



UNIVERSITY OF SOUTH BOHEMIA  
IN ČESKÉ BUDĚJOVICE



Faculty of Science

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

Name of the student: Michal Kamenický

Thesis title: Mass spectrometric analysis of tricarboxylic acid cycle metabolites

Supervisor: RNDr. Petr Šimek, CSc.

Reviewer: Ing. David Kahoun, Ph.D.

Reviewer` affiliation: Accredited Analytical Laboratory Aneclab

	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 extents of the thesis divisions</b> (recommended extent of the theoretical part is max. 1/3 of the total extent), <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	1
<b>Graphic layout of the text and of the figures/tables</b>	0-3	3
<b>Adequacy and clarity of the results and conclusions</b>	0-3	2
<b>Quality of the annotation</b>	0-3	3
<b>Language and stylistics, complying with the valid terminology</b>	0-3	3
<b>Accuracy and completeness of figures/tables legends</b> (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	2
<b>Formal requirements – points in total</b>		19
<b>(2) PRACTICAL REQUIREMENTS</b>		
<b>Clarity of the aims</b>	0-3	3
<b>Fulfillment of the aims</b>	0-3	2
<b>Discussion quality – interpretation of results and their discussion with the literature</b>	0-3	1
<b>Logic in the course of the experimental work</b>	0-3	2
<b>Completeness of the description of the used techniques</b>	0-3	2
<b>Experimental difficulty of the thesis, independence in experimental work</b>	0-3	1

\* Choose one

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

Quality of experimental data presentation	0-3	2
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	1
Formal requirements – points in total		17
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>51</b>	<b>36</b>

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

- 1) Page 9, Chapter 3.3.  
How did you obtain operation procedures for sample preparations? Did you perform any optimization?
- 2) Page 11, Chapters 3.4.3. – 3.4.5.  
How did you obtain GC-MS and LC-MS conditions? Did you perform any optimization?
- 3) Page 13, Chapter 4.1.1.: „*The oxaloacetic acid derivative was not detected in the GC-MS chromatograms although various reaction, extraction, and chromatographic conditions were examined with freshly prepared aqueous standard solutions.*“  
How did you perform this optimization and why did you not state these procedures and these results in this bachelor thesis?
- 4) What are typical values of concentration ranges of the examined TCAs in cell structures and biological matrices? Is the ECF-mediated derivatization-extraction method with GC-MS analysis sensitive enough? Is it possible to use the method for the detection and determination of the TCAs in cell structures without any other modifications (e.g. concentration steps)?

**Eventual mistakes, which have to be corrected:**

- 1) Page V, List of Abbreviations: “*EI electron impact*”  
This name is obsolete. It is recommended to use “electron ionization”.
- 2) Page 9, Chapter 3.1  
Succinic acid is not stated among chemicals.
- 3) Page 9, Chapter 3.1  
Preparation of stock solutions of examined acids is not described enough. There are not stated basic information e.g. weight, solvent volume, volumetric flask, pipettes, storage conditions, shelf life etc.
- 4) Page 13, Chapter 4.1.1., Table 2  
There is not stated the unit “[min]” of the parameter “RT”.
- 5) Page 16, Chapter 4.1.2., Table 3  
There is not stated the unit “[Da]” of the parameter “[M + NH<sub>4</sub>]<sup>+</sup>”.
- 6) Page 17, Chapter 4.1.2.: “*The proposed structures of the citric acid (1) and citric acid (2)*”

are shown in Fig. 8.“

There are not shown structures of citric acids but there are shown structures of their derivatives.

- 7) Pages 24 – 30, Chapter 8.: e.g. “Fig. 9: GC-MS analysis, ECF derivatization, EI spectrum - pyruvic acid“

Figure legends are written inaccurately. Chemical structure and mass spectrum show EI spectrum of pyruvic acid derivative.

- 8) Pages 31 – 33, Chapter 9.: e.g. “Fig. 23: LC-MS analysis, ECF derivatization, ESI positive spectrum - citric acid (1)“

Figure legends are written inaccurately. Chemical structure and mass spectrum show ESI positive spectrum of citric acid (1) derivative.

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

- 1) Do not leave one-letter words at the end of line and keep the number and the unit on the same line. Do not start a sentence with an abbreviation. These rules belong among basic typography standards.

- 2) Page 4, Chapter 1.2.: “The intermediates are present at various concentrations in a complex biological matrix, inside the cell, in the cell tissues or body fluids.“

The formulation is very vague. Typical values of concentration ranges should be stated.

- 3) Page 6, Chapter 1.4.: *If it is not possible, excess of the reagent can be added and moisture is removed from the sample by hydrolysis of water residues with the silylation reagent.*

Two prepositions “of” are used.

- 4) Page 6, Chapter 1.4.: “In some cases silylation reagent...“

There is no comma after the word „cases“.

- 5) Page 6, Chapter 1.4.: “The reaction time is in (minutes - hours) and...“

Words “is in” should not be stated here.

- 6) Page 9, Chapter 3.1

There are no information about purity of chemicals and reagents.

Unequivocal identification of chemical compounds using CAS registry number is recommended.

- 7) Page 9, Chapter 3.2.: “10 mM (10  $\mu$ mol/mL) stock aqueous solution of ...“

If possible, reword the sentence so it does not begin with a number e.g. „A stock aqueous solution of 10mM (10  $\mu$ mol/mL) of each compound was prepared by ...“

- 8) Page 10, Chapter 3.4.2.: „LC-MS analysis was carried out on a linear ion trap mass spectrometer ...“

Words “by” or “using” are more suitable in this case.

- 9) Page 11, Chapter 3.4.4.: “... a separation with mobile phase consisting of methanol (A) and water (B) ...“

Naming convention for reversed-phase liquid chromatography says, that mobile phase A contains little or no organic modifier than mobile phase B which contains a higher concentration of organic modifier.

10 Page 18, Chapter 4.2.: “*We tested liquid-liquid extraction ...*”

The results and discussion chapter is usually written using past tense, e.g. “*data were examined ...*” Notice that is written in the passive voice as well: this is used so that focus falls on *what* was examined and not on *who* did the examining.

11) Pages 20 – 22, Chapter 6.

References are not written uniformly. There are used both full title of journals and abbreviations of journals, different font sizes etc. It is necessary to write references uniformly and write it in compliance with ČSN ISO 690 or in compliance with requirements of some respected scientific journal (e.g. Journal of Chromatography A).

12) Page 23, Chapter 7.: “*Table 5: Analytes and their molecular weights, chemical formulas and structures*”

The letter “e” is missing in the word “chemical”.

### Conclusion:

In conclusion, I

recommend / ~~do not recommend~~\*

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

In České Budějovice date September 16, 2012



.....  
signature

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<sup>2</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.