REVIEW

from prof. Plamen Peikov, PhD

for the acquisition of the educational and scientific degree "Doctor" of MPharm Ivalina Valerieva Vassileva, PhD student in full-time study, doctorate program "Pharmaceutical Chemistry", field of higher education 7. "Health and Sport" and professional field 7.3. "Pharmacy", "New aspects in pharmaceutical analysis of Quinine and some of its oxidation products", Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University (MU) – Varna.

MPharm Ivalina Vassileva was born in 1982. She obtained the educational and qualification degree "Master Pharmacist" at the Faculty of Pharmacy, Medical University - Varna in 2015. In 2021, after successfully passing the exam, she acquired a specialization in "Analysis of medicinal products", Medical University-Sofia. From 2020, MPharm Vassileva is on a contract of employment at MU-Varna and is enrolled as a PhD student in full-time studies.

The dissertation thesis contains 94 pages, 4 tables, 41 figures and 48 diagrams. 159 literature sources are cited.

The structure of the work includes pharmaceutical analysis and pharmaco-analytical characterization of the alkaloids quinine ((R)-[(2S,4S,5R)-5-ethenyl1-azabicyclo[2. 2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol) and quinotoxin (3-[(3R,4R)-3-ethenylpiperidin-4-yl]-1-(6-methoxyquinolin-4-yl)propan-1-one). According to the European Pharmacopoeia, the therapeutic use of quinine is for the treatment of malaria and is used as salts: sulphate and hydrochloride. The S-isomer of quinine is quinidine, a medicinal product with antiarrhythmic activity, which is not used anymore. Quinotoxin, back in time was used as a vasodilator and with some antiplatelet activity. The topic is trendy, useful and dissertationable. It focuses on quinoline chemistry, basic structure of drug molecules and compounds with biological activity. The main hypothesis and aim of the study are to develop analytical methods for qualitative characterization of quinine and quinotoxin alkaloids and analysis of the corresponding analytical characteristics.

The literature review substantiates the aim and objectives of the doctoral thesis, which are set very precisely.

The PhD thesis focuses on the secondary alcohol functional group in the structure of quinine and quinidine. A simplified analytical approach is applied to determine this functional group. The analysis is based on the oxidation potential of molten sulfur (S_8) and the reactivity of the resulting H_2S towards $Pb(OAc)_2$. A methodology was also developed to estimate the limit of detection (LOD) of the studied alkaloids (~0.006 mg). The development of this analytical method has scientific and applied contributions in the determination of hydroxyl groups. The method is accurate, reproducible, and accessible, and no harsh reagents are used.

An analogous contribution is the qualitative analysis of the quinotoxin with respect to the piperidine fragment in its structure. The approach is described in the literature - secondary amine analysis. This approach has undergone literature development - analysis of cyclic amines but has not been applied to the characterization of natural compounds. The applicability of an analytical test in the qualitative analysis of the alkaloid quinotoxin, a compound containing a secondary amino group in the piperidine fragment of the molecule, has been established. The structure of the studied alkaloid was confirmed by spectral methods: infrared- and ultraviolet-visible spectroscopy (UV-Vis). The analytical response was recorded immediately with the introduction of the quinotoxin to a bromophenothiazine solution. The limit of detection (LOD) of the test is ~0.0075 mg. A reaction pathway for this *spot-test* reaction has been proposed. What is original about this approach is the analysis of the piperidine quinotoxin fragment rather than targeting the quinoline component of the molecule.

The Herapathite test developed by Herapath for quinine has been described in the literature. This test identifies quinine sulfate in tablet dosage forms. Herapathite has a capillary crystal form in which its iodine atoms are oriented in the direction of the major axis of the capillary crystal form. The reagents classically used are acetic acid, 95% alcohol, 10% sulfuric acid solution and 10% alcoholic solution of iodine. The PhD student optimized this classical test by applying potassium iodide instead of iodine. A sufficient number of experiments were carried out varying the ratios of the reagents: sulphuric acid; water; potassium iodide; acetic acid and ethanol. The amount of quinine sulphate analyzed was constant. When the samples were irradiated with sunlight, the amount of herapathite obtained in the samples increased and the size of the crystals increased. The quantitative significance of the individual reagents has been very successfully analyzed. By optimizing this test, the analytical result is achieved smoothly in one

hour and the procedure is easily feasible. This work is definitely an applied scientific contribution in the field of qualitative analysis.

