REVIEW

by prof. Alexander Borisov Zlatkov, DSci, PhD,

lecturer at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University – Sofia, appointed as a member of the scientific jury on the basis of Art. 4, para 1 and 2, ZRASRB, Decision of the Faculty Council of the Faculty of Pharmacy at MU – Plovdiv and Order No. P858 / 29.10.2024 of the Vice-Rector for Research and Development of Medical University – Plovdiv

of a dissertation for the award of the educational and scientific degree '**doctor**'

Higher education area *7. Healthcare and sports.*
professional field *7.3. Pharmacy.*
doctoral program *Pharmaceutical chemistry.*

Author: MS.-Pharm. Vanya Rangelov Kozhuharov

Form of doctoral studies: regular

Department: Pharmacognosy and Pharmaceutical chemistry

Topic: " **Modern guidelines and pharmacoanalytical approaches in doping prevention** "

Scientific advisor: Assoc. Prof. Kalin Valentinov Ivanov, PhD, Department of Pharmacognosy and Pharmaceutical Chemistry.

1. General presentation of the procedure and the doctoral student

The presented set of materials on paper / electronic media is in accordance with Art. 70 (1) of Section I. Acquisition of the educational and scientific degree "DOCTOR" and the scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv dated 28.01.2021 and includes the following documents:

- – Application to the Rector of MU-Plovdiv for opening the procedure for defending a dissertation
- – CV in European format with the signature of the doctoral student
- – Notarized copy of a higher education diploma
- – Orders for enrollment in doctoral studies, interruption of studies (due to maternity) and for continuation of studies; for withdrawal with the right to defend
- – Order for conducting an exam from the individual plan and a corresponding protocol for a passed exam or doctoral minimum in the specialty

– minutes of the department council for preliminary discussion of the doctoral thesis and the decisions made to open a procedure and to form a scientific jury
– dissertation
– abstract
– list of scientific publications on the topic of the dissertation
– copies of scientific publications
– list of participation in scientific forums
– list of noted citations
– declaration of originality and authenticity of the attached documents
– other documents related to the course of the procedure

The doctoral student has submitted 3 (three) scientific publications, of which 2 (two) in a journal with IF and 1 (one) in a refereed scientific publication, all related to the topic of the developed dissertation work..

I have no notes or comments on the documents.

2. Brief biographical data about the doctoral student

Vanya Rangelov Kozhuharov was born on 16.04.1990 in Pirot, Serbia. She obtained a Master's degree in Pharmacy at the Medical University of Plovdiv, 2015. In 2017, she joined the Department of Pharmacognosy and Pharmaceutical Chemistry. She was enrolled as a full-time doctoral student in the doctoral program "Pharmaceutical Chemistry" on 14.01.2021 with order No. P 58/14.01.2021. She was discharged with the right to defense with order No. P-2312/05.07.2024.

3. Relevance of the topic and appropriateness of the set goals and objectives

In recent years, unintentional doping has become a seriously discussed social and scientific problem. Numerous cases of unintentional doping have been registered due to the intake of food supplements containing undeclared prohibited substances. On the other hand, the use of food supplements is considered an indispensable element of the diet of professional and amateur athletes, and in recent years there has been a trend towards higher levels of intake of such products given the numerous benefits of their intake, such as shortening the recovery time between training sessions and a number of other.

Unfortunately, in recent years, the lack of strict rules and regulations regarding this type of product has led to the presence of food supplements contaminated with undeclared pharmacologically active substances, which is a huge problem not only for active athletes. In this sense, the development of doping prevention strategies is considered a current problem of urgent nature not only from an ethical point of view, but also with a view to preserving the health of athletes and active sports people of all ages.

One of the main elements of these strategies includes monitoring the quality of nutritional supplements in order to raise awareness among athletes and their teams about the "hidden" risks of unintentional doping.

