Quality Controlled Quantitative Analysis of Bile Acids in Plasma Samples Using Waters® ACQUITY UPLC® I-Class together with a Xevo® TQ-S mass spectrometer

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1 Introduction

Bile acids are considered not only as endogenous markers for liver cell functions, but also as signaling molecules regulating various metabolism processes and as mediators for gut microbiome status [1-6]. Figure 1 gives an overview over bile acids biosynthesis. The primary bile acids (cholic acid and chenodeoxycholic acid) are formed by oxidation from cholesterol in the liver and stored in the gall bladder, whereas the secondary bile acids (deoxycholic acid and lithocholic acid) are formed here by bacterial flora. The standardized and quality controlled quantitative analysis of individual bile acids and their conjugates can provide a powerful tool for large cohort studies in precision medicine as well as for applications in toxicology and clinical biomarker research. Hence, an (U)HPLC-ESI-MS/MS based Kit was developed, which comprises 20 individual bile acids and requires a sample volume of 10 µL. The Kit has been validated according to the EMA guidelines for human and rat plasma.

2 Materials and Method

A specially designed 96-well plate with filter spots and 10 µL of plasma samples are used for the Biocrates® Bile Acids Kit preparation (see User Manual). Two hours are needed for the sample preparation including pipetting, drying and extraction steps. Subsequently, 5 min analysis time per sample (UPLC, 10 µL of injection volume) is required. The metabolite panel of the Kit includes 20 individual bile acids, which are listed in Table 1. The quantification is based on an external 7 points calibration using 10 internal standards. Furthermore, 3 levels of quality control samples (QC, human plasma based) are included in the Kit.

Analysis of bile acids was performed on a Waters® Acquity UPLC® I-Class system coupled to a Xevo® TQ-S mass spectrometer (Waters Inc.). Bile acids were ionized in negative electrospray ionization mode and the MS instrument was operated in multiple reaction monitoring (MRM) mode.

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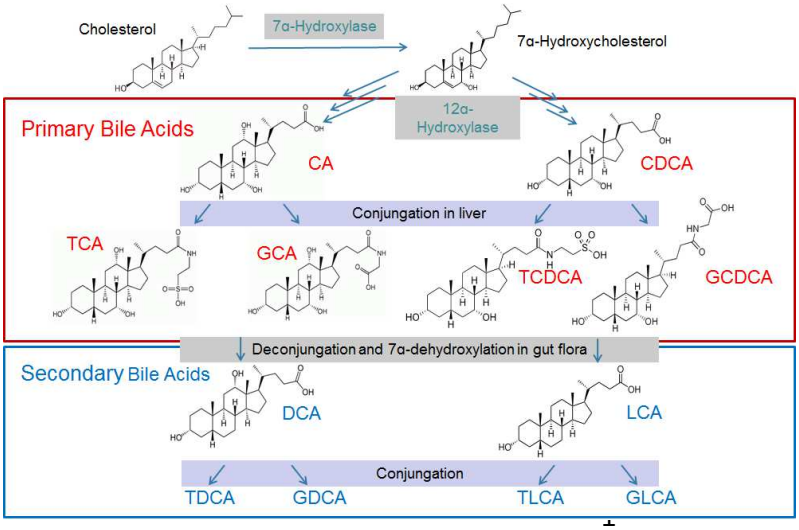

