

REVIEW

by prof. Maya Georgieva, PhD,

Department „Pharmaceutical chemistry“, Faculty of Pharmacy, Medical University – Sofia, designated as a member of a scientific jury on the basis of Art. 4, paras 1 and 2, ZRASRB and according to the Order № P-109-546/05.12.2023 of the Rector of the MU - Varna.

On a PhD thesis with topic: "New Aspects in the Pharmaceutical Analysis of Quinine and Some of Its Oxidation Products"

To acquire the scientific and educational degree "**doctor**" in

Area of higher education 7. *Health care and sports,*

Professional direction: 7.3. *Pharmacy*

Doctoral program: *Pharmaceutical Chemistry*

Author: master of pharmacy Ivalina Valerieva Vasileva, full-time doctoral student in the "**Pharmaceutical Chemistry**" doctoral program, professional direction 7.3. Pharmacy, enrolled by order No. P-109-391/09.10.2020 to the Department of Pharmaceutical Chemistry at the Faculty of Pharmacy, MU - Varna.

Scientific supervisors: assoc. prof. Svetlana Fotkova Georgieva, PhD.
assoc. prof. Ilian Nikolov Kolev, PhD.

I. General presentation of the procedure

The presented set of materials on paper and electronic media is in accordance with Art. 69 of the Regulations for the Development of the Academic Staff at the MU - Varna dated 21.11.2022 and includes the following documents:

- ✓ Dissertation work
- ✓ Abstract in Bulgarian and English
- ✓ Application to the Rector for the disclosure of a protection procedure;
- ✓ Autobiography signed by the doctoral student;
- ✓ Copy of a diploma for a completed higher education educational-qualification degree OKS "Master" with its annex;
- ✓ Copy of a diploma for a recognized specialty in Analysis of Liquor Products;
- ✓ Enrollment order;
- ✓ Minutes of an examination for the doctoral minimum;
- ✓ Minutes of a language test
- ✓ Minutes from the SC with a positive decision on the readiness for protection;

- ✓ Deduction order with right of defense;
- ✓ Declaration of originality;
- ✓ List of publications related to the topic of the dissertation;
- ✓ Copy of the publications related to the topic of the dissertation work;
- ✓ Declaration of authenticity of the submitted documents;
- ✓ Declaration for registration of profiles in scientific databases;
- ✓ Reference for similarity;
- ✓ Reference for an up-to-date scientific profile in Google Scholar and Orcid.
- ✓ Order to change the subject.

II. General presentation of the PhD student

In a short autobiographical form, Ivalina Valerieva Vasileva has provided the following information regarding the education she received so far, where she described that she graduated from the specialty "assistant pharmacist" at the Medical College of the Medical University - Varna and obtained a master's degree in "pharmacy" from the same University. In 2021, she acquired a specialty in "Analysis of Drugs" at the Medical University - Sofia. Since 2020, she has been enrolled as a full-time PhD student at the Department of Pharmaceutical Chemistry of the Faculty of Pharmacy at the University of Varna with Order No. R-109-391/09.10.2020 in the field of Higher Education "7. Health care and sports", professional direction: "7.3. Pharmacy", doctoral program: "Pharmaceutical Chemistry" with scientific supervisors assoc. prof. Svetlana Georgieva, PhD and assoc. prof. Iliyan Nikolov Kolev, PhD and Topic: "Analysis of some natural quinoline and pyridine toxins". By Order No. P-109-362/21.09.2022, the topic of Ivalina Valerieva Vasileva's dissertation was changed to "New aspects in the pharmaceutical analysis of Quinine and some of its oxidation products". By Order No. R-109-545/05.12.2023. is charged with the right of defence.

The brief form of presentation of the CV does not allow me to assess and analyze the working abilities of the PhD student and evaluate in detail her career development to date.

III. Dissertation structure

The presented dissertation is written on 94 pages, of which 1 page introduction, 28 pages theoretical part, 1 page goals and tasks, 10 pages experimental part, 38 pages results and discussion, 1 page conclusions, 1 page contributions, 1 page list of publications, related to the dissertation, 12 pages of references. The work includes 4 tables, 49 diagrams and 24 figures.

Spelling and stylistic errors are hardly noticeable.