The present work investigates analytical control and the creation of screening methods for detecting substances included in the doping lists, which makes it **relevant** with the possibility of deriving guidelines for optimizing the control of nutritional supplements used by active athletes.

4. Knowing the problem

The presented scientific work, as well as the scientific publications related to it, show the good awareness of the doctoral candidate on the problem she is developing. The presentation and the literature used show that the doctoral candidate has become familiar with a significant number of literary sources related to the nature of the scientific work.

5. Research methodology

The methodological set covers practically the full spectrum of instrumental analytical methods for the study of biologically active substances. The selected set of methods is adequate for the implementation of the tasks set out in the research program of the dissertation. The experimental setups do not raise any doubts and are a prerequisite for obtaining the discussed correct results.

6. Characteristics and evaluation of the dissertation work

The presented dissertation is written on 159 pages, of which 1 page is an introduction, 29 pages are a literature review, 1 page is a scientific hypothesis, goals and objectives, 13 pages are materials and methods, 65 pages are results and discussion, 3 pages are conclusions and future directions, 1 page is conclusions, 2 pages are contributions, 1 page is a list of scientific publications and participation in scientific forums in connection with the dissertation, 23 pages are literature, Appendices - 14 pages. The work includes 22 tables and 48 figures, as well as 3 pages of abbreviations used. The bibliography includes 249 literary sources, one of which is in Cyrillic. The bibliography contains 128 citations published in the last 10 years and 62 citations published in the last 5 years. There are also 15 citations on sites, such as the official website of the World Anti-Doping Agency, a list of banned substances, which has also been updated in the last 5 years.

The **goals** of the dissertation work – 2 in number – arise completely logically from the literature review. To achieve them, 8 **specific tasks** have been identified, formulated precisely and in a logical sequence.

Based on data from 50 studies that analyzed the purity of food additives and the % of undeclared substances, the doctoral candidate identified 875 cases out of 3132 studied additives of registering substances included in the WADA list of prohibited substances. A critical analysis of the type and quantities of registered prohibited substances was also made. This allows the research to be focused on creating:

1. HPTLC sibutramine screening method.
2. GC-MS sibutramine screening method
3. GC-MS anabolic steroids screening method
3. HPLC- UV anabolic steroids screening method
4. HPLC- UV diuretics screening method
5. LC-PDA higenamine screening method

All analyses were performed after proving the suitability of the system (system suitability test) to the respective analytical method. Analytical and chromatographic parameters were investigated, namely precision, specificity, linearity separately for quantification (for the interval 80-120%) and for determination of impurities (up to 120%), accuracy, limit of quantification for methods for determination of impurities, retention time and symmetry factor. The requirements of ICH and European Pharmacopoeia were met. Once their reliability has been determined, all chromatographic procedures can be used in comparative tests for quantification, purity and identity and the results obtained can be evaluated as a trend and statistical significance.

In addition, the doctoral candidate has developed and tested a questionnaire for health professionals regarding their readiness to conduct doping prevention campaigns. The target group of the study includes medical specialists (doctors and master pharmacists) and pharmacy and medicine students (III – VI year of study). The participants of the survey were recruited among pharmacy and medicine students studying at MU-Plovdiv.

The analysis of the results obtained from the survey shows the important role of medical professionals (including pharmacists) in educating athletes and the general public about the potential dangers of unintentional doping and the importance of making informed decisions. It is also concluded that training aimed at medical professionals would have a positive role in the fight against doping.

The **conclusions** (4 in number) are adequate and correctly reflect the results of the research conducted.

7. Contributions and significance of the development for science and practice

The contributions made in the dissertation work under development can be systematized as follows:

1. The pharmacologically active substances most often found to be contaminated with food additives (sibutramine, anabolic steroids and diuretics) have been systematized and described.

