REVIEW

by Dr. Zhana Yuliyanova Petkova

Associate Professor at University of Plovdiv „Paisii Hilendarski“

On PhD Thesis for awarding the educational and scientific degree “**Doctor**”

Area of Higher education: 4. Natural science, mathematics, and informatics;

Professional field 4.2. Chemical sciences;

Doctoral Program „Analytical chemistry“

Author: *Asya Dimitrova Hristozova*

Title: *Enhancement of the capabilities of gas chromatography - mass spectrometry by combination with "green" approaches for extraction and modeling*

Scientific supervisor: *Assoc. Prof. Dr. Kiril Kostov Simitchiev – University of Plovdiv „Paisii Hilendarski“*

1. General description of the submitted materials

By order No. РД-22-486 dated 21.02.2025, from the Rector of the University of Plovdiv "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury to oversee the defence procedure of a dissertation entitled „Enhancement of the capabilities of gas chromatography - mass spectrometry by combination with "green" approaches for extraction and modeling“ in the Area of Higher education: 4. Natural science, mathematics, and informatics, Professional field 4.2. Chemical Sciences in Doctoral Program „Analytical chemistry“. The author of the dissertation is Asya Dimitrova Hristozova, a full-time doctoral student in the Department of Analytical Chemistry and Computer Chemistry with a scientific supervisor Associate Professor Dr. Kiril Kostov Simitchiev from the University of Plovdiv "Paisii Hilendarski".

The set of paper and electronic materials presented by Asya Dimitrova Hristozova complies with Article 36 (1) of the Regulations for the Development of the Academic Staff at PU and includes the following documents::

- Application to the Rector of PU for the disclosure of the procedure for dissertation defence;
- CV in European format;
- Protocol from the Departmental Council addressing the reporting of the readiness to initiate the procedure and the preliminary discussion of the dissertation;
- Dissertation;

- Abstract in Bulgarian and English;
- List of scientific publications on the subject of the dissertation and a list of citations to the PhD student's work;
- Copies of the scientific publications;
- Declaration of originality and authenticity of the submitted documents;
- Statement of meeting the minimum academic requirements.

The doctoral student has submitted two publications in international journals, which are peer-reviewed and indexed in the global databases Web of Science and Scopus, with quartiles 1 (Q1) and 2 (Q2).

2. Brief Biographical data of the doctoral candidate

Asya Hristozova graduated with a bachelor's degree in Biology and Chemistry in 2005 from University of Plovdiv "Paisii Hilendarski". She continued her studies at the same university, earning a master's degree in Medicinal Chemistry in 2006, followed by a second master's degree in Spectrochemical Analysis in 2019. Since September 1, 2020, she has been a full-time doctoral student in the Department of Analytical Chemistry and Computer Chemistry at University of Plovdiv "Paisii Hilendarski".

Her professional journey began as a medical representative at Tchaikapharma High Quality Medicines Inc., where she worked in the Marketing Department. Later, she transitioned to the field of analytical chemistry, taking on the role of chemist in the Quality Control Department, eventually becoming an analytical chromatographer.

In 2020, Asya Hristozova shifted her career focus to academia, becoming an assistant professor in the Department of Analytical Chemistry and Computer Chemistry at University of Plovdiv "Paisii Hilendarski", where she teaches courses in “Analytical chemistry” and “Instrumental methods for analysis”. In parallel, since 2022, she has been working as a chemist in the Department of Pharmaceutical Sciences at the Medical University of Plovdiv, continuing her work in chromatographic techniques and the analysis of pharmaceutical substances.

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

The research is relevant due to the growing need for more precise, eco-friendly, and efficient analytical methods. Gas chromatography/mass spectrometry (GC-MS) is among the most reliable techniques for analyzing volatile and semi-volatile compounds; however, the identification process remains challenging because of sample complexity and the necessity for preliminary preparation. Additionally, the introduction of the principles of "green" analytical chemistry (GAC) and "green" sample preparation (GSP) is key to reducing the use of harmful solvents and optimizing resources.

The development of alternative "green" extraction approaches reduces the environmental footprint and facilitates the analysis of organic pollutants and natural products. The creation of chemometric models for predicting retention indices contributes to faster identification of unknown compounds, which is especially beneficial for essential oil analysis. The optimization of GC-MS/MS methods enhances the accuracy and reproducibility of analysis, which is crucial for chemical quality control in food, cosmetics, and environmental monitoring.

The goals and objectives outlined in the dissertation are entirely relevant and well-justified. The research is timely and scientifically grounded, addressing contemporary challenges in analytical chemistry. In the context of increasing environmental regulations and the pursuit of sustainable technologies, the development of more precise analytical methods with a clear "green" focus is an important contribution to both the scientific community and industry.