IV. Dissertability of the PhD thesis and analysis.

The dissertation explores various aspects of the qualitative analysis of the alkaloids quinine and quinotoxin - representatives of the general alkaloid set of cortex *Chinae*, where the literary data shows the presence of various derivatives thereof with completely different behavior and activity. This determines the importance of knowing every aspect of the analysis of these alkaloids, which will contribute to the knowledge of the analysis of this class of bioorganic compounds. In the current work, the PhD student presents adapted analytical methods, bearing their fundamental innovation and combining various interdisciplinary motives from the field of physics, photochemistry, fine organic synthesis. In this sense, the thesis may be considered dissertationable.

The theoretical part of a total of 28 pages, is based on 159 literary sources in Latin, of which 18 are from the last 10 years and 10 from the last 5. The theoretical part shows the awareness of the PhD student on the problem being developed. The detailed exploration of methods for obtaining quinine and its derivatives is impressive, as well as the classic qualitative methods for proving

quinine, quinotoxin and their compounds presented with understanding. Here I would like to point out the noticeable lack of citations for some of the classic qualitative reactions mentioned.

In general, the theoretical part shows a good knowledge by the PhD student of the physical, physicochemical and acid-base properties of both isolates from the general alkaloid set of cortex *Chinae*, as well as their salts and crystal hydrates.

Despite its spatiality and detail, the literature review thus presented does not provide enough information about the need to develop new qualitative methods for the analysis of the studied alkaloids quinine and quinotoxin, especially taking into account the fact that for *Quinine* and its salts *Quinine sulfate*, *Quinine hydrochloride*, as well as for *Quinidine* and *Quinidine sulfate* there are available corresponding analytical procedures described in the European Pharmacopoeia. Moreover, these molecules are used in modern practice relatively rarely, as evidenced by the fact that the main part of the literature data is from a period more than 10 years ago.

From this point of view, I have the following **recommendation** for the PhD student: At the end of the theoretical part, she should present a critical analysis of the available literature on the topic aimed at highlighting the need for the development and adaptation of new methods for qualitative analysis of the selected molecules.

The purpose of the dissertation, in its essence is correctly set. The tasks related to its implementation are 5 in number and are formulated precisely and in a logical sequence.

The experimental part related to the developed theme is detailed and well described. The manner of presentation of the methodology does not raise doubts and is a prerequisite for obtaining the correct results discussed below.

The "**Results and Discussion**" section describes the obtained experimental results in detail, simultaneously presenting their critical discussion. In its main part, it is divided into the following analytical developments:

1. S_8 in qualitative quinine analysis, where the PhD student describes a simple analytical qualitative technique for determining the presence of a secondary alcohol group in the composition of the medicinal enantiomeric pair – (-)-quinine and (+)-quinidine. The analysis is based on the oxidation potential of molten elemental sulfur (S_8) and the reactivity of separated H_2S towards $Pb(OAc)_2$. Thus, the proposed methodology represents a modification of Feigl's method aimed at expanding the potential of this approach, and with the proposed modification, a reduction in the time to reach the maximum level of analytical perception is achieved. In the course of the discussion, the PhD student also mentions some possible disadvantages of the proposed procedure, such as depositing the analyte outside the reaction medium, which will drastically reduce the analytical sensitivity as a value, as well as taking special precautions when evaporating the used solvent (acetone) in order to avoid localized overheating of the samples, as otherwise significant amounts of them may be forced out of the capillary volume.

Despite these observed negatives, the PhD student defines the presented analytical strategy as suitable for establishing the presence of secondary hydroxyl groups in the composition of the studied samples. As the established accuracy and reproducibility of this approach makes it fully applicable and suitable for the routine analysis of both alkaloids.

2. The quinotoxin applied as a precursor for the synthesis of "methylene blue" dye. The described methodology is aimed at developing a suitable analytical method for identifying the presence of a piperidine quinotoxin residue in the structure of a synthetically produced quinotoxin. The purpose of the present study is to establish the applicability of this test in the analysis of the alkaloid quinotoxin - a compound containing a secondary amino group in its piperidine residue, which will enable the detection of the presence of this product in a mixture with quinine. The presented test can be considered as a first attempt for the analysis of the piperidine quinotoxin

residue. Considering that other available methods target the quinoline residue that is common to this class of alkaloids, the presented test has the potential to be of choice to realize the intended purpose.

