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CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRALOVE

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Department: of Pharmaceutical Chemistry and Pharmaceutical Analysis

Master´s degree program in Pharmacy

Opponent´s review of Master´s thesis

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Student´s name: Panagiotis Michailellis

Mentor of the thesis: PharmDr. Petr Kastner, Ph.D.

Opponent of the thesis: Assoc. Prof. PharmDr. Radim Kučera, Ph.D.

Year of the thesis defense: 2022

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Title of the thesis:
HPLC evaluation of tyrosine and its metabolites

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Formal comments: number of pages: 74 number of figures: 18, number of tables: 3, number of references: 142.

Type of work: Literature review

a) The aim of the thesis is: Fulfilled

b) Language and graphic level: Very good

c) Processing of the theory: Very good

d) Methods description: Not commented, Literature review thesis

e) Results description: Not commented, Literature review thesis

f) Discussion and conclusions: Very good

I recommend Diploma thesis for the recognition as Rigorous thesis ☐.

Opponent´s comments: The diploma thesis by Panagiotis Michailellis deals with the literature review concerning the separation methods used for the catecholamine determination in different biological matrices. The student reviewed 117 scientific papers and summarized the data concerning sample preparation, separation conditions, and detection techniques. The theoretical aspects of sample preparation, separation, validation, and detection techniques connected to the analysis of catecholamines are also included. I would expect more information sources in this part. On the other hand, I appreciate very much the number of articles concerning catecholamines analysis, that the student has gone through and proceeded. The thesis is written relatively clearly without grammatical mistakes. However, some inaccuracies in terminology and incorrect conclusions were made.

Remarks and comments: p.12 - dopamine-decarboxylase is not the proper enzyme; p. 15, I prefer to put references also in the legend of the figure, not only in the text; p. 15 "pure biological material" is a confusing term; p. 17 – abstract terms are used, e.g., "low pH value for acids"; Fig. 3 is incomplete; Fig. 5 is mentioned before Fig. 4; p. 20. "Drug free real sample" is not commonly used; p. 23, HPLC factors, this term is not used; p. 24 – description of peak symmetry, the word line is missing; p. 28 "analytes absorb UV light (in UV or visible

region)"; p. 32, first sentence is confusing and inaccurate; p. 56 XAD is not defined; p. 59 – the term "lowest sensitivity" is confusing; p. 62 – I am missing the pH values of separation buffers as well as the references to the articles

Concerning the comments and conclusions, which are made in the theses, as a reader, I would appreciate the references to the statements to be able to find further details.

Questions: p. 16, 17 - can you name other precipitation agents? What did you mean by "LLE is more traditional sample preparation technique"? Can you explain the role of the pH adjustment in LLE?

p. 19, chapter. 3.2.4 – can you explain the first sentence?

p. 20 - is normal phase chromatography suitable for the separation of highly polar compounds? Is porous graphite suitable stationary phase for separation in normal phase chromatography?

p. 22 - which part of the chromatograph is missing?

p. 25 - which compounds are suitable for separation in CZE? Is the temperature control important or not in CZE? Is it possible to separate compounds in the direction from cathode to anode? What have you meant by "coated and uncoated capillary"?

p. 32 - can you explain this statement "ESI is more common than APCI as degradation is unusual"?

p. 34 – can you specify the peak height at LOQ in comparison to baseline noise?

p. 35 - is there a connection among conditions stated in tables 1, 2 and 3? Some references are mentioned only in some tables.

p. 58, chapter 5.1.3 - please explain the first two sentences. Is the choice of the extraction technique predetermined by the separation technique?

p. 59 – please explain this sentence: "Gradient elution in HPLC offered the ability to perform fast analysis in less than 8 minutes, with average run times among 10 to 15 minutes."

p.65 – can you explain this sentence: „An important factor is also the possibility of detecting isolated compounds."? What is the main disadvantage of CE? Is it really the high voltage? Why do you think is the high temperature dangerous in the GC?

p. 66 – why is LLE suitable in the case of GC in comparison with the other two techniques? I do not think that argon was used as a carrier gas.

p. 67 – can you characterize the micellar compounds which are analyzed by GC-MS?

Turnitin - the similarity with other documents 98 %. The similarity is seemingly high. This is due to a technical problem. The document was uploaded and checked before the submission and then it could not be possible to remove it from the system. Theses rated the overall similarity as 24 % and found a total of 36 similar documents. The largest agreement was rated by Theses as 4 %. From this point of view, the agreement can be evaluated as insignificant.

Despite the above-mentioned flaws, the diploma thesis is at an appropriate level and I can recommend it for defense.

Evaluation of Master´s thesis: Very good

Recommendations for the thesis defense: Recommended

In Hradec Kralove 29. 5. 2022   …………………………………
                                                                        Opponent´s signature