OPINION

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Assoc. prof. at Burgas State University "Prof. Dr Assen Zlatarov", in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics professional field: 4.2. Chemical Sciences scientific specialty: Analytical Chemistry

of a dissertation submitted for the award of the educational and scientific degree "**Doctor**" in the field of higher education: 4. Natural Sciences, 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 Modelling" Scientific advisor: Assoc. Prof. Dr. Kiril Kostov Simitchiev

1. General Overview of the Procedure and the Doctoral Candidate

By Order № P22-486 dated February 21, 2025, of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I was appointed as a member of the academic jury for the defense procedure of the doctoral dissertation entitled "Enhancement of the Capabilities of Gas Chromatography – Mass Spectrometry by Combination with "Green" Approaches for Extraction and Modelling", submitted in fulfillment of the requirements for the award of the educational and scientific degree "Doctor" in Higher Education Area 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.2. Chemical Sciences, Doctoral Program in Analytical Chemistry. The author of the dissertation is Asya Dimitrova Hristozova – a doctoral student at the Department of Analytical Chemistry and Computational Chemistry, under the scientific supervision of Assoc. Prof. Dr. Kiril Simitchiev from Plovdiv University "Paisii Hilendarski."

The set of materials submitted by the doctoral candidate complies with Art. 36 (1) of the Regulations for the Development of the Academic Staff at PU and includes all the required documents. Asya Hristozova holds a Bachelor's degree in Biology and Chemistry, a Master's degree in Medical Chemistry, and a second Master's degree in Spectrochemical Analysis from Plovdiv University "Paisii Hilendarski." Her professional background encompasses over 15 years of experience in the field of analytical chemistry and pharmaceutical analysis, including long-term work as a chromatographer and LIMS specialist at "Chaikapharma High-Quality Medicines" AD. Since 2020, she has served as an Assistant Professor and PhD student at the Department of Analytical Chemistry and Computational Chemistry at PU, and between 2022 and 2024, she held the position of chemist at the Faculty of Pharmacy, Medical University – Plovdiv. Her scientific activity includes publications in reputable international journals, participation in scientific conferences, and involvement in national and international research projects. The dissertation submitted by Asya Hristozova consists of 165 pages and is structured into seven main sections: Introduction (pp. 3–4), Literature Review (pp. 5–39), Materials and Methods (pp. 40–50), Results and Discussion (pp. 51–113), Conclusions (pp. 114–115), Contributions (pp. 115–116), and Scientific Communications (pp. 116–117). A total of 306 literature sources are cited, more than half of which have been published in the last 10 years. The experimental results are presented in 38 tables and illustrated with 25 figures. Four appendices are included as part of the dissertation.

2. Relevance of the scientific topic

The research problem addressed in Asya Hristozova's dissertation is of high relevance, as it is situated within a strategically important area of contemporary analytical chemistry – enhancing instrumental analysis through the integration of environmentally friendly extraction techniques and chemometric modelling. The topic fully aligns with current trends in the sustainable development of analytical methodologies, as defined by the frameworks of Green and White Analytical Chemistry. It reflects the growing need to implement innovative approaches aimed at reducing environmental impact, minimizing resource consumption, and improving both analytical efficiency and economic feasibility.

In the field of gas chromatography coupled with mass spectrometric detection (GC-MS) for the qualitative and quantitative analysis of volatile and semi-volatile organic compounds, conventional sample preparation methods often rely on toxic solvents, lengthy and energy-intensive procedures, and limited selectivity. In this context, the exploration and development of innovative extraction techniques, along with the application of chemometric modeling, significantly enhances the sensitivity and effectiveness of analytical processes. The validation of the methods using real-world samples confirms their applicability in areas such as food safety control, the analysis of natural products, and environmental pollutant monitoring, thus underlining the theoretical and practical significance of the dissertation work.

3. Familiarity with the scientific topic

The doctoral research is distinguished by a well-justified and timely choice of topic, which is in line with leading developments in modern analytical chemistry. The literature review is based on an analysis of 290 sources, a substantial portion of which have been published in the past decade. This demonstrates the candidate's strong orientation within current scientific advancements and provides a solid foundation for the formulation and implementation of the research tasks.

The literature review is carefully structured in accordance with the aims of the dissertation. The sources are analyzed with precision and critical awareness. In addition to summarizing wellestablished scientific findings, the review also highlights areas that remain underexplored in the existing literature. Noteworthy is the depth and balance of the doctoral candidate's analytical approach, which reveals a clear ability to systematize complex information, identify essential interrelations, and formulate well-grounded conclusions. This reflects not only the candidate's high level of scientific training but also the successful acquisition of the educational and research competencies envisaged in the doctoral program.

4. Research methodology

The dissertation demonstrates a clearly articulated and theoretically substantiated research objective – investigating the potential for combining GC-MS/MS with environmentally friendly ("green") approaches for qualitative and quantitative analysis. The nine research tasks – three related to the analysis of volatile components in essential oils and six focused on the quantitative determination of pesticides in various matrices – are specific, logically structured, and fully aligned with the overall aim of the study. Experimental protocols and conditions are described with precision, ensuring reproducibility and scientific rigor.