3. Photoinitiated synthesis of herapatite.

Despite the well-defined applicability of the quinine-herapatite test for qualitative analysis of quinine in medicinal form, from the critical analysis presented by the PhD student of her proposed modification, the reason for its implementation remains unclear to me. Even more so after the introduced change, in the words of the PhD student, the result "is realized smoothly, within a relatively short period of time (one hour)". The idea of using this approach for academic purposes is undeniably interesting, but at its core without the necessary justification it sounds self-serving.

4. Chemiluminescent quinine assay.

The reason for defining this development as a task in the present work is puzzling to me, due to the fact that in the method mentioned, quinine plays the role of a sensitizer only, but not an object of analysis, as the given topic and the resulting goal suggest. Of course, this does not diminish the importance of the work done on this task and the results obtained from it.

5. Sorption behavior of quinine-imprinted quartz crystal microbalance towards (+)- and (-)-carvone.

Combining the elements of the so-called sensing techniques, the PhD student successfully created a quinine-imprinted polymer layer on the surface of a QCM wafer (QI-QCM; quinine-imprinted QCM), introducing the use of the two carvone enantiomers – R-(+)-carvone and S-(-)-carvone as chiral probes based on the chirality of the quinine molecule. The obtained results confirm the ability of the created polymer layer to account for the subtle spatial/configurational differences in the two carvone isomers. The achievement can also be used as an additional indication of the successful preparation of a quinine imprint in the thus deposited polymer layer on a QCM surface. The obtained results are encouraging in the direction of applying this approach to other drug molecules, but in a liquid layer.

The conclusions (5 in number) correspond to the set tasks and well reflect and summarize the results of the conducted research.

V. Publications related to the dissertation work

The doctoral student has attached 2 (two) scientific papers related to the topic of the developed dissertation work, of which 1 (one) in a scientific periodical referenced and indexed in world-famous databases with scientific information and 1 (one) in a non-refereed edition with scientific review. In both publications M.Sc. Pharm. Vasileva is the lead author. The absence of one of the scientific supervisors as a co-author of the scientific publications accompanying the dissertation work is puzzling. There is no data on presentation of parts of the dissertation work at national and international scientific forums.

With regard to these scientometric indicators, the dissertation student meets the requirements for awarding the educational and scientific degree "Doctor", laid down in the Rules of the MU - Varna.

VI. Abstract

The accompanying **Abstract** has a total volume of 37 pages, accurately and sufficiently reflects the content of the dissertation work and is made according to the requirements.

VII. Critical notes and questions.

In summary, the work presented to me for review is interesting and concerns a modern topic from a theoretical point of view. Despite the many techniques and methods mastered by the doctoral student, the applicability of the developed analytical approaches in practice remains questionable for me. As a main critical note, I would emphasize the theoretical nature of the set goal in general.

I have the following questions for the PhD student:

1. What is the main reason for the need to develop methods for the qualitative analysis of quinotoxin, given its withdrawal, and what is the applicability of the modified methods for the analysis of this molecule?

2. How are the proposed modifications superior to the available and currently applied in practice qualitative methods for the analysis of quinine?

3. If she can indicate in more detail the new aspects in the analysis of quinine and products of its oxidation?

CONCLUSION

The dissertation mainly contains scientific and theoretical results, which in terms of content and essence meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Rules for the Implementation of ZRASRB and the Regulations of the Medical University - Varna. The presented materials and dissertation results correspond to the specific requirements adopted in connection with the Regulations of the Medical University - Varna for the application of the ZRASRB.

The dissertation shows that the doctoral student M.Sc. Pharm. Ivalina Valerieva Vasileva has acquired a diverse set of theoretical knowledge and skills for independent conduct of scientific research.

In view of the above, I consider the dissertation work submitted to me for review with topic: "New aspects in the pharmaceutical analysis of Quinine and some of its oxidation products" to be diserable and I suggest to the respected members of the Scientific Jury to vote positively for the awarding of the educational and scientific degree "doctor" by professional direction: 7.3. Pharmacy; doctoral program: Pharmaceutical Chemistry of M.Sc. Pharm. Ivalina Valerieva Vasileva.

Sofia

30 January 2024

Reviewer:

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

(prof. M. Georgieva, PhD)