



EVALUATION REPORT

on **THESIS** submitted for the acquisition of educational and scientific degree “**Doctor of Philosophy**” (PhD) **Scientific field: Medicine, scientific specialty: Hygiene**

Author: Daniela Dimitrova Stankova-Kostadinova

Analytical and Laboratory Activities Directorate, department Chemical factors, National Center of Public Health and Analyses

PhD title: Investigations on total mercury content in various media of importance for human health and environment

Scientific supervisor: Assoc. Prof. Rositsa Georgieva, PhD

Referee: prof. Irina Karadjova PhD, Faculty of chemistry and pharmacy, University of Sofia, St. Kliment Ohridski”

The review was prepared on the basis of the presented thesis, thesis summary, publication activity and participation in conferences. The presented materials meet the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation, and the Specific criteria for the acquisition of the requested scientific degree in National Center of Public Health and Analyses. The structure of the thesis meets the requirements of the rules of National Center of Public Health and Analyses.

Brief biographical data for the PhD candidate.

Daniela Stankova graduated from the University of Chemical Technology and Metallurgy, Sofia, with master degree in "Materials Technology and Materials Science". In 2019, she acquired the specialty "Medical Sanitary Chemistry" with a successfully evaluated state exam. Her scientific career is connected to the "Chemical Factors" Department of the National Center for Public Health and Analysis. Her research studies are related to the determination of chemical elements in various environments - cosmetic products, water, soil, sediments, food, air, chemical substances and mixtures, etc. The presented thesis is based on results obtained over many years and represents a complete summary of the development of

analytical methods, their successful application in the analysis of samples and conclusions about the behavior of a toxic chemical element such as mercury.

Parameters of the PhD thesis

The thesis is presented on 119 pages and contains 15 figures and 39 tables, 214 references are cited. The obtained scientific results are published in five articles. The PhD candidate is the first author of 3 of the five publications. The contribution of the candidate in terms of the conducted research, results discussion and manuscript preparation is undoubted. Results of the dissertation work have been presented at nine international conferences and national forums.

Evaluation of the PhD thesis

Undoubtedly the PhD thesis presented by Daniela Stankova is on the actual laboratory problems and in the spirit of modern analytics.

In the presented introduction part, Daniela Dimitrova Stankova-Kostadinova demonstrates a very good knowledge of the behavior of mercury, the regulatory documents related to the control of mercury content and the possibilities of instrumental methods for mercury determination. Mercury is a global, atmospheric pollutant of very high toxicity. The literature review successively presents the problems:

- related to the properties, toxic action, professional and non-professional exposure of mercury;
- behavior and distribution of mercury in the environment
- the content of mercury in various media of importance for human health and environmental quality.

The high toxicity and widespread distribution of mercury suggest serious control of mercury concentrations both in the environment and in products related to human health. The candidate demonstrates a very good knowledge of the international and national regulatory framework and the restrictions introduced.

The literature review summarises the methods applied for the determination of mercury in a wide range of samples. The presented critical analysis of the possibilities of analytical methods for mercury determination shows the high analytical culture of the candidate.

As a conclusion, the presented overview shows a very good qualification of the candidate and her possibilities for an informed choice of the most suitable method, as well as the possibilities for its optimization and application. Last but not least, the choice of the method

in the thesis is also determined by the aspiration to use a green method with minimal environmental load with harmful components. The final choice of a method with direct determination of mercury is logical and shows the relevance of both the posed problem and the proposed solution. The PhD candidate demonstrates a very good knowledge and reliably comments on the essential advantages of accepted option, but also indicates the disadvantages related to the requirements for high sample homogeneity because very small amounts of the sample are analyzed and the problems related to calibration - the need for certified reference materials in a wide concentration range of the certified element and also based on the analyzed matrix. Daniela Stankova knows the possibilities of the method for direct analysis of mercury in solid samples and correctly defines the tasks that must be solved in order to obtain reliable results. If the accepted approach of sample decomposition in a closed system is combined with mercury measurement by the cold vapor method, high sensitivity determination without loss of analyte will be realized, which will ensure accurate results at very low concentration level. The doctoral student optimized precisely this approach - determination of mercury through a direct mercury analyzer, i.e. direct analysis of solid samples. Obtaining accurate and reliable results in this variant of analysis, however, presupposes a very good optimization of the instrumental parameters on the one hand and the selection of an appropriate calibration method on the other. In this aspect, an approach is proposed to optimize the instrumental parameters and calibration methods in the analysis of different types of samples. The analytical approaches developed within the limits of the dissertation can contribute to the expansion of knowledge in the field of instrumental methods for the analysis of solid samples and might be directly applied in the analytical practice of laboratories in the country.

Within the limits of the implementation of the set goal, a methodical approach is proposed for the optimization of the instrumental parameters for the determination of mercury by a direct mercury analyzer. The influence of the matrix has been traced and suitable solutions have been proposed to eliminate possible interferences. A calibration approach is proposed for different matrices using certified reference materials. The validation of the proposed analytical procedure for each analyzed matrix includes the determination of repeatability and reproducibility, the definition of limits of detection and determination. The accuracy of the obtained results has been proven through successful participation in interlaboratory comparisons.

