REVIEW

by Prof. Dr. Stefan Leonidov Tsakovski

Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski"

of the dissertation 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: **"Analytical Chemistry"**

Author: **Asya Dimitrova Hristozova**
Topic: **ENHANCING THE CAPABILITIES OF GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION THROUGH COMBINATION WITH "GREEN" APPROACHES FOR EXTRACTION AND MODELING**
Scientific advisor: **Assoc. Prof. Dr. Kiril Kostov Simitchiev, Plovdiv University "Paisii Hilendarski"**

1. General Description of the Submitted Materials

By Order No. RD-22-486 dated 21.02.2025 of the Rector of Plovdiv University "Paisii Hilendarski", I was appointed as a member of the scientific jury for the procedure to defend the dissertation entitled **"Enhancing the Capabilities of Gas Chromatography with Mass Spectrometric Detection through Combination with 'Green' Approaches for Extraction and Modeling"** for the awarding of the doctoral degree in Higher Education Area 4: Natural Sciences, Mathematics, and Informatics; Professional Field 4.2: Chemical Sciences; Doctoral Program: Analytical Chemistry. The author of the dissertation is Asya Hristozova – a full-time PhD student at the Department of Analytical Chemistry and Computer Chemistry at the Faculty of Chemistry, with scientific advisor Assoc. Prof. Dr. Kiril Simitchiev from Plovdiv University "Paisii Hilendarski".

The submitted materials by Asya Hristozova comply with Article 36 (1) of the Regulations for the Development of Academic Staff at PU and include the following documents: (1) Application to the Rector to initiate the defense procedure; (2) European-format CV; (3) Departmental Council protocol related to readiness for initiating the procedure and preliminary discussion of the dissertation; (4) Dissertation; (5) Abstract; (6) List of scientific publications and conference participations; (7) Copies of two scientific publications; (8) Declaration of originality and authenticity of the documents; (9) Report on minimum requirements; (10) Statement from the scientific advisor.

A review of the materials shows that all legal requirements for conducting the procedure have been met.

2. Brief Biographical Data of the PhD Student

Asya Hristozova graduated with a Bachelor's degree in Biology and Chemistry and Master's degrees in Medical Chemistry and in Spectrochemical Analysis at PU "Paisii Hilendarski", Faculty of Chemistry, Department of Analytical and Computer Chemistry. In 2020, she was appointed as an assistant at the same department and enrolled in a PhD program. Her dissertation was reviewed and approved for defense by the extended departmental council on 03.02.2025. During her doctoral studies, Khristozova completed training courses carefully selected to support her research. She actively participated in a bilateral scientific project within which the dissertation research was conducted. Prior to starting her PhD, she held various positions at "CHAIKAPHARMA HIGH QUALITY MEDICINES" AD, Plovdiv (2005–2020).

3. Relevance of the Topic and Appropriateness of Goals and Tasks

Gas chromatography with mass spectrometric detection (GC/MS) is a widely used analytical technique for determining volatile and semi-volatile organic compounds in various matrices. It is routinely used for analyzing pesticides and persistent organic pollutants (POPs) in food and environmental samples. The dissertation focuses on expanding the potential of GC-MS/MS by integrating "green chemistry" approaches for qualitative and quantitative analysis and predicting linear retention indices. **This makes the research timely and promising.** Developed methods include qualitative analysis of volatile components in essential oils and quantitative analysis of 19 organochlorine and organophosphorus pesticides in fruit juices and spring waters.

The objective is clearly stated, and the tasks necessary for its achievement are well-defined.

4. Understanding of the Problem

The PhD student demonstrates very good understanding of all stages of developing and validating methods for qualitative and quantitative GC-MS analysis. The literature review covers numerous sources and follows the steps of analytical methodology, supporting accurate formulation of research objectives.

5. Research Methodology

The methodology is appropriate and allows for achieving the dissertation's objectives and solving the specific research tasks.

6. Characteristics and Evaluation of the Dissertation

The dissertation is 165 pages long, including 50 pages of introduction, literature review, objectives and tasks, apparatus and reagents; 64 pages of original research and discussion; conclusions and contributions. The bibliography includes 306 sources, providing an excellent overview of the field. Four appendices enhance transparency of the results.

