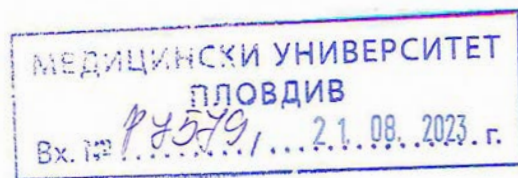


STATEMENT



By Assoc. Prof. Yordanka Ivanova Uzunova, PhD

Department of Bioorganic Chemistry, Faculty of Pharmacy, Medical University - Plovdiv

Subject: dissertation on the topic: **Determination of oxidative stress using liquid chromatography with mass spectrometric detection** for awarding the educational and scientific degree "**Doctor**"

Field of higher education: 7. Health care and sports

Professional field: 7.1 Medicine

Doctoral program: "Medical Biology"

With author Dr. Desislav Grozev Tomov

Form of doctoral studies: Independent study

Department of Medical Biology, Faculty of Medicine, Medical University-Plovdiv

Scientific supervisor: Prof. Dr. Dobrin Svinarov, MD

This statement was prepared pursuant to order P-2051/18.07.2023 of the Rector of the Medical University -Plovdiv and is in accordance with The Development of Academic Staff in the Republic of Bulgaria Act, the Rules for the Application of The Development of Academic Staff Act and the Rules of the Medical University-Plovdiv. under the defense procedure, Dr. Desislav Tomov has provided all the necessary materials.

1. Biography of the PhD student

Dr. Desislav Tomov was born on 12th March, 1975 in the city of Plovdiv. He graduated from secondary education in the city of Plovdiv in 1992, and a master's degree in medicine in 1999 at the Sofia Medical University. In 2012, he graduated with a master's degree in public health and health management and obtained a Clinical Laboratory specialty at the Medical University of Plovdiv. In the period 2018-2023, he increased his qualifications in the field of chromatographic analysis and the validation of analytical liquid chromatographic methods with mass detection and evaluation of the uncertainty of the methods, biochemistry and the human microbiome by participating in post-graduate studies and courses at Paisii Hilendarski University of Plovdiv and University of Tartu, Estonia, Massachusetts Institute of Technology, USA and Wageningen University & Research, Netherlands. After graduating, he worked as a resident doctor at Diagnostic consultative Center 5 and Cytomedika OOD. As a medical representative, he worked for Nestlé Bulgaria AD and Hygia AD. From 2004 to 2006, he worked in the Central Clinical Laboratory of the St. George University Hospital - Plovdiv. In the period 2006-2018, he performed management activities at Billboard AD and was a member of the Board of Directors. From 2013 to 2019, he worked as a doctor in clinical laboratories Chronolab, Synevo and Bodimed. From 2015 to 2019, he was the head of the laboratory for chromatographic mass spectrometric analysis at the Technological Center for Emergency Medicine-Plovdiv, and from 2019 to the present, he has worked at the Department of Bioorganic Chemistry of the University of Plovdiv in the laboratory for chromatographic mass spectrometry spectrometric analysis of the Scientific Research Institute of the Medical University-Plovdiv.

2. Relevance of the topic of the thesis

The modern lifestyle, associated with the consumption of processed food, exposure to a wide range of chemicals and reduced physical activity, plays an important role in the induction of oxidative stress, which in turn has a significant contribution to the pathogenesis of chronic diseases such as cardiovascular disease, diabetes, neurodegenerative diseases and cancer. The problem is extremely topical and studies in this direction are becoming more and more. The presented dissertation work is aimed at developing methods for determining a marker of oxidative stress in blood and saliva with the aim of application in diagnostic practice and/or clinical studies.

3. Knowledge of the subject.

The dissertation was written according to the requirements and contains the necessary sections. It was logically drawn up and structured. It was written in 109 standard pages as follows - table of contents - 2 pages, list of abbreviations used - 2 pages, list of tables and figures - 3 pages, introduction - 1 page, literature review - 25 pages, aim and objectives - 1 page, materials and methods- 16 pages, own results and discussion- 37 pages, conclusions -1 page, contributions 1 page, publications and participations-1 page. The bibliographic reference includes 159 sources, 3 of which are in Bulgarian and 60% published in the last 10 years.

