Přírodovědecká   Jihočeská univerzita
fakulta   v Českých Budějovicích
Faculty   University of South Bohemia
of Science   in České Budějovice

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student:	**Jan Tlačil**
Thesis title:	**"Determination of polyols in *Bombyx mori* larvae by GC-MS measurement"**
Supervisor:	Ing. Helena Zahradníčková, Ph.D.
Reviewer:	RNDr. Petr Kotas, Ph.D.
Reviewer's affiliation:	Department of Ecosystem Biology and Institute of Chemistry and Biochemistry, Faculty of Science, University of South Bohemia

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0–3	1
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0–3	1
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
Quality of the annotation	0–3	2
Language and stylistics, complying with the valid terminology	0–3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0–3	2
Formal requirements - points in total		12
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0–3	0
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0–3	2

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* Choose one

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

Discussion quality - interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	1
Experimental difficulty of the thesis, independence in experimental work	0-3	2
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	1
Practical requirements - points in total		13

POINTS IN TOTAL (MAX/AWARDED)	48	25

Comments of the reviewer on the student and the thesis:

The reviewed bachelor thesis is focused on the validation of the analytical determination of eleven polyols naturally occurring in the insect larvae (*Bombyx mori*) by GC-MS measurement of their silyl-derivatives. The GC-MS measurement of silyl-derivatives is currently the main analytical approach used for determination of mono- and disaccharides and other polyols in biological samples, the topic of reviewed bachelor thesis is therefore highly relevant and useful. However, I have serious concerns about the final quality of the thesis. Even though the use of language and stylistics is on a very good level, it seems that the thesis was written in hurry which strongly decreases its quality.

The introduction is logically divided into three sections. First attempted to describe the GC-MS instrumentation, second introduced the analysed polyols, and third focused on derivatization procedure which was used in the thesis. The first section is rather brief and omits some very important aspects of the GC-MS system such as injector and polarity of the GC column stationary phases, which is of crucial importance for the chromatographic performance. Uncomplete seems to me also the third section. Even though the student briefly described silylation, there is no mentioning of various silylation reagents. There is also no mentioning of the crucial methoximation step which must be conducted before the silylation step (it is very briefly mentioned in the experimental part).

The aims are completely missing in the thesis. Regarding the experimental part, the procedure of sample preparation is nicely described. But again, some important and indispensable details are missing, e.g. what was used as the internal standard, what column type (stationary phase) was used for GC separation, and how were the mass spectral data processed in the Xcalibur software (please include this information in the presentation during defense).

The result and discussion section spans only two pages. Even though it summarizes and briefly discusses the results, the broader discussion of results with literature is missing. The silylation of polyols is widely used technique for various types of biological samples, but the author did not attempt to confront his results with literature neither in terms of analytical procedure (e.g. Are there any other ways for analysing polyols? If yes, what advantages has the technique used in the thesis? Were the observed limits of detection comparable to other works?) nor in terms of polyol content in the experimental samples (e.g. Are the polyol contents similar in other insects?).

In summary, this bachelor thesis deals with a useful and relevant topic. The experimental

established analytical laboratory and the practical knowledge about sample preparation and analyses using the GC-MS instrumentation. Unfortunately, the thesis suffers by serious shortcomings resulting probably from a time-pressure during its writing.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Questions to the student:

Introduction
1) In section 1.2, lines 10-11, the author stated that "… derivatization is commonly used to prevent these non-required properties…" with respect to the inappropriateness of gas chromatography for measuring salts and macromolecules. How derivatization affects this? Could you please explain what did you mean by this statement?

2) Section 1.3.1.1, lines 4-5 – Could you explain what did you mean by the last sentence in this paragraph ("Limitations for these types of ionization are the relative volatility and thermal stability of the analyte")? Which compounds in terms of volatility and thermal stability are more suitable for electron and chemical ionization, respectively?

Experimental design and data evaluation:
3) The standard addition samples were prepared in various concentration ranges, but there is no justification for that. Why did you choose the given concentration ranges?

4) In both derivatization steps the DMF (dimethylformamide) was used. This is rather unusual. Why did you added DMF and why in both derivatization steps?

5) Were the samples prepared in replicates? There is no information about that in section 4, but the Table 4 shows means + SD.

6) It is not clear, how were the mass spectral data processed before the peak integration. It seems that the TIC (total ion current) data were used, but in the attachments are provided masses of 4-7 signature fragments for each compound. One of them is underlined, probably the base peak (the description of what the data means is not provided). What does it mean? Were the data processed based on TIC, on integration of all these fragments or on base peak integration?

7) Some of the examined polyols (fructose, glucose) are in the chromatograms represented by two peaks. How do you explain that? How did you calculate the concentration for these compounds?

8) In section 5.2, lines 6-7, the student mentioned impurities which interfered with fructose peaks. The co-elution is in the complex matrix a usual problem. However, the MS data and certain MS instrumentation offer some possibilities how to overcome this problem. What are these solutions and when could be applied? Could you please elaborate on this?

Results
9) The section 5.3 mentioned blank samples. How were the blanks processed (what type of blanks are you talking about here)?

10) Page 13, line 11. Was it really concentration or rather recovery here?

11) The larger between day accuracy values (page 13, lines 15-16) were explained by accidental mistakes during sample preparation. My impression was that the between-day accuracy was derived from re-measurement of the samples after three days (as given in section 4.5). Could you explain more thoroughly your explanation of higher between-day accuracy due to mistake during sample preparation?

Formal comments and suggestions for the student (no need to read this during the defense):

Methods:
Be careful about your statements - I don´t think that hydrogen, which is explosive when mixed with air, is absolutely "safe to work with" (section 1.2.1).
It is not appropriate to use different concentration units (mass versus molar) within the text.

Results and discussion:
Be careful about the content and don´t include methodological information into the result section (e.g. 1st paragraph in section 5.1). The main result tables are in the attachments (Table 4 - matrix samples Table 5 - validation parameters). Why???
Be careful about you statements - section 1.3.1, line 3 - I really don´t thing that there is something analysed inside the quadrupole - it rather works as a mass filter, the analyses is delivered by the detector behind the filter.

References :
The reference style should be unified (e.g. use of AND before the last author, use of dots in initials - W.A. versus WA etc., use of capitals and abbreviations in the article names and journal names)

Graphic layout:
The use of colors in the text should be uniform throughout the text (the section titles on page 3 are in blue while the rest is black!)

Tables and figures:
Should be understandable without the text (which is not true e.g. for Table 1 - missing units); in Table 2 are just averages of the three measurements??? Means + SD should be shown here.
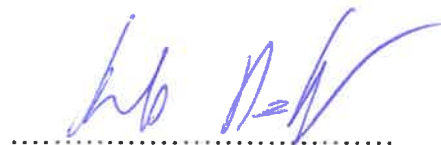
Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade   **Good (3)** .[2]

In   **České Budějovice**   date   **4.6.2018**

....
signature

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[2]   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).