**ABSTRACT**

of the thesis for the degree of Doctor of Philosophy (Ph.D.) on educational program «8D05301 – Chemistry»

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"Development of methods for the determination of pesticides in grapes using solid-phase microextraction"

**General characteristics of the work.** The study is aimed at optimizing two “green” methods for the determination of pesticides in grape samples based on vacuum headspace solid-phase microextraction (Vac-HS-SPME) and miniaturized headspace solid-phase microextraction (Mini-HS-SPME), followed by gas chromatography–mass spectrometric (GC–MS) detection.

**Relevance and degree of development of research.** Grapes (*Vitis vinifera*) are one of the key fruit and berry crops due to their high nutritional, biological, and economic value. Chemical pesticides are widely used for their protection; however, their regular application leads to the accumulation of residues, posing a potential risk to human health. Existing sample preparation methods for pesticide analysis are often multi-step, inefficient for samples with complex matrices, and require significant amounts of toxic solvents, generating considerable chemical waste, which contradicts the principles of “green” analytical chemistry.

An alternative to standard sample preparation methods is solid-phase microextraction (SPME) combined with gas (GC) or liquid chromatography (LC). SPME is based on the sorption of analytes onto a polymeric coating followed by thermal desorption directly into the injection system. The advantages of SPME include high selectivity, reduced matrix effects without the use of large volumes of solvents, the possibility of automation, and compliance with the principles of green chemistry, making this technique an optimal choice for rapid, accurate, and environmentally safe quality control of grapes. Yuchen Guo, Soleyman Moinfar, and Emanuela Gionfriddo with co-authors used headspace SPME (HS-SPME) for the extraction of organophosphorus pesticides and pyrethroids from grapes and grape juice. Erika Souza-Silva and co-authors applied SPME with direct immersion of the fiber into the sample for the analysis of pesticides in grapes. Hamid Hashemi-Moghaddam, Amir Ramezani, Füsun Okçu Pelit, and Shima Amini employed headspace SPME with nanocomposite coatings to determine organophosphorus pesticides in grapes and apples. The aforementioned studies demonstrate significant progress in the development of green, sensitive, and selective SPME-based methods for pesticide analysis in various fruit matrices. However, despite previous research using different coatings and SPME configurations, no studies up to date have focused on the application or optimization of vacuum-assisted headspace SPME (Vac-HSSPME) and miniaturized headspace SPME (Mini-HSSPME) for the extraction of pesticides from grape samples followed by GC–MS analysis. Therefore, the development and optimization of innovative SPME-based methods for pesticide analysis in grapes represent a novel and relevant research direction.

**The aim of the study.** The research focuses on the optimization of two “green” methods for the determination of pesticides in grape samples based on vacuum and miniaturized headspace solid-phase microextraction, followed by gas chromatography–mass spectrometric detection.

In accordance with the research objective, the following **tasks** were set**:**

1. To perform a comparative analysis of modern analytical methods for the determination of pesticides in grape samples using the GAPI tool.

2. Optimize the parameters of Vac-HSSPME for pesticides in grapes and establish the analytical characteristics for their quantitative determination.

3. Optimize the parameters of Mini-HSSPME for pesticides in grapes and establish the analytical characteristics for their quantitative determination.

4. To evaluate the proposed Vacuum-HS-SPME and Mini-HS-SPME methods using the GAPI, AGREEprep, and BAGI tools.

**Objects of the study.** Grape samples.

**The subject of the study.** Pesticides - metribuzin, oxyfluorfen, fluroxypyr, epoxiconazole, boscalid, quizalofop-methyl, atrazine, simazine, prometryn, cyanazine, tebuconazole.

**The methods of research.** Solid-phase microextraction followed by gas chromatography-mass spectrometric detection.

**Scientific novelty of the study.**

1. For the first time, the parameters for the determination of metribuzin, oxyfluorfen, fluroxypyr, epoxiconazole, boscalid, and quizalofop-methyl in grape samples were optimized and established using Vac-HSSPME combined with GC-MS detection, and the analytical characteristics were determined.

