CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Michaela Hříbková**
Thesis Tutor:	PharmDr. Lukáš Lochman, Ph.D.
Consultant:	Larissa Silva Maciel, Ph.D.
Opponent:	RNDr. Ondřej Horáček, Ph.D.
Thesis title:	**Miniaturized solid-phase extraction in analysis of amino compounds by LC-MS/MS**

Scope of work, number of 91 pages, 23 figures, 8 tables, 62 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Excellent
b)	The complexity of the methods used:	Excellent
c)	Preparation of the methodological part (clarity, comprehensibility):	Very good
d)	The quality of the experimental data obtained:	Excellent
e)	Processing of results (clarity):	Excellent
f)	Evaluation of results, including statistical analysis:	Excellent
g)	Discussion of results:	Excellent
h)	Clarity, conciseness, and adequacy of conclusions:	Excellent
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Excellent
l)	Formal level of the work (text structure, graphic design):	Excellent

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

Michaela Hříbková completed her Master's thesis at the University of Tartu in Estonia within the Erasmus+ program, under the supervision of Larissa Silva Maciel, Ph.D., who also served as a consultant for the thesis. The supervisor at the Department of Pharmaceutical Chemistry and Pharmaceutical Analysis was Dr. Lukáš Lochman. The aim of the thesis was to develop a PT-μ-SPE sample preparation method with subsequent derivatization and LC-MS/MS analysis for the identification and quantification of 42 amino compounds in honey. Student carried out a lot of experimental work that will be invaluable in further research and developments of PT-μ-SPE sample preparation method.

In terms of the formal aspects of the work, I observed a minimal number of typographical errors and ambiguous terms. For example, the in-text tables in the experimental section lack proper captions, and the abbreviation FLD is not explained. Regarding terminology, the phrases "…precursor ions with an added proton…" and "…[M+H]+ forms adducts with protons." should be revised to "protonated molecule" and "…forms adducts with atoms or molecules," respectively.

The extensive theoretical section of the thesis is clearly written. The experimental section was comprehensible, except for the preparation of standard solutions. The calibration curve equation is shown only for arginine; in my opinion, the work should include parameters for all calibration curves. Since some analytes were purchased as racemic mixtures and others as pure enantiomers, it would be appropriate to include this information in the chemical structures in Annex 3. The results were clearly presented in tables or graphs and discussed with reference to previously published methods. The references were consistently styled and properly cited, with the exception of a minor error in the DOI of reference 23, which refers to a different publication than intended.

Theses has found 58 similar documents with similarity 4% at most. Turnitin reports an overall similarity of 31%, but the similarity of each document is less than 2%. All the similarities relate to general terms used in this type of theses. Therefore, Michaela Hříbková's Master's thesis is her original work, and I highly recommend it for defense.

Questions and comments to student:

1. In Table 2, the abbreviation HBL is used. What does it stand for?

2. Units of concentration for the stock solution are mg/kg. These solutions are prepared in a mixture of MeOH:H2O with 0.1M HCl. Why did you choose this unit instead of the more suitable mg/mL? Could you give a brief overview of how the standard solutions were prepared?

3. How did you prepare the dispersion of 2g of sorbent in 10 mL of MeOH, and how did you calculate to take 165 µL of dispersion to get 25 mg of sorbent?

4. For quantification, how many replicates were prepared for one calibration level? Is there a disadvantage in using calibration curve with external standards for quantification in honey samples? What other quantification methods could be used to obtain more accurate results?

5. The derivatization agent was prepared in 0.75M borate buffer at pH 9.00. How did you prevent the borate buffer from entering the ion source of the mass spectrometer?

6. The retention times of the peaks in Figure 21 do not match the retention times in the caption. Are some peaks invisible due to low signal intensity, or is the caption under the figure incorrect?

7. In your thesis, you chose derivatization after the SPE procedure. Why? Is there an option to use derivatization prior to the SPE procedure? What sorbent would you use?

Evaluation of the thesis: Excellent		**For the defense: Recommend**
In Hradec Králové	9. září 2024	signature of the opponent