Standardized Quantitative Bile Acids Phenotyping in Plasma Samples Using Waters® ACQUITY UPLC® I-Class together with Xevo® TQ-S micro MS

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1 Introduction

Bile acids are known as endogenous markers for liver cell functions, as signaling molecules regulating various metabolism processes and as mediators for gut microbiome status [1-6]. The primary bile acids, cholic acid and chenodeoxycholic acid, are formed by oxidation from cholesterol in the liver and stored in the gall bladder. The secondary bile acids, deoxycholic acid and lithocholic acid, are formed here by bacterial flora (see Figure 1). In order to provide reliable results in large cohort studies in precision medicine, toxicology, and clinical biomarker research, a standardized and quality controlled analysis of individual bile acids and their conjugates is needed. An (U)HPLC-ESI-MS/MS based Kit was developed which comprises 20 individual bile acids and requires a sample volume of 20 µL. The Kit has been validated according to the EMA guidelines for human and rat plasma.

2 Materials and Method

20 µL of plasma samples are used for the Biocrates® Bile Acids Kit preparation (see User Manual). Two hours are needed for the sample preparation (robust, simple, 96-well plate extraction) and subsequently 5 min analysis time per sample (UPLC, 10 µL of injection volume). The metabolite panel of the Kit includes 20 individual bile acids (see 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.

A Waters® UPLC® I-Class system was coupled to a Xevo® TQ-S micro mass spectrometer (Waters Inc.). Peak integration and quantitation were performed with TargetLynx (Waters). Our in-house developed software tool (Met***IDQ***™) was used for sample registration, technical validation, and statistical analysis.

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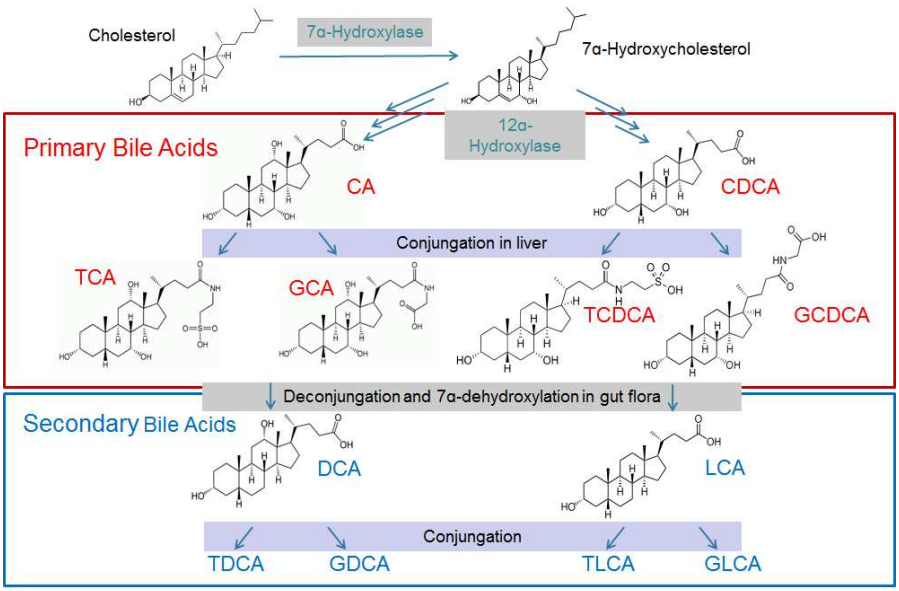

Figure 1: Bile acids biosynthesis: cholesterol catabolism

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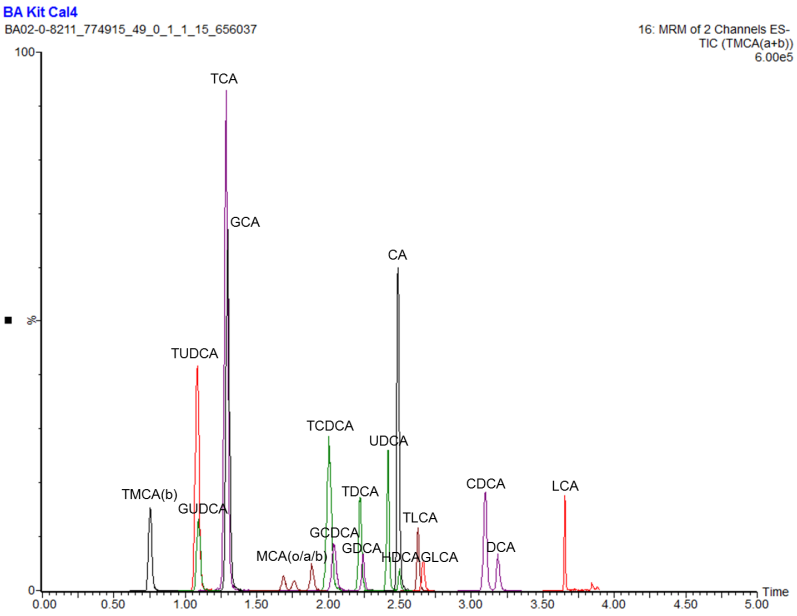