2. For the first time, the parameters for the determination of atrazine, simazine, prometryn, cyanazine, tebuconazole, metribuzin, fluroxypyr-methyl, epoxiconazole, boscalid, and quizalofop-methyl in grape samples were optimized and established using Mini-HSSPME combined with GC-MS detection, and the analytical parameters were determined.

3. For the first time, the environmental efficiency of existing methods and two optimized methods based on Vac-HSSPME and Mini-HSSPME combined with GC-MS detection was assessed using the greenness evaluation tools GAPI, AGREEprep, and BAGI simultaneously, which resulted in the development of a comprehensive environmental efficiency metric for the grape matrix.

**Validity and reliability of the results obtained.**

The reliability of the experimental data is confirmed using controlled testing conditions: during the optimization of the methods, one or two independent variables were varied while the remaining parameters were kept constant. The dependent variables characterized the analytical performance of the developed methods, including limits of detection, recovery, response linearity, accuracy, and reproducibility. All measurements were conducted in a series of parallel experiments (n=3–5), ensuring the statistical reliability of the obtained data. For the identification and quantitative analysis of pesticides, an extremely sensitive gas chromatography system coupled with mass spectrometric detection was used, which ensured the selectivity and accuracy of the results.

**Theoretical and practical significance of the study.**

The **theoretical significance** of this study lies in advancing the comprehension of mechanisms and scientific principles underlying the use of vacuum and miniaturized headspace solid-phase microextraction for the quantitative determination of pesticides in grapes. For the first time, the influence of parameters such as sorbent fiber type, temperature, extraction and desorption time, pre-incubation time, salt effect, medium pH, vacuum duration, and sample volume was thoroughly investigated. Optimal values of these parameters were established, which significantly improved the sensitivity and efficiency of the analysis. For the first time, Vac-HSSPME was adapted and optimized for the determination of semi-volatile pesticides in grape matrices, enhancing sensitivity without increasing resource consumption, thereby broadening the applicability of this approach.

For the first time, a comprehensive assessment of the greenness and practical feasibility of the developed methods was carried out using modern tools of green analytical chemistry - GAPI, AGREEprep, and BAGI. Thus, this work contributes to the advancement of green analytical chemistry principles in food quality control and expands the theoretical basis for the transferability and automation of HSSPME in complex food matrices.

The **practical significance** is confirmed by the implementation of the optimized methods in the activities of an analytical laboratory responsible for grape quality control. Based on the research results, an implementation act for the two methods was issued at the Kazakh Scientific Research Institute of Fruit and Vegetable Growing LLP, and a draft measurement procedure (MP) was prepared for subsequent certification. The application of improved approaches ensures rapid and

environmentally safe determination of pesticides in grapes, meets modern requirements, and contributes to strengthening food security. The obtained results and the developed MP can be used in the development and certification of national measurement procedures and in updating standards in the field of food quality control, thereby contributing to food security assurance.

**The main provisions to be defended.**

1. The optimal parameters of solid-phase microextraction (Vac-HSSPME) for the determination of metribuzin, oxyfluorfen, fluroxypyr, epoxiconazole, boscalid, and quizalofop-methyl in grape samples by GC-MS are as follows: extraction temperature – 70°C, extraction time – 40 minutes, sorption fiber based on polyethylene glycol (PEG, 60 μm), liquid sample volume – 1 mL, vacuuming time – 60 seconds (pumping rate 170 L/min), pH – 10. The limits of detection (LOD) and quantification (LOQ) for the analyzed pesticides are in the ranges of 0.02–1.3 ng/mL and 0.1–4.4 ng/mL, respectively (using the Vac-SPME Arrow setup).

2. The optimal parameters of solid-phase microextraction (Mini-HSSPME) for the determination of atrazine, simazine, prometryn, cyanazine, tebuconazole, metribuzin, fluroxypyr-methyl, epoxiconazole, boscalid, and quizalofop-methyl in grape samples by GC-MS are as follows: extraction temperature – 90 °C, extraction time – 30 minutes, pre-incubation time – 10 minutes, sorption fiber based on polydimethylsiloxane (PDMS, 100 μm), sample volume – 200 μL with salt addition, pH – 6. The limits of detection (LOD) and quantification (LOQ) for the analyzed pesticides are in the ranges of 0.05–3.0 ng/mL and 0.2–10.1 ng/mL, respectively.

