ANNOTATION

dissertation for the degree of Doctor of Philosophy (PhD)
specialty “8D07502- Standardization and certification (by industry)”

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FOOD QUALITY CONTROL BY SOLID-PHASE MICROEXTRACTION IN COMBINATION WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

The development of metrology, standardization, and certification in Kazakhstan is imperative to meet international standards, enhance confidence in the country's products and services, and facilitate international integration. Kazakhstan actively participates in international metrology organizations, promoting mutual recognition of measurement results. Modern methods, measuring instruments, improved equipment ensure the accuracy and reliability of measurements across various fields. As measurements become more complex, it is essential to view measurement technologies as a sequence of actions to obtain high-quality measurement information.

In line with Resolution No. 903 of July 2, 2001, titled "On the draft Decree of the President of the Republic of Kazakhstan 'On the State Program of the Republic of Kazakhstan 'Drinking Water' for 2001-2030," and the Government of the Republic of Kazakhstan's Resolution dated March 31, 2022, No. 178, which approved the "Food Security Plan" for 2022–2024, the objectives are to enhance water quality, ensure food safety, and improve the regulatory framework. To solve the problems of ensuring food safety and improving the quality of water used, in this dissertation, research was carried out in an expanded range of determinable indicators of product quality, such as endocrine disruptors characterizing the quality of drinking water and biomarkers of honey. Most food analysis uses general-purpose chromatographic methods that provide reliable identification of components, allow large volumes of data to be collected without retention time calibration, and can be used to determine volatile and non-volatile components, including major ingredients, impurities and their residues.

In the last two decades, organic compounds have become widely used in various fields, and as confirmed by recent scientific publications in the field of ecology, this leads to serious environmental pollution. Due to the emergence of new substances and persistent organic pollutants, there is a need to update analysis methods. Of particular concern is the contamination of water resources with steroid hormones and alkylphenols, which have a detrimental effect on the endocrine system of living organisms. In this regard, the development of an environmentally safe method for the analysis of endocrine disruptors in water based on the modern method of solid-phase microextraction is relevant throughout the world to ensure a safe water supply and environmental protection.

Kazakhstan is a major producer of honey and actively exports its products. However, falsification of honey can undermine the country's reputation in the world

market and infringe on the interests of honest producers. To solve this problem, it is necessary to tighten control over the production and quality of honey, as well as to develop and implement a method for analyzing honey products using a highly sensitive express method of solid-phase microextraction.

In this work, two rapid and sensitive methods for analyzing the most commonly consumed food products have been developed. Miniaturization of solid-phase microextraction with preliminary concentration of analytes through adsorption-desorption experiments and with a significant reduction in the number of analyzed objects was carried out.

Connection of the topic with government initiatives and research plans.

The research was carried out in accordance with the approved research plan, the Ministry of Education and Science of the Republic of Kazakhstan and within the framework of grant funding projects for scientific research: “Improvement and development of highly sensitive methods to ensure food safety in Kazakhstan (2020-2022, code AP08857501, state registration number 0120RK00611) and “Effective development of highly sensitive methods for food analysis based on miniaturized solid-phase microextraction" (2021-2023, code AP09058561, state registration number 0121PK00061).

The purpose of the dissertation work is to develop a method for the effective determination of endocrine disruptors in drinking water and a method for the determination of biomarkers in honey. In accordance with the goal, the following main **tasks** are set:

1. Optimize the physical parameters for the determination of endocrine disruptors in drinking water using mini-SPME/GC-MS: selection of the optimal extraction coating, optimal extraction parameters: temperature, extraction time and preincubation time.

2. Standardize and develop a method for determining endocrine disruptors in drinking water, establish the metrological characteristics of the developed methods, including accuracy, linearity, detection limit.

3. Optimize the physical parameters for determining honey biomarkers using the vacuum-SPME/GC-MS method: selection of the optimal extraction coating, optimal extraction parameters: temperature and extraction time, pre-incubation time.

