CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of analytical chemistry

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Mykyta Starovoit**
Thesis Tutor:	PharmDr. Juraj Lenčo, Ph.D.
Consultant:	-
Opponent:	doc. PharmDr. Hana Sklenářová, Ph.D.
Thesis title:	**A high-temperature LC-MS method for bottom-up proteomic analyses with reduced artifacts**

Scope of work, number of 62 pages, 17 + 5S figures, 2S tables, 112 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):	Excellent
d)	The quality of the experimental data obtained:	Excellent
e)	Processing of results (clarity):	Very good
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):	Very good

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

The diploma student has prepared detailed survey and proved the trap-elute concept to decrease types and quantity of artifacts produced in the separation column using high temperature and longer gradient elution for bottom-up proteomic analyses.The obtained results are based on quite huge number of experiments and are evaluated with stress to trap-elute set-up compared to direct injection using different retentivity and temperatures in both trap and analytical columns. The text is well written with high level of English and high level of understading to the mentioned topic.

Similarity check showed 7% (Theses) and 27% (Turnitin) but as the English text was found in grate number of theses such high number corresponds to many documents with the highest similarity of 3% and thus problems were not found.

Questions and comments to student:

I have just several comments that do not change the high level of this diploma thesis.

Comments:

Abstract in Czech include translation that is not the best one - vyhledávací jednonástřiková analýza - then I understand that English text is better to describe the selected topic.

Reference formating in the text can be modified, e.g. [10-11] or [14, 41] as it is usual in such theses and manuscripts as well.

Figures S1, S2, and S3 should be included into the text and not to supplement - such supplementary figures are commonly used in manuscripts where number of figures is limited, but in 60 pages text is not convenient to find figures at the end of the document. Additionally, Figure S1 is not cited in the text and I have found it just by mistake.

p. 23 - analytes in Prodigy standard can be mentioned in the Experimental part.

p. 24 - the freezer temperature was -80°C.

p. 27 - you have mentioned separation of peptides instead of the analytes in Prodigy standard mixture - is it true?

p. 42 - Figure 16B - the axes in this figure can be reversed, increase can be over the line and decrease bellow the line - this will be more logic to understand what you try to show

References 22, 23, and 41 from years 1974, 1979, 1963 were accessed as original articles or different way?

Questions:

1. Why did you compare different temperatures for trap-elute and direct injection approaches (35 and 30°C)?

2. You have mentioned two possibilities to elute the injected zone from the trap column - the same direction or opposite. Did you find such application of opposite direction elution in the literature and can you describe what will be expected in such system?

Evaluation of the thesis: Excellent		**For the defense: Recommend**
In Hradec Kralove	17. května 2024	signature of the opponent