The literature review examines in detail the main types of reactive oxygen and nitrogen particles that are formed in the body and their interaction with proteins, carbohydrates, nucleic acids and lipids. Oxidative stress has been considered as a factor in the initiation and development of many diseases and the candidate has managed to convey its role in some of them. The formation of 5-, 8-, 12- and 15-F2 isoprostanes of arachidonic acid, as well as malondialdehyde and 4-hydroxynonenal as products of lipid oxidation, was presented in detail. Isoprostanes are found in all body fluids and their concentration can serve as a marker of oxidative stress. In the part describing the methods for the analysis of oxidative stress, the analysis of isoprostanes in various biological matrices with chromatographic and immunochemical methods was emphasized and their advantages and disadvantages were indicated. From the review analysis, it is logical to conclude that the development of new, highly sensitive methods for the analysis of oxidative stress products and in particular 8-isoPGF2-alpha is necessary.

The aim and tasks are formulated correctly and realistically in view of the equipment used and the data accumulated so far on the problem.

The research methodology describes in detail two methods developed by the dissertation for the analysis of 8-isoPGF2-alpha in blood plasma and in saliva and includes procedures for sample preparation, calibration and validation, the equipment and consumables used. The statistical methods of analysis and the numbers of the necessary permits for working with patients were indicated. The chosen methods and equipment are suitable for the implementation of the tasks and the achievement of the goal of the dissertation work.

The results were presented in such a way that they follow the logically set tasks. They were set out in 26 tables and 31 figures and reflect the development and validation of two analytical methods that use liquid chromatography with mass detection for the analysis of 8-isoPGF2-alpha in two different biological matrices - blood and saliva. Sample preparation procedures are optimized and conditions were defined that allow maximum extraction of 8-isoPGF2-alpha in a short time. Validation procedures were precisely executed. Linearity of the method, parallelism of the calibration curves in a surrogate and real matrix, accuracy and reproducibility, limit of detection, limit of quantification, detectability, selectivity, stability of the solutions were determined, carry over was evaluated. All this

is a guarantee of the reliability of the methods. The doctoral student coped with these tasks in an excellent way.

The method for the analysis of 8-isoPGF2-alpha in blood plasma was applied to patients with Hashimoto's autoimmune thyroiditis. 95 patients and 21 control individuals were studied.

The method for the analysis of 8-isoPGF2-alpha in saliva was applied to evaluate local oxidative stress before and after placement of prosthetic structures in the oral cavity. The study included 35 patients.

Conclusions were drawn logically from the obtained results. **Contributions** are original and confirmatory in nature.

The following original contributions of the dissertation can be highlighted:

1. Two new highly specific liquid chromatographic methods with tandem mass spectrometric readout have been developed and validated for the determination of isoprostane 8-isoPGF2-alpha in blood plasma and saliva, combined with a precise sample preparation procedure allowing high analyte yield.

2. The methods were applied to target groups of patients and a connection was established between local oxidative stress in the oral cavity and local inflammatory processes, as well as the effect of the treatment. The relationship between local oxidative stress and the application of CoCr alloys in the oral cavity was established.

The **author's summary** was written according to the requirements and reflects the results achieved in the dissertation.

The doctoral student has presented three publications in third quartile Q3 scientific journals (Scopus), and two of them are in journals with an impact factor. The results were also reported at 5 scientific forums.

Conclusion

The *dissertation contains scientific and scientific-applied results, which represent an original contribution to science and meet the requirements* of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of the Medical University - Plovdiv.

The dissertation shows that the doctoral student, Dr. Desislav Grozev Tomov, **has** in-depth theoretical knowledge and professional skills in the Medical Biology doctoral programme, demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my *positive assessment* of the conducted research and *propose to the members of the respected scientific jury to award the educational and scientific degree "doctor" to Dr. Desislav Grozev Tomov.*

Plovdiv
21. 08. 2023

Prepared by: 
Assoc. Prof. Yordanka Uzunova, PhD

Заличено на основание
Чл.5 §1, 6."В" Регламент (ЕС)2016/679