Figure 2: Chromatogram of a bile acids calibration standard level 4.

3 Results and Discussion

Validation: Overview

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

These ranges correspond to calibration standards 1–7 for most bile acids; for HDCA the LLOQ was calibration standard 2. Excellent correlation coefficients (R^2) above 0.996 were obtained for all analytes.

The Bile Acid Kit proved as highly suitable for quantitative determination of the individual bile acid profile in human and rat plasma. HDCA and MCAs were absent in human plasma, whereas the analysis of GUDCA and TUDCA was interfered by an endogenous compound in rat plasma samples. Overall, the expected physiological bile acids concentration ranges were well covered.

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

No	Bile acid	Validity in human plasma	Validity in rat plasma	LR (µM–µM)	R^2
1	CA	✔	✔	0.03–75	0.999
2	CDCA	✔	✔	0.02–30	0.999
3	DCA	✔	✔	0.02–10	0.999
4	GCA	✔	✔	0.03–75	0.999
5	GCDCA	✔	✔	0.02–20	0.997
6	GDCA	✔	✔	0.01–10	0.996
7	GLCA	✔	✔	0.01–5	0.996
8	GUDCA	✔	[b]	0.01–10	0.997
9	HDCA [a]		✔	0.02–5	0.998
10	LCA	✔	✔	0.01–5	0.999
11	MCA(a) [a]		✔	0.005–5	0.998
12	MCA(b) [a]		✔	0.01–10	0.999
13	MCA(o) [a]		✔	0.005–5	0.999
14	TCA	✔	✔	0.02–50	0.999
15	TCDCA	✔	✔	0.01–20	0.999
16	TDCA	✔	✔	0.01–10	0.999
17	TLCA	✔	✔	0.01–5	0.996
18	TMCA(a+b)	✔	✔	0.01–10	0.999
19	TUDCA	✔	[b]	0.01–15	0.999
20	UDCA	✔	✔	0.02–30	0.998

[a] absent in human plasma samples (MCAs: rodent-specific bile acids)

[b] partially coeluting with unknown matrix compound in rat plasma

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

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Validation: Interday precision and accuracy (n = 4)

Tables 2 and 3 show the performance at endogenous, diluted and spiked concentration levels of human and rat plasma samples on the Xevo® TQ-S micro system.

Excellent interday precision and accuracy values below 15% and between 85-115%, respectively, were obtained for the majority of analytes.

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 cal6
CA	n.d.	n.d.	15.3	8.9	3.5
CDCA	n.d.	22.6	6.9	7.7	6.6
DCA	11.4	4.3	6.5	6.6	6.2
GCA	n.d.	n.d.	1.7	7.4	4.9
GCDCA	6.0	5.1	3.4	2.9	12.7
GDCA	10.4	7.7	4.5	12.4	12.4
GLCA	n.d.	n.d.	n.d.	5.0	13.2
GUDCA	n.d.	11.8	9.5	17.9	2.2
HDCA	Absent in human plasma samples				
LCA	n.d.	n.d.	n.d.	15.7	3.1
MCA(a)	Absent in human plasma samples – rodent-specific bile acids				
MCA(b)					
MCA(o)					
TCA	n.d.	n.d.	n.d.	9.5	5.0
TCDCA	n.d.	11.4	7.6	13.8	4.0
TDCA	n.d.	11.8	10.7	5.4	5.0
TLCA	n.d.	n.d.	n.d.	7.1	14.2
TMCA(a+b)	n.d.	n.d.	n.d.	8.3	3.4
TUDCA	n.d.	n.d.	n.d.	18.7	3.0
UDCA	n.d.	n.d.	12.9	5.8	7.9

Rat plasma:

Bile Acids	1:5 diluted	1:2 diluted	Endogenous level	Spiked with cal2	Spiked with cal6
CA	3.3	7.2	5.0	6.6	4.6
CDCA	7.6	7.8	3.9	4.2	4.4
DCA	12.4	5.1	8.0	9.3	3.5
GCA	8.4	6.7	2.7	8.8	5.7
GCDCA	7.7	1.7	4.0	11.0	7.0
GDCA	n.d.	9.2	14.8	8.3	7.3
GLCA	n.d.	n.d.	n.d.	19.9	7.5
GUDCA	Interference in rat plasma				
HDCA	0.8	1.5	6.5	4.4	9.5
LCA	n.d.	23.4	18.0	12.1	1.8
MCA(a)	2.5	7.3	6.0	3.9	5.6
MCA(b)	10.5	5.1	6.7	7.1	5.2
MCA(o)	n.d.	24.8	13.0	14.5	5.4
TCA	8.2	4.1	6.6	3.7	4.0
TCDCA	14.4	10.7	4.1	9.0	5.9
TDCA	n.d.	5.5	3.6	4.9	6.1
TLCA	n.d.	n.d.	n.d.	18.9	6.0
TMCA(a+b)	5.0	3.7	2.8	6.2	4.3
TUDCA	Interference in rat plasma				
UDCA	n.d.	10.2	4.0	10.8	6.4

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 cal6
CA	n.d.	n.d.	106.4	108.4
CDCA	n.d.	111.6	103.4	109.6
DCA	86.1	96.8	97.1	110.2
GCA	n.d.	n.d.	102.3	120.9
GCDCA	101.5	97.1	110.9	116.7
GDCA	104.5	102.7	116.4	107.9
GLCA	n.d.	n.d.	133.9	109.8
GUDCA	105.7	129.0	108.6	97.7
HDCA	Absent in human plasma samples			
LCA	n.d.	15.7	102.4	116.6
MCA(a)	Absent in human plasma samples – rodent-specific bile acids			
MCA(b)				
MCA(o)				
TCA	n.d.	n.d.	111.3	110.7
TCDCA	n.d.	97.7	102.8	112.3
TDCA	n.d.	115.7	122.7	101.0
TLCA	n.d.	n.d.	120.2	116.3
TMCA(a+b)	n.d.	n.d.	104.0	92.1
TUDCA	n.d.	n.d.	120.6	114.0
UDCA	n.d.	n.d.	100.7	117.0

Rat plasma:

Bile Acids	1:5 diluted	1:2 diluted	Spiked with cal2	Spiked with cal6
CA	99.8	98.8	93.3	115.7
CDCA	98.4	99.4	95.3	118.6
DCA	92.0	98.5	99.1	115.9
GCA	97.0	93.1	92.7	114.3
GCDCA	105.5	99.3	101.0	120.9
GDCA	101.4	90.4	103.1	121.2
GLCA	n.d.	n.d.	123.8	120.3
GUDCA	Interference in rat plasma			
HDCA	96.4	91.3	99.1	112.9
LCA	n.d.	105.0	94.5	121.2
MCA(a)	99.5	97.1	93.7	103.6
MCA(b)	102.2	99.6	94.4	114.4
MCA(o)	n.d.	111.7	89.2	113.4
TCA	99.3	93.7	91.1	118.7
TCDCA	98.4	96.7	95.6	117.5
TDCA	n.d.	101.8	94.7	117.9
TLCA	n.d.	n.d.	122.2	117.5
TMCA(a+b)	108.8	102.6	95.3	96.8
TUDCA	Interference in rat plasma			
UDCA	109.7	96.5	93.2	118.1