Figure 1: Bile acids biosynthesis: cholesterol catabolism

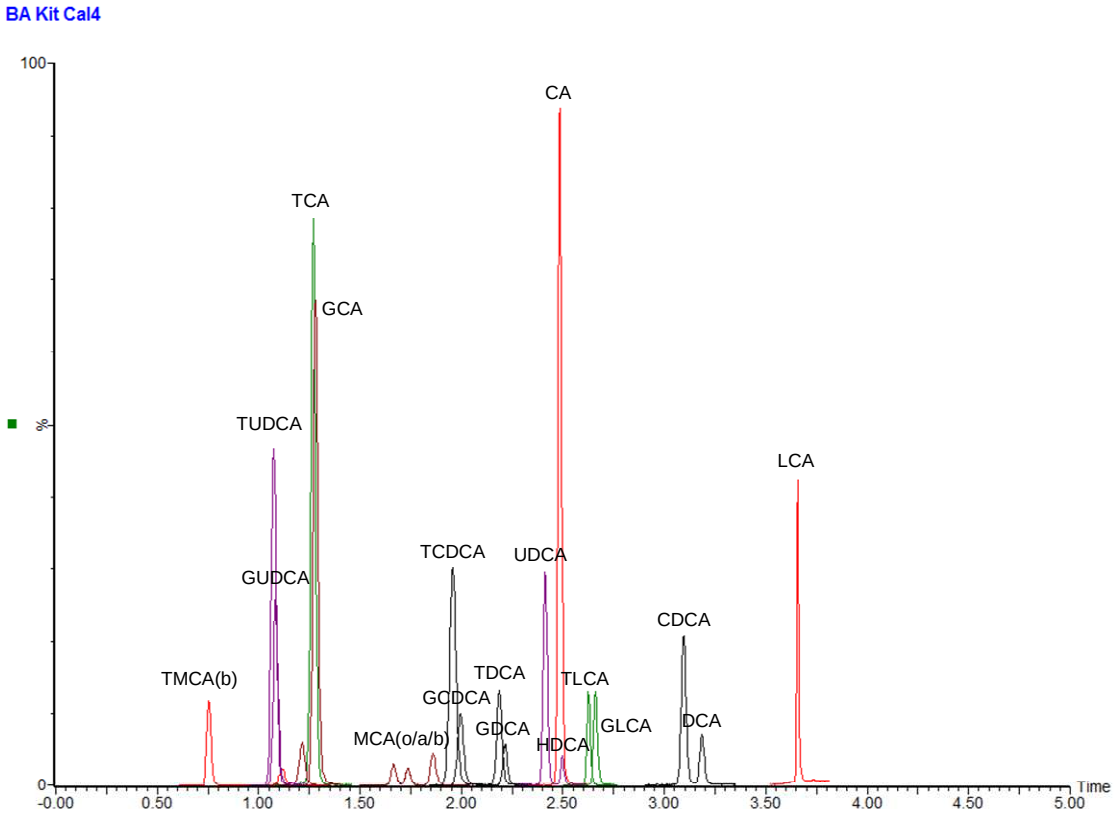

Figure 2: Chromatogram of a bile acids calibration standard level 4.

Data analysis was performed with TargetLynx (Waters) and our in-house developed Biocrates® Met*IDQ*™ software. Our multifunctional software tool was used for sample registration, and for technical validation and statistical analysis based on the concentration values from TargetLynx.

3 Results and Discussion

Optimization of sample volume and MRM transitions was performed. As opposed to the Waters® Xevo® TQ-MS and Xevo® TQ-S micro mass spectrometers, a smaller sample volume of 10 µL instead of 20 µL could be used due to the excellent detection sensitivity of the Xevo® TQ-S instrument (see later section).

Validation: Overview

Figure 2 shows a TIC chromatogram of calibration standard level 4. Table 1 gives an overview of the bile acids panel (full and short name, respectively), the validity of the individual metabolites in human and rat plasma and the calibration ranges (LLOQ[1] – ULOQ[2]) for analysis on the Xevo® TQ-S system.

The obtained calibration ranges well contained our calibration standards 1–7, which covered the expected physiological bile acids concentration ranges. The calibration curves exhibited excellent correlation coefficients (R^2) above 0.997 for all bile acids. Throughout the validation process, which included the determination of intraday (within batch) and interday (batch-to-batch) precision and accuracy as well as detection sensitivity and selectivity, the Bile Acid Kit proved as highly suitable for quantitation of the individual bile acid profile in human and rat plasma. All MRM transitions of external and internal standards were selective as revealed by the analysis of individual solutions.

[1] *lower limit of quantification*
[2] *upper limit of quantification*

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Table 1: Analytical validity of bile acids in human plasma and rat plasma on the Xevo® TQ-S system.

