UNIVERSITY OF SOUTH BOHEMIA
IN ČESKÉ BUDĚJOVICE

Faculty of Science

STATEMENT OF THE BACHELOR/~~DIPLOMA~~[*] THESIS REVIEWER

Name of the student:	Monika Litviňuková
Thesis title:	Profiling of steroid conjugate metabolites in body fluids of pregnant women by liquid chromatography-mass spectrometry (LC-MS)
Supervisor:	RNDr. Petr Šimek, CSc.
Reviewer:	Ing. David Kahoun, Ph.D.
Reviewer`s affiliation:	University of South Bohemia – Faculty of Science

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced extents of the thesis divisions** (recommended extent of the theoretical part is max. 1/3 of the total extent), **logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	1
Graphic layout of the text and of the figures/tables	0-3	2
Adequacy and clarity of the results and conclusions	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	2
Formal requirements – points in total		18
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	2
Discussion quality – interpretation of results and their discussion with the literature	0-3	1
Logic in the course of the experimental work	0-3	3

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[*] Choose one
[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

3) Page 27, Chapter 4.2.2: "*The results were evaluated as a percentage out of original sample amount before the extraction and analysis. The original value was 100pmol/μl.*"

How did the author evaluate LC-MS results? Which parameter was used for comparison of the SPE experiments exactly – concentration of analyte (as the text implies), peak area of analyte or different parameter? If concentration of analyte was used, no information about calibration curves is given.

4) Page 28, Chapter 4.2.2: "*Many values were found to be reaching over 100% which might be caused by the matrix effect or by other interferences*".

How significant were contributions of matrix effects on SPE recoveries of evaluated analytes? What information gave results of blank solutions which were analysed (see page 17, Chapter 3.4, last paragraph, last sentence). Were these contributions of blank solutions subtracted from results of SPE extractions of standard solutions presented in Table 5, Table 6 and Table 7?

Eventual additional comments of the supervisor on the student and the thesis:

This part does not have to be read during the bachelor's thesis defence due to time reasons. These comments are made especially for the author as recommendations for future work.

1) Abstract, page 9, 10, ...: Insert one space between a numerical value and its unit.

2) List of Abbreviations: APCI does not mean Atmospheric-Chemical Ionization but it means Atmospheric Pressure Chemical Ionization.

3) Pages 1 – 31: Do not use a full point after last number of a chapter.

4) The thesis contains some mistakes in English language:

   a) "*endoplasmatic reticulum*" instead of "*endoplasmic reticulum*"

   b) "*transfered*" instead of "*transferred*"

   c) "*nuclephilic*" instead of "*nucleophilic*"

   d) "*column were connected*" instead of "*column was connected*"

   e) etc.

5) Pages 4 – 7 and pages 24 – 26: Table headings are not repeated on subsequent pages.

6) Page 7, Table 1: The number of atoms in the molecular formula of cortisol is not written using subscript text.

7) Page 8, Table 2: Structural formula of 17α-Hydroxyprogesterone is relatively blurred.

8) Pages 9, 15, 16, ...: Do not use small letter (l) for the symbol for volume unit (liter) but use capital letter (L) only.

5) Page 10 and page 19: Do not leave a number at the end of a line and its unit on the beginning of the following line.

6) Page 11, Chapter 1.6: "*In that case, pre-concentration is necessary to achieve suitable analytical sensitivity.*"

The sensitivity of an analytical method is often used to refer to the limit of detection of an analytical method but this use is not generally approved. The sensitivity is the capability of the method to discriminate small differences in concentration or mass of the analyte. For detailed information see The Fitness for Purpose of Analytical Methods published by EURACHEM or see Compendium of Chemical Terminology published by IUPAC.

7) Page 11 and page 12, Chapter 1.6.3: This chapter contains two absolutely identical paragraphs (see the first paragraph and the third paragraph).

8) Page 16 and page 17: Do not leave a one-letter word at the end of a line.

9) Page 17, ...: Use the term "*optimization*" instead of the term "*optimalization*".

10) Page 24 and page 25, Table 3 and Table 4: There is not stated any unit of the parameter "*MW*".

11) Pages 27 – 29, Tables 5 – 7: Do not begin a title of a table with a small letter.

12) Which standard for citations and references is used? If standard ISO 690:2010 is used (what it seems), citations and references are not written correctly. For detailed information see ISO 690:2010 or use e.g. Microsoft Word 2010, which can generate citation and bibliography automatically and correctly based on the source information that you provide for the document.

Citations:

- Full point is written before number of a citation (e.g. pages 2, 3, 4, ...).

- Citations in the text are not sorted in ascending order (e.g. the first citation is citation [1], second citation is citation [4], the third citation is citation [7], ...).

References:

- References are not written correctly.

- Reference items of some references are not written in required order and style.

- Journal titles are not written in italics.

- Using Czech equivalents "a" (for the English word "and"), "s" (for the abbreviation of the English word "pages").

- Using of different font styles and font sizes (e.g. references [23] and [23]).

- If Digital Object Identifier (DOI) is used, Uniform Record Locator (URL) is not necessary to be used subsequently (e.g. reference [8]).

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis	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 filed and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Formal requirements – points in total		20

POINTS IN TOTAL (MAX/AWARDED)	**51**	**38**[2]

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

1) How did the author evaluate the mobile phase optimization (see Chapter 4.1 and Chapter 5)?

   Page 17, Chapter 3.4: "*For the mobile phase optimalization, variety of mobile phases was used to determine the most fitting criteria concerning the ionization and separation of the conjugates.*"

   Page 17, Chapter 3.5: "*After the analysis, the obtained peaks were integrated using Thermo Scientific X-calibur system to determine the intensity of each measured steroid in given mobile phase*".

   Chapter 4.1 deals with mobile phase optimization but no single specific parameter was selected, because "*intensity of each measured steroid*" could be expressed e.g. as Peak Area, Peak Height, Signal-to-Noise Ratio etc. Moreover, no numerical evaluations or comparisons of any parameter (instead of the parameter Retention Time) were done. Finally, the parameter "*intensity*" could not be used for evaluation of the chromatographic separation.

   Emotional words (e.g. "*quite nice*", "*nicely symmetric shape*" or "*unattractive*") or non-objective expressions (e.g. "*the peak seemed to be slightly unsymmetrical*") were used for evaluation only. Use objective suitable parameters which could be expressed numerically whenever is possible. Description of the peak shape or characteristics of the chromatographic separation should be expressed by parameters which are defined in guidelines of well respected organizations e.g. in United State Pharmacopeia General Chapter <621> Chromatography published by the U. S. Pharmacopeial Convention (e.g. Symmetry Factor, Peak Width at Half Height, Resolution, Retention Factor, Separation Factor, ...), in Compendium of Chemical Terminology published by the International Union of Pure and Applied Chemistry, ...

2) Page 18, Chapter 4.1.1: "*Concerning the pure methanol, the results were not as coherent as expected*".

   Which results did the author expect? No expected results were stated, no reference was given, no comparison was done.

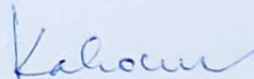---

2   Enter the number of points awarded.

Conclusion:

In conclusion, I
<u>r e c o m m e n d</u> / ~~do not recommend~~*

the thesis for the defense and I suggest the grade   2 .[3]

In **České Budějovice** date **8 September 2014.**

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Signature