

# WRITTEN STATEMENT

**from assoc. prof. Evgeni Evgeniev Grigorov, MScPharm, PhD**

**Senior lecturer at the Department of Organization and economics of pharmacy,**

**Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov"- Varna**

*(Internal member of the Scientific Jury, approved by Order No. P-109-92/24.02.2022 of the Rector of MU-Varna)*

**Subject:** Procedure for the defence of a dissertation for obtaining educational and scientific degree "Doctor", FHE 7. Healthcare and sports, PF 7.3. Pharmacy, in the doctoral program " Pharmacology /incl. pharmacokinetics and chemotherapy/".

**Topic of the dissertation:**

*"Isolation and analysis of methylxanthine fraction, catechin fraction and total Bancha green tea extract and study of their influence on the pharmacokinetics of sildenafil in rats"*

**Author of the dissertation:**

assist. prof. MAYA PETROVA RADEVA-ILIEVA, MScPharm - PhD student in full-time education at the Medical University-Varna.

**Supervisor:**

Assoc. Prof. Kaloyan Dobrinov Georgiev, MScPharm, PhD, DSc

**Significance of the topic:** In recent years, interactions between medicinal products and herbal preparations have posed a serious problem for clinical practice due to the increasing use of natural remedies for the prevention and / or treatment of diseases. Concomitant use of medicines and phytopreparations may lead to pharmacokinetic and / or pharmacodynamic interactions. This, in turn, may result in an increased incidence of side effects or therapy failure, especially when herbal preparations are taken without first consulting a doctor.

**Relevance of the developed topic:** The dissertation work of assist. prof. Maya Radeva-Ilieva is dedicated to the study of potential interactions between sildenafil and Japanese Bancha green tea extract, catechins and methylxanthines. It is well known that green tea is one of the most consumed beverages in the world due to its many health benefits, such as antioxidant, anti-inflammatory, antimicrobial activity, cancer prevention, weight loss and others. At the same time, a number of studies have reported that the active ingredients contained in green tea modulate the activity of medicine-metabolizing enzymes, which may reflect on the pharmacokinetics of simultaneously taken medications. On the other hand, sildenafil is a substrate of CYP450 enzymes and is involved in pharmacokinetic interactions. After reviewing numerous scientific publications on the problem,

the current data provided by the author unequivocally prove that the developed topic is in a direction that in the last decade has been developing extremely actively in scientific terms. The PhD student was able to summarize the more significant interactions observed so far between medicinal products and frequently used phytopreparations, as well as between medicines and green tea extract / EGCG / caffeine.

Conducting studies to assess potential interactions between herbal preparations and conventional medicines can significantly reduce the incidence of adverse reactions due to interactions and increase the safety of herbal products.

**Structure of the dissertation:** The dissertation work of assist. prof. Maya Radeva-Ilieva is very well formed and structured in terms of its main parts and fully complies with the Regulations for Development of the Academic Staff of MU-Varna. It starts with an introduction and contains the following sections:

- Literature review;
- Aim and objectives;
- Materials and methods;
- Results;
- Discussion;
- Conclusions;
- Contributions;

Then the bibliography is placed. The dissertation covers a total of 139 standard typewritten pages, including 27 figures and 14 tables. 392 literary sources are cited, of which, however, only 7 are in Bulgarian.

**The literature review** contains a sufficient amount of summarized and analyzed scientific material, which reflects a lot of data and information about research on the topic so far. It is systematically presented and reveals a very good knowledge of the subject related to drug interactions and justifies the choice of topic for the dissertation. The literature review is divided into several parts, in which consequently are discussed: green tea and its main biologically active substances, drug interactions and interactions between drugs and phytopreparations / food / beverages, as well as methods for studying drug interactions.

**The main aim** of the dissertation is to evaluate the influence of methylxanthine fraction, catechin fraction and total extract of Japanese Bancha green tea on the pharmacokinetics of sildenafil. The PhD student formulates a hypothesis for potential interactions when taking sildenafil and green tea extract, EGCG or caffeine.

**The tasks** are 6 in total and result from the set goal. They are formulated precisely and are logically justified, so their implementation allows the author to achieve the defined goal.

**The object of the study** are 198 white male Wistar rats, provided by the Vivarium to MU-Varna. A virtual population of healthy volunteers, men aged 60 to 65 years (10 groups of 10 people), was used to predict potential human interactions.

**The research methods** used are classic and are selected in view of its specifics:

- Extraction methods;
- Liquid chromatographic methods for analysis;
- Methods for studying the pharmacokinetics of sildenafil in experimental animals;
- Development of physiologically based pharmacokinetic models for predicting human interactions;
- Statistical methods;

The overall design of the study is evidence of the in-depth knowledge and mastery of the PhD student of modern methodological approaches in pharmacology. It makes a good impression that the methods used are diverse and in different directions.