The developed analytical approaches have been applied to the analysis of a wide range of samples - samples essential to human health and environmental samples. After each section

discussing the results obtained for mercury content in samples studied, a summary follows, making the dissertation a very informative read.

Most likely, the dissertation presents one of the most serious studies on mercury content in Bulgarian cosmetics - commercial products produced in the country. About 823 samples were examined including: hair cosmetics (n=237), face cosmetics (n=234) and body cosmetics (n=352). The conclusions that all samples meet the requirements of regulation 1223/2009 and that only 2.9% of the samples had a mercury content above the limit of determination is relevant for cosmetics manufacturers. The results are essential because mercury content in cosmetic products is not allowed at all due to its potential to accumulate in the human body (with the exception of mercury compounds used as effective preservatives). The conclusion that mercury is presented in cosmetic products as an impurity accompanying the raw materials used is important for quality control of final product.

Research on mercury content in surface, drinking and bottled waters is significant from the point of view of human health. The data presented in the thesis show that there is practically no product that does not meet the requirements of the relevant legislation. The research conducted on a significant number of drinking waters from different regions of the country (863 water samples from the water supply network of 17 regions in Bulgaria) shows that all results are much below the permissible value and close to the detection limit are only 3% of all analyzed samples. An important conclusion, on the one hand, for the health of consumers, on the other hand, for future assessments of possible contamination of drinking waters in the country.

The mercury content of fish is considered a bioindicator of mercury contamination of water bodies, mainly due to the atmospheric transport possibilities. The results obtained in the frame of the thesis for mercury content in fish are above the environmental quality standard for surface water, according to Directive 2013/39/EU. The result is expected and can be used to assess the chemical status of surface waters. At the same time, the results show that there is no mercury content in fish above the permissible limit for human consumption.

Mercury is a highly toxic element that accumulates in the body, and therefore results for mercury content in foods, nutritional supplements, sugar, sweeteners and beverages are essential. The fact that practically all the obtained results are below the detection limit or close to it shows that no health problems are expected, the products consumed in the country are safe for consumers.

Research on mercury contents in soils and WWTP sludges shows the influence of anthropogenic pollution and the possibilities to reduce mercury levels in the future. These

results are the basis for the assessment of background concentrations and sources of mercury that must be limited to achieve the requirements for global reduction of mercury concentrations in the environment.

The requirements for the quality of packaging materials and packaging waste from polymeric materials require the determination of mercury content in 94 samples from four groups of polymeric materials: polystyrene (n=44), polypropylene (n=28), polyethylene (n=14) and polyvinyl chloride (n=8). The results show that all tested samples meet the requirements.

Scientific achievements

The PhD candidate has carried out a considerable amount of experimental work and has shown extreme precision in the interpretation of the obtained results, a large number of samples has been analyzed. A very good impression is made by the variety of matrices that have been analyzed in order to guarantee the credibility of both the quantitative results and the conclusions of the developed methodical approach. The presented summary of mercury content in samples relevant to human health and environmental quality shows mercury concentrations in the country and might be used to assess the need for measures to control mercury emissions from potential emitters - coal thermal power plants, metallurgical productions, cement plants.

The developed analytical procedure for the determination of mercury could be applied in a wide range of samples. An optimization of instrumental parameters is presented, which can be used by laboratories in the country in the analysis of a wide range of samples for mercury content.

Scientific contributions can be characterized as new scientific results with very good practical application.

The text of the dissertation is very well organized, with a concise description of the scientific problems and scientific-applied aspects of the proposed approaches and quantitative results obtained.

Critical remarks

I have no fundamental critical remarks about the research and the chosen approach to solving the problem of mercury determination by direct mercury analyzer. The PhD candidate has taken into account the critical remarks in the preliminary presented review and has presented the optimization of the instrumental parameters, accompanied by a short discussion - an approach how to choose the optimal amount of sample, the drying temperature, retention times all based on the influence of the matrix; in addition, approach to find the most suitable

calibration method. A good visualization of the results for mercury content in a large number of samples is achieved, with the ability to quickly assess the levels and compare them with the permissible values.

Conclusion

In conclusion, I believe that the dissertation work represents a completed scientific study with an actual topic - from the development of a green method for the determination of mercury to its application to a wide range of samples and a summary of the obtained results. Significant scientific contributions of interest to the analytical audience and laboratory practice have been achieved.

From the view point of actuality, volume and quality the presented thesis and the published scientific articles fully meet the requirements of the the Act on Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation, and the Specific criteria for the acquisition of the requested scientific degree in National Center of Public Health and Analyses. In addition, I consider that Daniela Stankova is a researcher with a high professional level of research work and results that represent a certain scientific achievement, which gives me the reason to give a positive assessment and recommend to the Scientific Jury to vote positively for the awarding of the educational and scientific degree "doctor" to Daniela Stankova in professional field 7.1 Medicine, scientific specialty "Hygiene".

Sofia, 19.02.2024 г.

Referee: **Irina
Karardjova**

Digitally signed by Irina Karardjova
DN: cn=Irina Karardjova, o=Faculty of
chemistry and pharmacy, ou=University
of Sofia, email=karardjova@chem.uni-
sofia.bg, c=BG
Date: 2024.02.19 09:33:14 +02'00'
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