4. Standardize and develop a method for determining honey biomarkers and establish the geographical origin of honey samples using statistical methods.

The objects of the study are drinking water contaminated with endocrine disruptors, as well as honey samples purchased directly from producers.

Research methods

The analysis of objects will be carried out using methods based on solid-phase microextraction in combination with gas chromatography-mass spectrometry (GC-MS).

Scientific novelty lies in the fact that for the first time:

For the first time, the optimal physical parameters of mini-solid-phase microextraction gas chromatography-mass spectrometry (mini-SPME/GC-MS) for determining endocrine disruptors in drinking water have been established. The mini-SPME/GC-MS technique indicates that the use of a

divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fiber provides the best extraction results for target analytes. The identified optimal parameters include an extraction temperature of 80°C, an extraction time of 60 minutes for steroid hormones, and 30 minutes for alkylphenols. Additionally, pre-incubation times of 20 minutes and 5 minutes are recommended for steroid hormones and alkylphenols, respectively.

For the first time standardized method for establishing the authenticity of honey has been introduced, based on the vacuum-solid-phase microextraction gas chromatography-mass spectrometry (vacuum-SPME/GC-MS) technique. Optimal physical parameters for determining honey biomarkers using vacuum-SPME/ GC-MS were determined as follows: an extraction time of 30 minutes, an extraction temperature of 60 ℃, and an incubation time of 30 minutes for honey biomarker analysis. Geographical origin determination of honey samples was achieved through principal components and hierarchical cluster analysis. Notably, it was revealed that steppe honey samples contain aldehydes in the range of 28.0-72.2%, while mountain honey samples contain aldehydes in the range of 1.4-20.4%. The lower aldehyde content in mountain honey is attributed to the fact that in mountainous areas, the average annual air temperature does not exceed 30℃.

Provisions to be defended:

1. The developed method miniaturized solid-phase microextraction in combination gas chromatography-mass spectrometry for determining endocrine disruptors in drinking water, exhibits optimal performance under the following physical parameters: a temperature of 80 ℃, an extraction time of 60 minutes, and a pre-incubation time of 20 minutes. The extraction coating, comprising divinylbenzene/carboxene/polydimethylsiloxane, is identified as optimal.

2. The linear range for quantifying endocrine disruptors in drinking water is established as 10.0–1000 μg/ml. The detection limits for steroid hormones range from 0.02 to 0.08 μg/ml, while for alkylphenols, the range is also 0.02 to 0.08 μg/ml. The linearity function for steroid hormones is represented by R^2 = 0.96 -0.994, and for alkylphenols R^2=0.96–0.994.

3. The developed method vacuum solid-phase microextraction in combination with gas chromatography and mass spectrometry for determining honey biomarkers, demonstrates optimal performance under the following physical parameters: an extraction time of 30 minutes, an extraction temperature of 60 ℃, and a pre-incubation time of 30 minutes.

4. The standardized method for identifying honey biomarkers facilitates the determination of the geographic origin of honey. The content of aldehydes in steppe honey ranges from 28.0% to 72.2%, whereas in mountain honey samples, it varies from 1.4% to 20.4%.

Theoretical and practical significance of the work

The results obtained in the dissertation work can find application in both theoretical, practical, and legal metrology, since they represent a set of interrelated and interdependent general rules, approaches, requirements and norms aimed at ensuring the uniformity of measurements and the uniformity of measuring instruments. The results of the work can be used in the field of ensuring the quality

of food products and are the basis for the certification of methods in the RSE "KazStandard" and the introduction of the developed methods into the additional clause of TR CU 021/2011 "On safety of food products" and TR EAEU 044/2017 "Safety of Bottled Water Including Natural Mineral Water".