**The results obtained** are clearly presented and well structured for each aspect of the study, accompanied by numerous figures and tables. The quantitative and percentage content of catechins (EGCG and (+)-catechin), caffeine and gallic acid in the obtained plant extracts were calculated. The lower quantitative content of the listed biologically active substances in the Japanese Bancha green tea has been confirmed. At the same time, the results clearly confirm the potential of green tea to interact with medicines that are metabolized by CYP450 enzymes.

Among the more important results, I would highlight those obtained from the original studies on potential pharmacokinetic interactions between sildenafil and green tea extract, catechin and methylxanthine fractions in experimental animals, and the development of physiologically based pharmacokinetic models to predict human interactions. The obtained results are original and reliable, supported by a large volume of analyzed and summarized scientific material.

**The discussion** is well structured as the PhD student consistently and in detail discusses each of the results obtained in the research. It makes a very good impression that every statement or assumption is supported and substantiated by scientific information.

**Conclusions.** Specific conclusions from the conducted research have been formulated, which have a practical orientation and are in accordance with the collected and analyzed data. Given the results of the studies, it is reasonable to say that there is a risk of interactions when sildenafil and green tea extract / catechins / methylxanthines are co-administered. I believe that all the conclusions made objectively reflect the results obtained by the PhD student.

## Contributions to the dissertation

### ORIGINAL CONTRIBUTIONS:

- *TE, CF and MF from Japanese Bancha green tea have been isolated and analyzed for the first time in Bulgaria.*
- *For the first time it was performed a pharmacokinetic study to assess potential interactions when sildenafil is taken after repeated administration of TE, CF and MF from Japanese Bancha green tea in rats and it was proven their influence on sildenafil pharmacokinetics.*
- *For the first time it was performed a pharmacokinetic study to assess potential interactions when sildenafil is taken after a single dose of MF from Japanese Bancha green tea in rats and it was proven its influence on sildenafil pharmacokinetics.*
- *For the first time, static and dynamic PBPK models have been developed and implemented to predict potential interactions between sildenafil and CF or MF from green tea, both alone and in combination in humans.*

### CONTRIBUTIONS OF SCIENTIFIC AND APPLIED NATURE:

- *A selective and reliable HPLC-UV method for qualitative and quantitative determination of EGCG, (+)-catechin, gallic acid and caffeine in samples of Japanese Bancha green tea has been developed and validated. The proposed analytical approach can be used in the analysis of other types of tea, as well as other plant extracts that are believed to contain these compounds.*
- *A sensitive and reliable HPLC-UV method for qualitative and quantitative determination of sildenafil in plasma samples from experimental animals has been developed and validated. The proposed analytical method can be used in the analysis of sildenafil in health facilities or other institutions performing an analysis of drugs in biological samples.*

### CONFIRMATIVE CONTRIBUTIONS:

- *The lower quantitative content of EGCG and caffeine in Bancha green tea has been confirmed.*

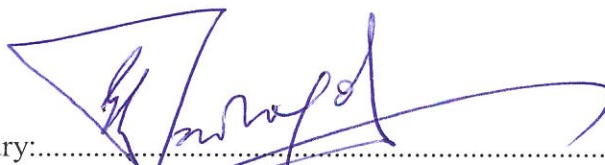
The presented contributions are divided into those with original, scientifically applied and confirmatory nature.

**Abstract and publications.** The abstract is prepared in accordance with the requirements of the Regulations for the Development of the Academic Staff of the Medical University of Varna and correctly reflects the results obtained and the scientific contributions of the dissertation. Three publications in scientific journals and three participations in scientific conferences related to the topic of the dissertation are presented. In all articles the PhD student is the first author, which shows his personal participation in the development, discussion and presentation of the results. In addition, two of the articles have been published in international journals with IF, which is indisputable proof of the importance of the studies conducted.

**Conclusion.** I positively evaluate the dissertation work of assist. prof. Maya Radeva-Ilieva and I believe that in terms of content and scientific contributions it fully meets the requirements of Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of MU-Varna. The good methodological preparation, the in-depth theoretical knowledge and the accumulated practical experience of the PhD student in the

field of Pharmacology are an excellent prerequisite for his future successful development as a scientist. All this gives me reason to convincingly recommend to the members of the respected Scientific Jury to award **MAYA PETROVA RADEVA-ILIEVA** the educational and scientific degree "**Doctor**".

Varna  
7 April 2022



Member of the Scientific Jury:.....

(Assoc. prof. Evgeni Evgeniev Grigorov, MScPharm, PhD)

*By signing here, I declare that I am not related to the doctoral student, and that I have no private interest that could affect the impartial and objective implementation of the opinion in the current procedure for acquiring ESD "Doctor".*