The original research is presented in two parts:

1. Creation of a QSRR model for predicting linear retention indices

The modeling of retention indices is detailed and includes essential oil analysis, molecular descriptor calculations, and selection of independent variables. A stepwise multiple linear regression model is developed and compared with published models. The proposed QSRR model with 14 molecular descriptors can be used to identify essential oil components in nonpolar stationary phases by predicting LRI.

2. Optimization of GC-MS/MS conditions for organochlorine and organophosphorus pesticide analysis

This section focuses on developing two new "green" procedures: MW-CPE and NADES-DLLME for separation and concentration of 19 pesticides. Matrix effects are studied, and both one-factor and multi-factor optimization approaches are used. The multi-factor optimization scheme includes: (i) Screening (Plackett-Burman design), (ii) Determining optimal values (CCD plan), and (iii) Target function for compromise factor values regarding all analytes signals.

The methods are successfully applied to fruit juices and spring water. Detection limits are significantly below regulatory thresholds.

7. Contributions and Significance for Science and Practice

The stated contributions are valid and can be summarized as:

- A regression model for predicting LRIs in essential oils using GC-MS and GC-MS/MS.
- A GC-MS/MS protocol for analyzing 19 pesticides with optimized GC and MS parameters.
- Improved methods for separation and concentration compatible with gas chromatographic analysis.

The dissertation would benefit from outlining future development directions for the proposed methods.

8. Evaluation of Publications

Two peer-reviewed publications related to the dissertation have been published in reputable journals (*Talanta*, *Acta Chromatographica*) with impact factors and Q1 and Q2 rankings. Six citations confirm scientific recognition. Results have been presented at ten scientific forums. **Thus, national requirements for awarding the doctoral degree are convincingly met.**

9. Personal Contribution of the PhD Student

Although the research is team-based, in line with modern practice, **I have no doubt that the dissertation is the PhD student's own work.** Her position as first author in the publications and the advisor's positive statement confirm this.

10. Abstract

The abstract successfully summarizes the key contributions and reflects the main results accurately.

11. Critical Notes, Recommendations, and Questions

I have the following remarks and questions for the PhD student:

1. As a rule, the Partial Least Squares (PLS) method generally yields better results than multiple linear regression. PLS addresses the issue of collinearity among the independent variables, provides an assessment of their importance (e.g., variable importance parameter, selectivity ratio), and is easier to interpret compared to various types of neural networks, support vector machines, and others. I recommend that the PhD student consider this approach in her future research.

2. A common practice in model validation involves three steps: (i) calibration ($RMSE_{Cal}$); (ii) cross-validation ($RMSE_{CV}$); and (iii) prediction ($RMSE_{PR}$). In the validation of the QSRR model, I do not see the cross-validation step. $RMSE_{CV}$ is an important indicator of the model's internal predictive ability, and the ratio between $RMSE_{Cal}$ and $RMSE_{CV}$ is a key indicator of potential overfitting.

3. What approach was used to determine the composition and size of the training and validation sets?

4. Which stepwise method—forward or backward—was used in the multiple linear regression?

12. Personal Impressions

I have no personal impressions of the PhD student. However, I am well acquainted with the rigorous and thorough work of the Department of Analytical Chemistry and Computer Chemistry,

which has undoubtedly helped Asya Hristozova gain valuable knowledge and skills as an independent researcher.

CONCLUSION

The dissertation contains **applied results representing an original contribution to science and meets the legal requirements** for awarding the doctoral degree in Bulgaria. The work demonstrates that the PhD student possesses deep theoretical knowledge and professional skills in analytical chemistry and can conduct independent research.

Therefore, **I give my positive assessment** of the dissertation, abstract, publications, and achievements, **and recommend that the scientific jury vote in favor of awarding the degree of "Doctor" to Asya Dimitrova Hristozova** in Higher Education Area 4: Natural Sciences, Mathematics, and Informatics, Professional Field 4.2: Chemical Sciences, Doctoral Program "Analytical Chemistry".

09.05.2025 г. Reviewer:

/Prof. Dr. Stefan Tsakovski/