During the dissertation work, “Method for determining endocrine disruptors in drinking water using mini-SPME/GC-MS” and “Method for identifying honey biomarkers using vacuum-SPME/GC-MS” were developed. The result of the development of a method for determining endocrine disruptors in drinking water was introduced into the activities of the Institute of Hydrogeology and Geoecology named after U.M. Akhmedsafin" to the laboratory of chemical analytical research. The implementation certificate has been received (Appendix O). The result of the development of a methodology for determining biomarkers was introduced into the activities of the Research Institute "Food Safety" at JSC "Almaty Technological University" and an implementation certificate was received (Appendix R).

Validity and reliability of the result obtained in the study, is supported by the use of selective, precise and modern analytical methods, as well as the scientific method. To ensure reliability and repeatability, all experiments were carried out in several identical parallels.

List of published works

The research results are reflected in the following scientific papers, including:

Scientific publications with a high impact factor included in the international database Scopus and Web of Science

1. Alimzhanova, M., Mamedova, M., Ashimuly, K., Alipuly, A., & Adilbekov, Y. (2022). Miniaturized solid-phase microextraction coupled with gas chromatography-mass spectrometry for determination of endocrine disruptors in drinking water. Food Chemistry: X, 14, 100345. https://doi.org/https://doi.org/10.1016/j.fochx.2022.100345 (Q1 Web of Science и 78% Scopus)

2. Mamedova, M., Alimzhanova, M.B. Determination of Biomarkers in Multifloral Honey by Vacuum-Assisted Headspace Solid-Phase Microextraction. Food Anal. Methods 16, 1180–1190 (2023). https://doi.org/10.1007/s12161-023-02499-0 (Q2 Web of Science и 92% Scopus)

Scientific publications recommended by Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan for publication of the main results of scientific activities

1. Мамедова М.Р., Орынбасар А.Б., Алимжанова М.Б. Определение биомаркеров гречишного меда методом твердофазной микроэкстракции с целью установления подлинности. Вестник Казахстанско-Британского технического университета. 2022;19(3):23-32. https://doi.org/10.55452/1998-6688-2022-19-3-23-32

2. Mamedova, M., Ibraimov, A., Ashimuly, K., Yegemova, S., & Alimzhanova, M. (2023). Validation of the methodology for the analysis of endocrine destructors in water. Научный журнал «Доклады НАН РК», 345(1), 265–281. https://doi.org/10.32014/2023.2518-1483.200

Publications in collections of abstracts of reports based on the results of International scientific conferences

1. Alimzhanova M.B., Mamedova M.R. Determination of biomarkers in multifloral honey by vacuum-assisted headspace solid-phase microextraction". «Качество продуктов питания, безопасность и общество» // International conference Food BioTech, 2021. С. 4.

2. Орынбасар А., Мамедова М.Р. Хромато-масс-спектрометриялық тәсілмен балды талдау әдістемесінің валидациясы// Международная научная конференция студентов и молодых ученых «Фараби Әлемі», 2022. – С. 328.

3. Муса А.Қ,, Мамедова М.Р. Определение качества меда методом вакуумной твердофазной микроэкстракции // Международная научная конференция студентов и молодых ученых «Фараби Әлемі», 2023. – С. 275.

4. Alimzhanova M.B., Mamedova M.R. "Monofloral honey analysis using vacuum assisted HS-SPME" 25th International Symposium on Advances in Extraction Technologies (ExTech 2023).

The author's personal contribution lies in the independent planning and execution of the experimental part of the study, as well as the interpretation and processing of the data obtained, the development of final methods for the determination of endocrine disruptors in drinking water and biomarkers of honey, the establishment of geographical origin using statistical methods of modern software and the calculation of metrological characteristics. The author also worked with scientific advisors to define the objectives and discuss the results of the study.

Structure and scope of the dissertation. The dissertation consists of 133 pages and an introduction, 6 sections, a conclusion, a list of sources used (152 titles) and 14 appendices. The work contains 19 tables and 41 figures.