No	Bile acid	Short name	Validity in human plasma	Validity in rat plasma	LLOQ (µmol/L)	ULOQ (µmol/L)	R^2
1	Cholic acid	CA	✔	✔	0.03	75	0.998
2	Chenodeoxycholic acid	CDCA	✔	✔	0.02	30	0.999
3	Deoxycholic acid	DCA	✔	✔	0.02	10	0.999
4	Glycocholic acid	GCA	✔	✔	0.03	75	0.999
5	Glycochenodeoxycholic acid	GCDCA	✔	✔	0.02	20	0.999
6	Glycodeoxycholic acid	GDCA	✔	✔	0.01	10	0.999
7	Glycolithocholic acid	GLCA	✔	✔	0.01	5	0.999
8	Glycoursodeoxycholic acid	GUDCA	✔	[b]	0.01	10	0.999
9	Hyodeoxycholic acid [a]	HDCA [a]		✔	0.01	5	0.999
10	Lithocholic acid	LCA	✔	✔	0.01	5	0.999
11	Muricholic acid, alpha [a]	MCA(a)		✔	0.005	5	0.999
12	Muricholic acid, beta [a]	MCA(b)		✔	0.01	10	0.999
13	Muricholic acid, omega [a]	MCA(o)		✔	0.005	5	0.999
14	Taurocholic acid	TCA	✔	✔	0.02	50	0.997
15	Taurochenodeoxycholic acid	TCDCA	✔	✔	0.01	20	0.999
16	Taurodeoxycholic acid	TDCA	✔	✔	0.01	10	0.999
17	Taurolithocholic acid	TLCA	✔	✔	0.01	5	0.999
18	Tauromuricholic acid, alpha and beta sum concentration	TMCA(a+b)	✔	✔	0.01	10	0.999
19	Tauroursodeoxycholic acid	TUDCA	✔	[b]	0.01	15	0.999
20	Ursodeoxycholic acid	UDCA	✔	✔	0.02	30	0.999

[a] absent in human plasma samples (MCAs: rodent-specific bile acids)
[b] partially coeluting with unknown matrix compound in rat plasma

Analysis of various human and rat plasma samples revealed that HDCA and MCAs were absent in human plasma, whereas the quantitation of GUDCA and TUDCA was interfered by an unknown compound in rat plasma samples.

Validation: Interday precision and accuracy (n = 4)

Tables 2 and 3 show the performance of the Acquity UPLC® I-Class Xevo® TQ-S system in the analysis of human and rat plasma samples at endogenous, diluted and spiked concentration levels.
Excellent interday precision and accuracy values below 15% and between 85-115%, respectively, were obtained for the majority of analytes. GLCA and TDCA fulfilled the accuracy requirements easily in human and rat plasma, whereas the quality tests were narrowly passed on the Xevo® TQ-S micro system.

Table 2: Interday precision [%] of human and rat plasma samples (n = 4).

Human plasma:

Bile Acids	1:5 diluted	1:2 diluted	Endogenous level	Spiked with cal2	Spiked with cal4
CA	n.d.	n.d.	8.6	3.1	0.9
CDCA	n.d.	3.8	2.9	4.6	0.9
DCA	2.4	2.7	1.0	5.2	0.9
GCA	n.d.	9.3	7.0	1.9	4.1
GCDCA	8.4	4.2	1.1	1.6	4.2
GDCA	2.4	5.7	2.8	3.5	3.7
GLCA	n.d.	n.d.	6.8	3.6	4.8
GUDCA	5.6	2.4	3.9	3.8	2.9
HDCA	Absent in human plasma samples				
LCA	n.d.	n.d.	27.0	14.4	5.1
MCA(a)	Absent in human plasma samples – rodent-specific bile acids				
MCA(b)					
MCA(o)					
TCA	n.d.	n.d.	n.d.	4.0	2.5
TCDCA	n.d.	6.1	4.7	2.7	3.1
TDCA	n.d.	11.8	5.5	3.8	3.9
TLCA	n.d.	n.d.	n.d.	6.5	3.9
TMCA(a+b)	n.d.	n.d.	n.d.	3.9	4.0
TUDCA	n.d.	n.d.	n.d.	7.8	4.0
UDCA	n.d.	11.6	9.4	5.4	6.9

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Table 2 continued.