4. Knowledge of the problem

Doctoral student Asya Dimitrova Hristozova demonstrates a high degree of understanding of the problem. A detailed literature review has been conducted, including an extensive analysis of modern methods for gas chromatography/mass spectrometry, extraction methods, identification of components in essential oils, and pesticide analysis. The citation of current scientific sources indicates a deep understanding of previous research and its development.

The doctoral student clearly formulates issues related to the identification of compounds with similar spectral characteristics, the necessity for more efficient and environmentally-friendly extraction methods, and the optimization of chromatographic analysis. The inclusion of mathematical modeling for predicting linear retention indices (LRI) and the optimization of analytical procedures shows a high level of problem awareness and the ability to utilize modern statistical and machine-learning methods.

Asya Hristozova demonstrates profound comprehension of the subject matter, familiarity with contemporary analytical approaches, and the ability to integrate various scientific disciplines to achieve more precise and effective results.

5. Research methodology

To achieve the set objectives of the dissertation, namely to study the capabilities of GC-MS/MS for combining with "green" approaches for qualitative and quantitative analysis, nine main tasks were defined. Regarding the qualitative analysis of volatile components in essential oils, the instrumental conditions of a GC-MS/MS were optimized for the identification of volatile components in rose, lavender, and peppermint essential oils during injection of liquid samples. A total of 49 components in lavender oil, 51 compounds in two commercial brands of rose oil, and 32

compounds in peppermint oil were identified. In total, 103 unique compounds were selected to create a database. Using the obtained experimental data, the linear retention indices of the identified compounds were calculated, and a list of their molecular descriptors was compiled. A multiple linear regression algorithm for predicting linear retention indices was developed and validated. Additionally, the procedure for preliminary sample preparation through headspace solid-phase microextraction (HS-SPME) was optimized.

Two new "green" methods have been proposed and developed for the group separation and concentration of a total of 19 organochlorine and organophosphorus pesticides: microwave-assisted cloud point extraction (MW-CPE-GC-MS/MS) and dispersive liquid-liquid microextraction based on a natural hydrophobic deep eutectic solvent (NADES-DLLME-GC-MS/MS), which have been successfully combined with GC-MS/MS analysis. The instrumental conditions for analysing organochlorine and organophosphorus pesticides via GC-MS/MS have been optimised. The procedure for cloud point extraction (CPE) has been adapted for the analysis of pesticides using GC-MS or GC-MS/MS. The solubility of the most commonly used surfactants for cloud point extraction (Triton X-100 and Triton X-114) in organic solvents – hexane, isooctane, cyclohexane and ethyl acetate – has been evaluated. The stability of GC-MS/MS to solutions containing Triton X-100, as well as its non-spectral matrix effect, has been investigated. The method of cloud point extraction in combination with re-extraction in organic solvent has been optimized. Food samples (lemon juice and red apple juice) have been analyzed using the developed MW-CPE-GC-MS/MS method. The compatibility of GC-MS/MS with the natural hydrophobic deep eutectic solvent (NADES) as a matrix component and its effects on the chromatographic system have been assessed, along with an evaluation of the non-spectral matrix effects in GC-MS/MS. The stability of the chromatographic system in presence of NADES has been studied. The methodology of dispersive liquid-liquid microextraction has been optimized. Real samples from three commercial brands of bottled spring water have been analyzed using the developed method. The "greenness" of both developed methods for determining organochlorine and organophosphorus pesticides has been evaluated using the AGREEprep.

6. Characteristics and evaluation of the thesis

The dissertation consists of 165 pages, including 25 figures, 38 tables, 4 appendices (containing 1 table and 8 figures), and references to 306 literature sources, 178 of which are published in the last 10 years. The dissertation includes main sections such as: Introduction (2 pages), Literature Review (35 pages, 21.2% of the total dissertation volume), Goals and Research Objectives (1 page), Equipment, Reagents, and Preparation of Solutions (10 pages), Results and

Discussion (63 pages, 38.2% of the total dissertation volume), Conclusions and Summary (2 pages), Contributions to the Dissertation (1 page), Scientific Communications on the Dissertation Topic (2 pages), Literature Sources (26 pages), and 4 appendices (16 pages).

The Literature Review is comprehensive, covering a broad range of sources (290 in total) and is well-structured, which clearly demonstrates the depth of the doctoral student's knowledge on the dissertation topic.

The section on Equipment, Reagents, and Preparation of Solutions provides detailed descriptions of the equipment used, the operating conditions of the analyses, the reagents used, certified reference materials, as well as the preliminary procedures for sample preparation.

In the "Results and Discussion" section, the development and optimization of the following are described in detail:

- Methods for identifying volatile components in rose, lavender, and peppermint essential oils, both during injection of liquid samples and after preliminary preparation by headspace solid-phase microextraction HS-SPME;

- Methods for combining the cloud point extraction (CPE) with GC-MS/MS;

- Methods for combining the dispersive liquid-liquid microextraction (DLLME), based on natural deep-eutectic solvents (NADES), with GC-MS/MS.

