



Přírodovědecká  
fakulta  
Faculty  
of Science

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice

## STATEMENT OF THE BACHELOR/~~DIPLOMA~~\* THESIS REVIEWER

**Name of the student:** Felix Leibetseder

**Thesis title:** THE BIOCHEMISTRY OF BILE ACIDS AND THEIR HPLC-MS ANALYSIS

**Supervisor:** Petr Šimek PhD  
Petra Berková PhD

**Reviewer:** Pavla Fojtíková PhD

**Reviewer's affiliation:** University of South Bohemia in České Budějovice  
Faculty of Science, Institute of Chemistry and Biochemistry

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
<b>Extent of the thesis</b> (for bachelor theses min. 18 pages, for masters theses min. 25 pages), <b>balanced length of the thesis parts</b> (recommended length of the theoretical part is max. 1/3 of the total length), <b>logical structure of the thesis</b>	0-3	3
<b>Quality of the theoretical part (review)</b> (number and relevancy of the references, recency of the references)	0-3	2
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
<b>Graphic layout of the text and of the figures/tables</b>	0-3	1
<b>Quality of the annotation</b>	0-3	3
<b>Language and stylistics, complying with the valid terminology</b>	0-3	2
<b>Accuracy and completeness of figures/tables legends</b> (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
<b>Formal requirements – points in total</b>		14
<b>(2) PRACTICAL REQUIREMENTS</b>		
<b>Clarity and fulfillment of the aims</b>	0-3	2
<b>Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions</b>	0-3	2
<b>Discussion quality – interpretation of the results and their discussion with the literature</b> (absence of discussion with the literature is not acceptable)	0-3	3

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		22
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>48</b>	<b>(36)<sup>2</sup></b>

### Comments of the reviewer on the student and the thesis:

The thesis is well structured. The student was able to fulfill the aims of the work and also to give visions and tasks for further experiments. Results and discussions are based on intensive experimental work using very sophisticated technique. The results of the work are well presented as a logical sequence of steps. The text contains quite a lot of formal mistakes thus reducing the quality of the work. The discussion within the whole work should be given in more details and supported by objective assessments and acceptance criteria.

### Suggestions and questions, to which the student has to answer during the defense.

#### Mistakes, which the students should avoid in the future:

- 1) Formal mistakes within the whole document: page numbering, wrong and not uniform citing of the references (e.g. the number of reference before/after full stop), inconsistency in writing of units (e.g. [nM] or / nM; g/mol or nmol\*ml<sup>-1</sup>), varying margins, number at the end of line and unit at the beginning of the next line, style of writing [M+H]<sup>+</sup>, [M+H]<sup>-</sup> instead of [M+H]<sup>+</sup> and [M+H]<sup>-</sup>, missing units (e.g. Table 6), missing axes descriptions (e.g. Fig. 15, 18, 21)).
- 2) List of used abbreviations and symbols should be placed either above the introduction or after references but not on the same page as acknowledgement. The list is incomplete (missing e.g. SIM, CID, SRM, LoD, LoQ, symbols from tables k, d, R<sup>2</sup>).
- 3) Chapter 1: *“The aim of this work was .... to develop a HPLC-MS method for the qualification and quantification of the bile acids.”* This aim was not achieved and later on in Conclusions was marked as task for further research. On the other hand, according to annotation the aims of the work were fulfilled.
- 4) Chapter 3.2: No clear explanation about data given in Table 3, what is *“amount left”* and so on.
- 5) Chapter 3.4.: Please explain why you did not use the internal standard in the preparation of calibration solutions and real samples.

<sup>2</sup> Enter the number of points awarded.

- 6) Ordering in chapters during the explanation of methods should be the same as the order of the corresponding chapters in results. See the chapters 3.6. and 3.7. which are in opposite order than the chapter 4.1. and 4.2.
- 7) Chapter 4.3.: “*An adequate separation of all examined BAs and their well shaped peaks were obtained. ... The separation of all analytes was satisfactory.*” Objective expressions should be used. How can be evaluated the peak shape and what parameter can be used to describe the chromatographic resolution? Please compare these results with general acceptance criteria of these parameters.
- 8) Chapter 4.4.: “*The calibration equation and coefficients of determination are all in a usable range and therefore the calibration can be used for the real samples.*” Please explain what is the **usable range**.
- 9) Chapter 4.4.: “*The calibration equation and coefficients of determination are all in a usable range and therefore the calibration can be used for the real samples. ... The obtained results in these experiments are only valid for clean methanol standard solutions, the situation in a real sample matrix could be entirely different.*” There are two opposite meanings given in one chapter, please explain.
- 10) In chapter 4.4., it is written that the limit of the detection for most of the studied BAs is probably below the used concentrations. How can be determined the detection limits and the limits of quantification?
- 11) Chapter 4.7.: The discussion of BAs measurements with a UHPLC-HRMS is very short.
- 12) Missing list of appendixes.
- 13) Appendixes: The formatting of tables is not very good – missing units, inconsistent usage of capital letters, various formats of numbers, not appropriate cell width in the tables resulting in missing some digits.

#### Eventual mistakes, which the students should avoid in the future:

This part does not have to be read during the bachelor's thesis defense. These comments are made especially for the author.

- 1) Chapter 2.2.1.: Fig. 4: Not the appropriate size of the figure.
- 2) Chapter 2.3: Paragraph one and two seems to be almost identical.
- 3) Chapter 2.4.1.: The text has very big left alignment.
- 4) Chapter 3.2: The usage of the appropriate cell width (Table 1, the cell with the word “Compound”), use the same indication of compounds (CA versus CA 98%) consistently. Missing information about the type of used analytical balances.
- 5) Chapter 3.6.: “*After a good separation was achieved in the HPLC, ...*” this should not be written at the beginning of this paragraph. According to Chapter 4, the optimization of ESI temperature for BAs was done before the optimization of HPLC conditions.
- 6) Chapter 4.1.: Table 4: Do not let any empty cell in a table.
- 7) Chapter 4.2.: Fig. 10: There is two times written Fig.
- 8) Chapter 4.4.: Fig. 15, 16, 17: Both axes must have description, there is no need to put values of concentration on x-axes with two decimals.
- 9) Chapter 4.4.: Table 6 – it is necessary to describe the meanings of symbols and to put the units.
- 10) Chapter 4.5.: “*The chromatograms for the plasma can be seen in Fig. 21-23.*” The references should probably be Fig. 18 – 20.
- 11) Chapter 4.5.: Fig. 18, 19, 20: Both axes must have a description.
- 12) Chapter 4.6.: Fig. 21, 22, 23: Please define or express in a better way what is plotted. Both axes must have a description.

13) Chapter 4.7.: Table 7: Incompleted table description.

**Conclusion:**

In conclusion, I

**r e c o m m e n d / ~~d o n o t r e c o m m e n d~~\***

the thesis for the defense and I suggest the grade 2 .<sup>3</sup>

In České Budějovice date 4.6.2018

*Paula Fojtíková*  
.....

signature

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<sup>3</sup> You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).