Rat plasma:

Bile Acids	1:5 diluted	1:2 diluted	Endogenous level	Spiked with cal2	Spiked with cal4
CA	3.6	4.8	2.6	1.9	0.7
CDCA	4.0	4.6	3.3	2.4	1.2
DCA	4.7	6.4	3.0	4.3	0.9
GCA	3.5	6.1	4.3	2.9	4.4
GCDCA	15.8	12.7	6.1	4.6	2.4
GDCA	2.7	7.4	5.4	1.9	4.5
GLCA	n.d.	n.d.	n.d.	6.9	3.0
GUDCA	Interference in rat plasma				
HDCA	1.7	4.4	5.3	8.4	16.2
LCA	n.d.	16.5	14.9	2.7	4.0
MCA(a)	1.9	3.5	3.3	2.2	2.1
MCA(b)	1.3	3.6	2.2	3.0	2.0
MCA(o)	5.4	9.7	6.5	7.3	3.2
TCA	2.8	4.1	3.6	2.7	2.9
TCDCA	7.2	6.4	3.2	3.7	3.3
TDCA	11.5	5.2	4.1	4.2	3.2
TLCA	n.d.	n.d.	n.d.	3.6	3.0
TMCA(a+b)	4.1	4.2	2.8	1.6	1.9
TUDCA	Interference in rat plasma				
UDCA	8.0	5.0	2.6	5.4	9.6

n.d. … not determined because value < LLOQ

Table 3: Interday accuracy [%] of human and rat plasma samples (n = 4).

Human plasma:

Bile Acids	1:5 diluted	1:2 diluted	Spiked with cal2	Spiked with cal4
CA	n.d.	n.d.	95.7	103.1
CDCA	n.d.	106.1	99.6	109.1
DCA	93.9	98.1	93.8	108.1
GCA	n.d.	104.3	101.8	114.0
GCDCA	100.1	98.8	93.6	106.1
GDCA	94.1	95.5	95.0	105.1
GLCA	n.d.	n.d.	108.6	104.6
GUDCA	106.2	97.8	97.3	100.1
HDCA	Absent in human plasma samples			
LCA	n.d.	n.d.	118.3	112.6
MCA(a)	Absent in human plasma samples – rodent-specific bile acids			
MCA(b)				
MCA(o)				
TCA	n.d.	n.d.	n.d.	108.2
TCDCA	n.d.	103.9	102.4	107.1
TDCA	n.d.	114.3	109.1	113.1
TLCA	n.d.	n.d.	n.d.	116.7
TMCA(a+b)	n.d.	n.d.	n.d.	99.3
TUDCA	n.d.	n.d.	n.d.	111.4
UDCA	n.d.	129.1	100.3	110.6

Table 3 continued.

Rat plasma:

Bile Acids	1:5 diluted	1:2 diluted	Spiked with cal2	Spiked with cal4
CA	98.1	100.8	95.7	101.0
CDCA	102.0	101.5	97.2	103.9
DCA	97.9	102.6	101.5	107.9
GCA	100.1	102.3	97.8	102.0
GCDCA	107.6	103.1	98.5	105.3
GDCA	113.7	107.8	101.2	108.6
GLCA	n.d.	n.d.	n.d.	107.1
GUDCA	Interference in rat plasma			
HDCA	95.1	98.7	101.9	106.6
LCA	n.d.	107.4	99.2	110.2
MCA(a)	103.7	103.2	96.0	96.5
MCA(b)	104.0	101.6	95.4	103.2
MCA(o)	104.5	97.2	93.1	102.1
TCA	100.9	101.6	95.5	106.5
TCDCA	103.8	102.6	97.7	104.8
TDCA	121.2	106.7	97.1	107.3
TLCA	n.d.	n.d.	n.d.	108.8
TMCA(a+b)	104.2	104.0	96.3	95.3
TUDCA	Interference in rat plasma			
UDCA	123.5	108.2	98.4	107.1

n.d. … not determined because value < LLOQ

Xevo® TQ-S versus TQ-S micro: interday precision of low calibration standards (n = 4)

During validation of the Bile Acids Kit on the Xevo® TQ-S micro mass spectrometer, CVs > 20% of low concentrated calibration standards were observed in intraday and interday experiments. Since the maximum injection volume of the Flow-Through-Needle (FTN) autosampler is 10 µl, the sample volume was increased to 20 µl whereas the injection volume of 10 µL was maintained.