A thorough assessing of the ecological evaluation of the developed analytical methods was performed using quantitative metrics. Additionally, the developed methods were applied to the analysis of organochlorine and organophosphorus pesticides in water and food samples.

7. Contributions and Significance of the Research for Science and Practice

The dissertation presents a total of seven scientific and scientific-applied contributions:

- A database of experimentally determined linear retention indices on a non-polar gas chromatography column for 122 compounds included in the composition of essential oils has been created;

- A new easily applicable regression model for predicting linear retention indices has been proposed, which can be used in the identification of volatile components in essential oils by GC-MS and GC-MS/MS;

- A protocol has been created for performing GC-MS/MS analysis of 19 organochlorine and organophosphorus pesticides – optimization of both the parameters affecting the gas chromatographic separation and the mass spectrometric registration of the analyzed substances has been performed;

- The application of cloud point extraction for preliminary separation and concentration of organochlorine and organophosphorus pesticides has been expanded. A procedure is proposed, the "greenness" of which is enhanced by the use of microwave radiation. The procedure has been applied to the analysis of real samples – fruit juices;

- Knowledge of combining cloud point extraction with gas chromatographic analysis has been enriched. Evidence has been provided that the introduction of a surfactant into the gas chromatographic system not only does not have a negative impact on instrumental analysis, but conversely can lead to an increase in sensitivity and a decrease in detection limits. An important advantage in implementing the combination of cloud point extraction and gas chromatographic analysis is the possibility of performing using matrix-matched calibration;

- An innovative approach to dispersive liquid-liquid extraction is proposed, based on the use of a deep eutectic solvent of natural origin as a method for preliminary sample preparation in the analysis of organochlorine and organophosphorus pesticides. Operating conditions were established, ensuring simultaneous extraction of 19 target analytes, while completely eliminating the use of toxic organic solvents. The procedure was applied to the analysis of real samples – bottled spring waters;

- The possibilities of combining dispersive liquid-liquid microextraction using a deep eutectic solvent as an extractant with gas chromatography analysis were studied. It was found that the final phase obtained after extraction can be injected directly into GC-MS/MS. In the analysis of organochlorine and organophosphorus pesticides, it was observed that the deep eutectic solvent (menthol: decanoic acid) acts as a protectant of the analytes, which leads to an increase in the sensitivity of instrumental detection and a decrease in the detection limits.

8. Assessment of the publications on the dissertation

Two publications in international journals have been presented as part of the dissertation. These journals are peer-reviewed and indexed in global databases such as Web of Science and Scopus, with quartiles 1 (Q1) and 2 (Q2). The total score for the presented publications is 45, significantly exceeding the national minimum requirements for obtaining a Doctoral degree and the requirements of the Regulations of the University of Plovdiv "Paisii Hilendarski" for the development of academic staff (30 points required). The doctoral student is the first author of the publications, which fully reflect the essence of the work conducted in the dissertation.

9. Personal Contribution of the PhD student

Doctoral student Asya Hristozova is the first author of the submitted publications, which indicates her active participation in the conducted research, analysis of the results, and the development of the dissertation. She has attended ten scientific conferences, presenting the research findings through oral reports at two of them and through poster presentations at the remaining ones. This highlights her active role in disseminating the results of her dissertation.

10. Abstract

The abstract consists of 32 printed pages, 11 tables, and 13 figures. It is presented in both Bulgarian and English and illustrates the main points and contributions of the dissertation. The submitted abstract fully complies with the requirements of the regulations of University of Plovdiv "Paisii Hilendarski."

11. Critical remarks and recommendations

I have no critical remarks or recommendations regarding the conducted research and the presented set of materials.

12. Personal Impressions

My impression of the presented dissertation is that a tremendous amount of work has been carried out. The literature review and the research results are systematically organized and presented clearly and concisely. The doctoral student presented the dissertation to the expanded departmental council in a clear and professional manner.

CONCLUSION

The dissertation presents scientific-applied and applied results, representing an original contribution to science and meeting all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the relevant Regulations of the University of Plovdiv "Paisii Hilendarski."

The dissertation demonstrates that the doctoral candidate, Asya Dimitrova Hristozova, possesses in-depth theoretical knowledge and professional skills in the doctoral program „*Analytical chemistry*“ by showcasing qualities and skills to independently conduct scientific research.

Consequently, I confidently give my positive assessment of the conducted research, presented in the above-reviewed dissertation, abstract, achieved results, and contributions. I propose that the honorable scientific jury award the educational and scientific degree of "Doctor" to Asya Dimitrova

Hristozova in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics, professional field 4.2. Chemical Sciences, doctoral program „*Analytical chemistry*“.

17.04.2025 г. Reviewer: .....

Assoc. Prof. Dr. Zhana Petkova