n.d. … not determined because value < LLOQ

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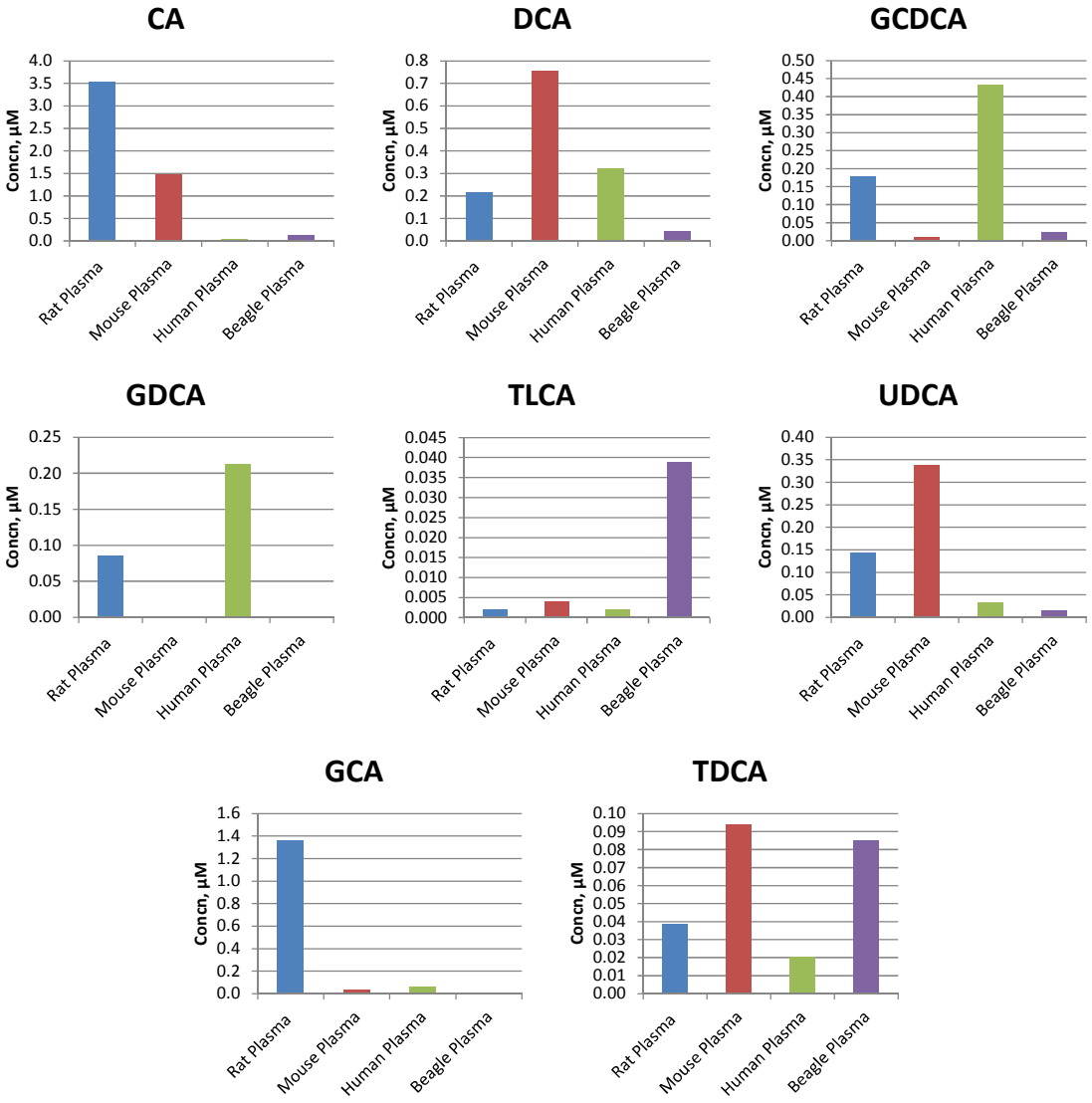

Figure 3: Differences in bile acids concentration levels between plasma samples from rat, mouse, human and beagle.

Phenotyping

The analyses of plasma samples from mouse, rat, beagle and human indicate the suitability of the Kit across species. It was revealed that the bile acids profile differs for each species (Figure 3). These findings might be of high interest for translational medicine and toxicological studies, e.g. in drug development.

4 Conclusions

Quantification of bile acids was performed using the Biocrates® Bile Acids Kit in combination with Waters® ACQUITY UPLC® I-Class Xevo® TQ-S micro MS systems.
The Kit is based on an easy to perform and quick sample extraction on a 96-well patented filter plate. Only 3 steps are needed to

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complete the sample preparation process. The whole plate including 80 samples can be analysed within 14 hours (from sample registration to the final concentration table), hence, enabling high sample throughput.
The Biocrates® Bile Acids Kit was successfully validated for human and rat plasma according to EMA guidelines. It enables quantification of bile acids with high accuracy and precision over a broad linear range covering the expected physiological concentrations.
In summary, the Biocrates® Bile Acids Kit in combination with a Waters Xevo® TQ-S micro instrument is a powerful targeted metabolomics assay to discover bile acids profiles in plasma samples.

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