Chemiluminescence is the phenomenon in which matter emits light of a certain wavelength without giving off heat and returns to a basically excited state after having absorbed external energy from an electromagnetic wave, heat, friction, electric field, or chemical reaction. The focus in this research on the reduction of Se(IV) to Se(III) ions and the release of electromagnetic radiation is of contributory importance. It is a chemiluminescent system for the analysis of drug molecules in dosage forms. The source of Se(IV) is $(NH_4)_8[Ce_2(SO_4)_8]$-$4H_2O$ (CAS), given its reactivity and considering the structure of quinine as a sensitizer. The specific study is focused on the ability of the alkaloid to interact with the oxidant. After n number of experiments were performed, the metal ions from the metal injection needle were found to be introduced into the analyzed solution with the introduced quinine solution. Their catalytic action is experimentally proved. A scheme for the oxidation of quinine with CAS is proposed. A photoemission spectrum reflecting the interaction of 4-aminophenol with CAS in the presence of quinine is presented. Thus, the possibility of this method, specifically for the proof of 4-aminophenol, as a potential impurity in the synthesis of paracetamol is demonstrated.

The PhD student directed the research towards the quartz crystal microbalance (QCM/Quartz Crystal Microbalance) technique by implementing a quinine-imprinted polymer layer on the QCM wafer surface (QI-QCM/quinine-imprinted QCM). The chirality of the quinine molecule provides a clue to the chirality of its molecular imprints in the polymer layer. The analysis was performed with two carvone enantiomers: R-carvone and S-carvone. The larger adsorption potential of the QI-QCM surface is towards the (S)-enantiomer. The sorption capacity of this layer exceeds approximately 7 to 10 times that of the (R)-enantiomer. The developed polymer layer can register the subtle spatial/configurational differences in the two carvone enantiomers, further demonstrating the quinine fingerprint of the polymer layer on the QCM surface. This work is potentially applied in nature.

There are some inaccuracies and omissions in the doctoral thesis. Its structuring is a bit messy. There is a mixing of the results and discussion with the experiment and review elements. The results in the area of analysis are not fully validated according to the International

Conference on Harmonization`(ICH). There is a lack of uniformity in the spelling of the literature. Inappropriate terms and words are used in the text. These observations do not reduce the amount of work done by the PhD student.

Scientific research work

The PhD thesis fully covers the PhD program in "Pharmaceutical Chemistry" and meets the requirements of the Regulations for the Development of Academic Staff at MU-Varna and the Minimum Science Metric Requirements of MU-Varna (Indicator D, 7. Publications and reports published in scientific journals, refereed and indexed in world-known databases of scientific information and 8. Publications and reports published in non-refereed peer-reviewed journals or published in edited collective volumes). Scientific publications related to the PhD work are two, in Bulgarian Chemical Communications and Scripta Scientifica Pharmaceutica.

The Abstract book completely covers the thesis.

Conclusion

This is serious scientific research in the field of pharmaco-analytical characterization of drug molecules. MPharm Ivalina Valeriyeva Vassileva has mastered and developed various methodologies in the field of pharmaceutical analysis. The scientific hypothesis and the aim of the doctoral thesis have been fulfilled, the experiment is sufficient in scope, the conclusions are accurate, and the contributions are mainly of scientific and applied nature. The nature of the scientific contributions is the enrichment of existing knowledge and the potential application of these scientific advances in practice.

I propose to award the educational and scientific degree "Doctor" to MPharm Ivalina Valeriyeva Vassileva.

My assessment is convincingly POSITIVE.

10.01.2024

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

(prof. Peikov, PhD)