexpress.com)	99.93
Hyodeoxycholic acid	HDCA	MedChemExpress (https://www.medchemexpress.com)	99.87
Glycohyodeoxycholic acid	GHDCA	MedChemExpress (https://www.medchemexpress.com)	98.06
Taurohyodeoxycholic acid	THDCA	MedChemExpress (https://www.medchemexpress.com)	≥98.0

Instrumentation

Agilent 1100 Infinity LC system; API 4000 tandem mass spectrometer from AB Sciex.

Chromatography and detection

Column: Zorbax SB-C8 (150 × 4.6 mm, particle size 3.5 µm). Temperature: 60 ºC. Mobile phase: 0−0.5 min,5mM Ammonium Acetat in 10% Acetonitrile pH 4. 50% Acetonitrilel/50% 2propanol; 0.5−5 min, 2.0 mM ammonium Acetate; 6−10 min, 50% Acetonitrilel/50% 2propanol (linear gradient across time gaps). Injections: (15 µL) each 12min.

Compounds

Compound	Retention time (min)	Detection mode	Ion pairs (m/z)	
			precursor	product
C4	8.70	pos; MRM	401	177
CA	6.45	neg; MRM	407	343
GCA	5.35	neg; MRM	464	74
TCA	4.70	neg; MRM	514	80
CDCA	7.50	neg; MRM	391	391
GCDCA	6.00	neg; MRM	448	74
TCDCA	5.15	neg; MRM	498	80
HCA	5.80	neg; MRM	407	405
GHCA	5.18	neg; MRM	464	74
THCA	4.09	neg; MRM	514	80
TaMCA	3.47	neg; MRM	514	80
DCA	7.60	neg; MRM	391	391
GDCA	6.15	neg; MRM	448	74
TDCA	5.25	neg; MRM	498	80
LCA	8.15	neg; MRM	375	375
GLCA	7.30	neg; MRM	432	74
TLCA	5.76	neg; MRM	482	80
LCAsulf	5.86	neg; MRM	455	97
isoLCA	7.95	neg; MRM	375	375
UDCA	6.50	neg; MRM	391	391
GUDCA	5.35	neg; MRM	448	74
TUDCA	4.60	neg; MRM	498	80
HDCA	6.70	neg; MRM	391	391
GHDCA	5.45	neg; MRM	448	74
THDCA	4.70	neg; MRM	498	80

Internal standards

IS	Retention time (min)	Detection mode	Ion pairs (m/z)	
			precursor	product
d5CA	6.45	neg; MRM	412	85
d4TCDCA	5.15	neg; MRM	502	81
d4LCA	8.15	neg; MRM	379	379

Method performance

Compound/IS	Linear range (nmol/L)	Linearity (r^2)	CV (%)[1]		LOD (nmol/L)
			br ('wd')	bd	
C4	0–300	0.9971	6.85	6.84	20
CA d4CA	0–30000	0.9989	2.31	1.82	50
GCA	0–10000	0.9956	2.47	2.61	25
TCA	0–10000	0.9936	4.14	5.31	15
CDCA	0-10000	0.9987	4.46	3.76	25
GCDCA	0-150000	0.9998	3.73	4.19	300
TCDCA d4TCDCA	0–5000	0.9873	4.60	2.32	30
HCA	0–500	0.9992	4.68	2.92	5
GHCA	0–500	0.9948	3.33	2.89	2
THCA	0–200	0.9991	3.94	4.15	0.75
TaMCA	0–7500	0.9844	6.38	4.44	0.75
DCA	0-30000	0.9982	4.75	2.16	25
GDCA	0–10000	0.9978	5.86	8.25	20
TDCA	0–5000	0.9990	7.63	4.46	10
LCA d4LCA	0–400	0.9798	3.33[2]	6.25[2]	7
GLCA	0–500	0.9967	3.26	3.75	2
TLCA	0–300	0.9914	5.40	4.20	2
LCAsulf	0–200	0.9977	4.28	6.97	5
isoLCA	0–400	0.9948	(14.0)[3]	(20.3)[3]	5
UDCA	0–4000	0.9978	4.33	5.18	10
GUDCA	0–5000	0.9946	4.47	3.92	10
TUDCA	0–2500	0.9956	4.39	2.41	10
HDCA	0–500	0.9963	5.80	5.90	15
GHDCA	0–3000	0.9972	3.29	3.58	15
THDCA	0–400	0.9928	4.76	6.68	5

[1] Mean CVs across three different concentration levels (low, medium, high) are estimated from a single-site validation study using the 20x2x2 design recommended by *CLSI EP05-A3* (https://clsi.org/standards/products/method-evaluation/documents/ep05/) and calculated from the variance component analysis using the R package *VCA* v.1.4.5 (https://cran.r-project.org/web/packages/VCA/vignettes/VCA_package_vignette.html).
[2] Calculated from Q21 only.
[3] Write here 'under optimization' until we have performed additional analyses with a new ISTD.
br, between-run CV (aka within-day CV without the residual CV); bd, between-day CV.