2. An effective comprehensive strategy for the prevention of unintentional doping is proposed, through different approaches that can be practically applied in different laboratories. Key points in this strategy for the prevention of unintentional doping are the monitoring of the quality of FS before they are included in the diet of professional athletes and the support of athletes by accessible health professionals. At present, it is believed that the main cause of unintentional doping is contaminated food supplements. Another cause of unintentional doping can be considered plant/extracts and FS, which naturally contain prohibited substances such as higenamine and methylhexamine. In order to prevent such cases, constant training of professional athletes on the risks of doping and its prevention is necessary. The study conducted within the framework of the dissertation emphasizes that master pharmacists could effectively support professional athletes with their competencies and could be effectively included in programs aimed at doping prevention.

3. An innovative analytical method based on HPTLC for the detection of sibutramine has been developed, which is characterized by high sensitivity and allows simultaneous monitoring of over 20 samples. This method can also be applied to HPTLC systems equipped with autosamplers, and then simultaneous analysis of 72 samples can be achieved. The method is characterized not only by high precision, accuracy and speed, but also by easy sample preparation.

4. A rapid and sensitive GC-MS method with high selectivity has been developed for monitoring sibutramine in FS.

5. A sensitive HPLC-UV-based method for the detection of diuretics has been developed, which can be successfully applied to the quality monitoring of FS and DP.

6. For the first time, a rapid and highly sensitive LC-PDA method for the analysis of higenamine has been developed, which can be successfully applied for the identification and quantification of higenamine in both plant extracts and FS.

8. Assessment of dissertation publications

In connection with the dissertation work, 3 publications have been attached, in which the doctoral student is the first author of each of them. Two of the publications are in journals with an impact factor and one in a refereed journal. The total number of citations in Scopus is 45, of which one article has been cited 41 times.

Parts of the dissertation research have been presented at two scientific conferences – 1 national and 1 international. In connection with the development of the dissertation work, an intra-university project (No. 11/2022) has been developed

on the topic: "Modern guidelines and pharmacoanalytical approaches in doping prevention", with the supervisor Assoc. Prof. Kalin Ivanov, Ph.D.; and the lead researcher: doctoral student Vanya Kozhuharov.

The total number of points for the doctoral student is 47 points, which covers the minimum national requirements for group of indicators D of the Regulations for the Implementation of the ZRASB.

9. Personal participation of the doctoral student

The presented scientific work, as well as the scientific publications related to it, clearly demonstrate the personal participation of the doctoral student in the research conducted, with the formulated contributions and obtained results being largely her personal merit.

10. Abstract

The abstract (total volume 57 pages) is prepared according to the requirements and accurately and sufficiently reflects the content of the dissertation work.

11. Critical remarks and recommendations

I have no critical remarks or recommendations.

12. Personal impressions

I have no personal impressions.

13. Recommendations for future use of the dissertation contributions and results

There is an obvious need for urgent changes in legalization to ensure safe and quality products. Preliminary market regulations for safety and efficacy are needed. However, this is a long and slow process that cannot be implemented simultaneously on a global scale, but at the national level the contributions of the dissertation work under consideration can be applied to prepare recommendations for the introduction of analytical control of food supplements (carried out only in accredited laboratories) before they are included in the regimen of professional athletes.

CONCLUSION

The dissertation work submitted to me for review contains *scientific, applied and applied results that represent an original contribution to science* and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the

relevant Regulations of MU - Plovdiv. The submitted materials and dissertation results fully comply with the specific requirements of MU - Plovdiv.

The dissertation shows that the doctoral student, MS. (Pharm.), Vanya Kozhuharov, possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Pharmaceutical Chemistry, demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my *positive assessment* of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results and contributions, and I *propose to the esteemed scientific jury to award the educational and scientific degree of 'doctor'* to the full-time doctoral student, MS. (Pharm.), Vanya Rangelov Kozhuharov in the doctoral program in Pharmaceutical Chemistry..

15 November 2024.

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(prof. Al. Zlatkov, DSci, PhD)