The research is grounded in the application of innovative extraction techniques, including headspace solid-phase microextraction, microwave-assisted extraction at cloud point temperature, and dispersive liquid–liquid microextraction using natural deep eutectic solvents. The studies were conducted using advanced analytical instrumentation (GC-MS/MS, GC-MS, UV-VIS spectrophotometer, microwave-assisted extraction system, etc.), with optimized chromatographic conditions tailored for the analysis of both essential oils and organochlorine and organophosphorus pesticides. Additionally, retention index calculations, the development of multiple linear regression models, and the implementation of chemometric techniques reflect a rigorous analytical approach and robust statistical justification of the findings. In summary, the methodological framework of the dissertation is scientifically sound and adheres to high contemporary standards in analytical chemistry.

5. Characteristics and evaluation of the dissertation and its contributions

The comprehensive study is distinguished by a well-structured design, theoretical depth, and clarity of scientific purpose, with each chapter contributing logically to the overall achievement of the research objectives. Grounded in a well-reasoned methodological sequence and the application of high analytical standards, the work ensures strong reliability of the results and validity of the conclusions drawn. The integration of modern instrumental methods, innovative approaches, and analytical precision provides a robust foundation for the formulation of the following scientific and applied contributions, summarized as follows: i) A database of linear retention indices has been established for 122 compounds present in essential oils, and a new, user-friendly QSRR model for their prediction using GC-MS and GC-MS/MS has been proposed; ii) An optimized protocol has been developed for the determination of 19 organochlorine and organophosphorus pesticides by GC-MS/MS, integrating two innovative extraction techniques - microwave-assisted extraction at cloud point temperature and dispersive liquid – liquid microextraction with a natural deep eutectic solvent; iii) The validated methods exhibit a pronounced "green" character and high analytical sensitivity, including evidence of the positive effect of the extractant phases on instrumental detection and limits of quantification; iv) The practical applicability of the developed methods has been confirmed through successful analysis of real-world samples – fruit juices and bottled spring waters. The significance of these contributions lies in the sustainable transformation of sample preparation processes through the elimination of toxic solvents and the implementation of eco-friendly alternatives with proven analytical performance. The proposed models and procedures not only broaden the application potential of GC-MS/MS but also offer reliable tools for future studies in the context of green analytical chemistry.

6. Assessment of the publications and the doctoral candidate's individual contribution

The results presented in the dissertation are reflected in two scientific publications in peerreviewed journals – Talanta (Scopus, SJR 2023 = 0.956, Q1) and Acta Chromatographica (Scopus, SJR 2023 = 0.344, Q2). In both publications, the doctoral candidate is listed as the first author, indicating her significant personal contribution to the research and the preparation of the manuscripts. To date, these publications have received six independent citations, further affirming the scientific relevance, timeliness, and international visibility of the research.

In accordance with the provisions of the Higher Education Act, the Law on the Development of Academic Staff in the Republic of Bulgaria, the Law on Scientific Degrees and Academic Ranks and its Implementing Regulations, as well as the internal regulations of Paisii Hilendarski University of Plovdiv, the submitted materials not only fully satisfy but in certain aspects exceed the scientometric criteria required for the award of the educational and scientific degree "Doctor". The dissertation, the abstract, and the accompanying documentation are characterized by a high degree of completeness, methodological accuracy, and strict structural organization, which contributes to the professional and well-substantiated presentation of the overall research contribution.

7. Abstract

The abstract (31 pages) is consistent with the content of the dissertation and presents a concise yet substantive summary of its essence. It objectively reflects the achieved results and the conducted analyses, highlighting the main scientific contributions. The English translation (30 pages) further enhances the international visibility of the research.

8. Recommendations for the future application of the dissertation's contributions and results

Although I do not know the doctoral candidate personally, the topical relevance of the research, the high scientific level of the dissertation, and the related publications serve as compelling indicators of her ambition, scientific maturity, and ability to conduct high-quality research. The analytical and methodological competencies demonstrated clearly reveal her potential for sustained academic development in one of the most dynamically evolving areas of modern analytical chemistry. I strongly recommend that this line of research be further pursued and expanded within the framework of a multidisciplinary scientific team, which would enhance both its applicability and scientific added value.

CONCLUSION

The dissertation presents original scientific and applied research results that fully comply with the requirements stipulated by the Law on the Development of Academic Staff in the Republic of Bulgaria, the Law on Scientific Degrees and Academic Ranks and its Implementing Regulations, as well as the internal regulations of Paisii Hilendarski University of Plovdiv. Through the research conducted, the doctoral candidate Asya Dimitrova Hristozova demonstrates in-depth theoretical knowledge and a high level of scientific competence in the field of analytical chemistry, convincingly proving her ability to carry out independent scientific investigations at a professional level. Based on the presented dissertation, the abstract, the achieved results, and contributions, I express my unequivocally positive evaluation and recommend that the esteemed academic jury award the educational and scientific degree "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".

03.05.2025 г.

Prepared by:

(Assoc. prof. L. Gonsalvesh, PhD)