Table 4 shows the CVs of calibration standards cal 1 – cal 3 with sample volumes of 10 µl and 20 µl (n = 4) analysed on the Xevo® TQ-S micro MS. Better CVs were obtained for most bile acids with 20 µL sample volume.

On the contrary, the small sample volume of 10 µL could be used for the bile acid analysis on the Xevo® TQ-S instrument due to its excellent detection sensitivity. This resulted in even lower CVs than those obtained with 20 µL on the Xevo® TQ-S micro MS (Table 4).

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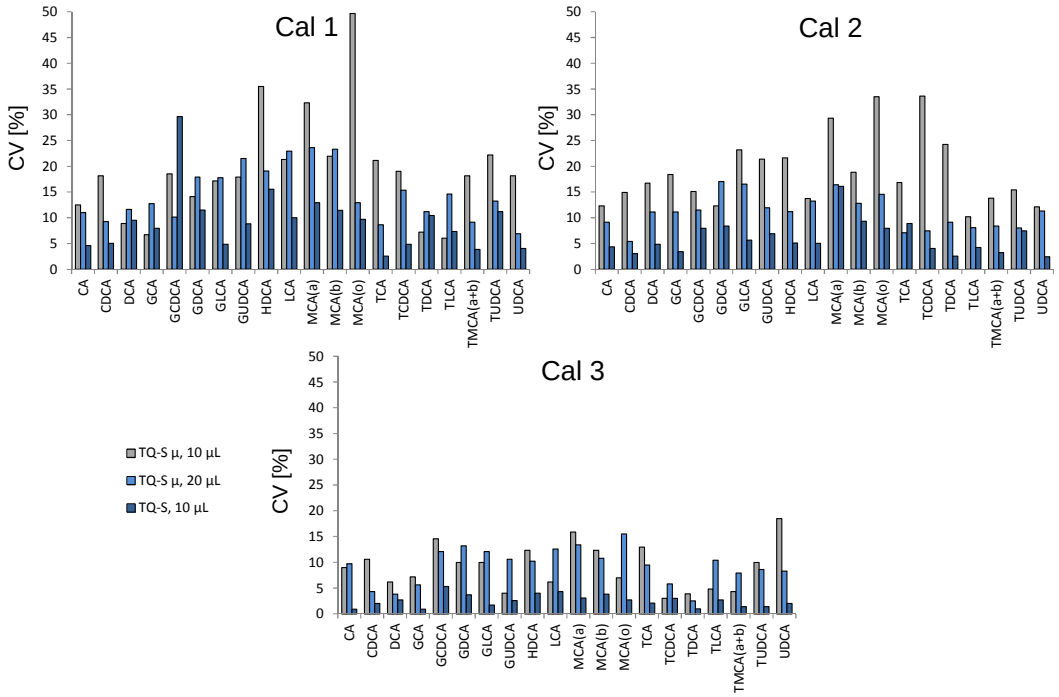
Table 4: Interday precision [%] of low concentrated calibration standards cal1 – cal3 (n = 4) analyzed by Acquity UPLC® I-Class coupled to Xevo® TQ-S micro versus TQ-S mass spectrometers. 10 µL/20 µL and 10 µL of sample were used, respectively.



4 Conclusions

Using the Biocrates® Bile Acids Kit together with Waters® ACQUITY UPLC® I-Class coupled to a Xevo® TQ-S mass spectrometer, accurate and precise quantification of bile acids in human and rat plasma samples is feasible.

The Kit is based on an easy to perform and quick sample extraction on a 96-well patented filter plate. Only 3 steps are required to complete the simple and robust sample preparation process. The total analysis time for one plate including 80 samples is 14 hours (from sample registration to the final concentration table). Hence, high sample throughput is achieved.

A total of 16 and 18 bile acids in human and rat plasma, respectively, proved fully valid. A smaller sample volume of 10 µL than previously 20 µL for the Xevo® TQ-S micro instrument could be used. A broad quantification range was covered including the expected physiological bile acid concentrations.

In summary, the Biocrates® Bile Acids Kit in combination with a Waters® Acquity UPLC® I-Class Xevo® TQ-S system is a powerful targeted metabolomics assay to discover bile acids profiles in low-volume plasma samples.

References

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