3. The scores calculated using the AGREEprep and BAGI tools for the Vacuum-HSSPME-based method were 0.67 and 60.0 points, respectively; for the Mini-HSSPME-based method, they were 0.79 and 67.5 points, respectively.

**Main results of the study.**

1. Based on the literature review of the **first article**, “Modern Analytical Methods for the Analysis of Pesticides in Grapes: A Review”, it was established that the most common sample preparation methods for determining pesticide residues in grapes are QuEChERS (44%), solid-phase extraction (18%), and solid-phase microextraction (18%). The solvents most frequently used are acetonitrile, ethyl acetate, and acetone. Among analytical methods, liquid chromatography predominates (56%), followed by gas chromatography (33%). The main types of detectors are tandem mass spectrometry (44%), mass spectrometry (28%), and tandem quadrupole time-of-flight mass spectrometry (19%). These methods provide detection limits ranging from 0.01 to 10 mg/kg with extraction efficiencies of 70–120%.

Currently, there is no universal method for the analysis of pesticide residues in grapes, due to the diversity of chemical classes and differences in laboratory equipment. A promising direction remains the development of rapid, accurate, sensitive, cost-effective, and environmentally friendly analytical methods.

2. In the **second article**, “Greenness Evaluation of Sample Preparation Methods by GAPI for the Determination of Pesticides in Grapes: A Review”, a systematic analysis is presented, showing that in recent years, the assessment of the environmental friendliness of sample preparation methods for pesticide analysis in grapes has become a priority task in analytical chemistry. Tools such as GAPI, Eco-Scale, NEMI, and AGREE are applied, considering solvent volume and toxicity, waste generation, energy consumption, number of steps, and operator hazard.

Using GAPI, 42 sample preparation methods were evaluated, including liquid–liquid microextraction, QuEChERS, pressurized liquid extraction, solid-phase extraction, single-drop microextraction, and solid-phase microextraction. It was found that the most environmentally friendly methods are miniaturized techniques with low solvent volumes (less than 1 mL), non-toxic reagents, and simplified procedures, particularly SPME and single drop microextraction combined with GC. Therefore, the development and optimization of more miniaturized, simple, environmentally friendly, and precise SPME-based methods remain highly relevant.

3. Based on the previously described reviews, the task was set to optimize an alternative approach for pesticide analysis in grapes. The **third article** presents the results of optimizing the vacuum headspace solid-phase microextraction (Vac-HSSPME) method. The optimal conditions include: sample volume - 1 mL, pH 10, extraction temperature - 70 °C, extraction time - 40 minutes, vacuum - 900 mbar for 60 seconds, fiber coating - polyethylene glycol (PEG, 60 μm), without salt addition.

Using a Miniert vacuum valve within a linear range of 0.05–1.92 μg/mL, limits of detection of 0.11–0.61 μg/mL were achieved with correlation coefficients ranging from 0.960 to 0.999. With a Vac-SPME Arrow valve within a linear range of 5–1000 ng/mL, detection limits were 0.02–1.3 ng/mL with correlation coefficients ranging from 0.992 to 0.999. The Vac-HSSPME/GC-MS method showed higher efficiency compared to classical HSSPME/GC-MS: 8–9 times for quizalofop-methyl, 3 times for boscalid, and 2–2.5 times for fluroxypyr, metribuzin, and epoxiconazole.

The method's limitations include the need for special vacuum valves, which do not always provide stable pressure, and the requirement for a certain level of operator qualification. Therefore, further optimization of simpler, more reliable, and environmentally friendly analytical methods remains relevant.

4. In the **fourth article**, considering the limitations of the Vac-HSSPME method, an alternative approach based on miniaturized headspace solid-phase microextraction (Mini-HSSPME) was optimized for the analysis of various classes of pesticides in grapes, with an emphasis on green chemistry principles. The optimal parameters are: extraction temperature – 90 °C, extraction time – 30 minutes, sample volume – 200 μL, pH 6, incubation – 10 minutes, fiber – polydimethylsiloxane (PDMS, 100 μm). The Mini-HSSPME/GC-MS method provided limits of detection of 0.09–3.0 ng/mL and quantification limits of 0.2–10.1 ng/mL. Recoveries for the target pesticides ranged from 60–112%.

The greenness and practicality of the method assessed using GAPI, AGREEprep (0.79), and BAGI (67.5), confirmed its low environmental impact and high applied value. Thus, Mini-HSSPME/GC-MS represents a sensitive and environmentally safe approach for pesticide analysis in grapes.

The four presented articles demonstrate internal coherence and logical sequence, forming a comprehensive study. The experimental data obtained fully corresponds to the stated objectives and confirms the achievement of the main goal - the development of accurate and environmentally friendly methods for pesticide analysis.

**Approval of the practical results of the research.**

The main research results were presented at the following conferences:

1) International Scientific Conference "International Conference Food BioTech", Saint Petersburg, Russia, August 23–24, 2021;

2) International Scientific Conference for Students and Young Scientists "Farabi Alemi", Almaty, Kazakhstan, April 6–8, 2022;

3) International Scientific Conference for Students and Young Scientists "Farabi Alemi", Almaty, Kazakhstan, April 6–7, 2023;

4) International Scientific Conference "25th International Symposium on Advances in Extraction Technologies (ExTech)", Tenerife, Spain, July 18–21, 2023;

5) International Scientific Conference "The International Symposium on Chromatography (ISC)", Liverpool, United Kingdom, October 6–10, 2024;

6) International Scientific Conference "XXIII European Conference on Food Chemistry", Bratislava, Slovakia, June 11–13, 2025;

7) International Scientific Conference "XXII European Conference on Analytical Chemistry (EuroAnalysis)", Barcelona, Spain, August 31 – September 4, 2025.

**Publications and the author's contribution to the research work.**

The results of the completed work are reflected in the following scientific publications:

1) Y. Syrgabek, M. Alimzhanova, Modern Analytical Methods for the Analysis of Pesticides in Grapes: A Review, Foods. 11 (2022) 16–23. https://doi.org/10.3390/foods11111623 (WoS - Q1, Impact Factor 5.2; Scopus - 97th percentile, CiteScore 5.8 according to 2022 data). CRediT: methodology, formal analysis, writing - original draft, data curation and visualization.

2) Y. Syrgabek, M. Alimzhanova, P.A. García-encina, J. Jose, L. Rebeca, Greenness evaluation of sample preparation methods by GAPI for the determination of pesticides in grape: A review, Trends in Environmental Analytical Chemistry. 39 (2023). https://doi.org/10.1016/j.teac.2023.e00206 (WoS - Q1, Impact Factor 11.1; Scopus - 99th percentile, CiteScore 21.2 according to 2023 data). CRediT: writing - original draft, data curation and visualization, methodology.

3) Y. Syrgabek, M. Alimzhanova, S. Yegemova, S. Batyrbekova, Vacuum-assisted headspace-solid phase microextraction of pesticides in grape samples, Advances in Sample Preparation. 11 (2024) 1–15. https://doi.org/10.1016/j.sampre.2024.100123 (WoS - Q1, Impact Factor 6.5; Scopus - 82nd percentile, CiteScore 7.5 according to 2024 data). CRediT: writing - original draft, experimentation.

4) M. Alimzhanova, Y. Syrgabek*, P.A. García-encina, Eco-Friendly Miniaturized Solid Phase Microextraction for Pesticide Analysis in Grapes, Journal of Separation Science. 48 (2025). https://doi.org/10.1002/jssc.702041of14 (WoS – Q2, Impact Factor 2.8; Scopus – 66th percentile, CiteScore 6.1 according to 2024 data). CRediT: validation, writing – original draft, experimentation.

**Relevance of the dissertation to research and government programs.**

This study was conducted within the framework of:

- grant funding for scientific and/or scientific-technical projects for 2020-2022 under the topic “Improvement and development of highly sensitive methods to ensure food safety in Kazakhstan” project IRN AP08857501;

- grant funding for scientific and/or scientific-technical projects for 2023-2025 under the topic “Development of a biological product to increase the yield of grapes” project IRN AP19680563;

- grant funding for research by young scientists under the “Zhas Galym” project for 2024-2026 on the topic “Development of highly sensitive "green" methods for the analysis of residual amounts of pesticides in grapes” project IRN AP22685606.