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NEW APPROACHES FOR MICROEXTRACTION OF XENOBIOTICS FOR THEIR DETERMINATION IN FOOD PRODUCTS

Scientific specialty: 1.4.2. Analytical chemistry

THESIS

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CONTENT

LIST OF ABBREVIATIONS AND SYMBOLS

BAA – biologically active additive;

CE – capillary electrophoresis;

CE-CD – capillary electrophoresis with conductometric detection;

CE-UV – capillary electrophoresis with ultraviolet photometric detection;

CMC – critical micelle concentration;

Cryo-SEM – Cryo-scanning electron microscopy;

DES – deep eutectic solvents;

DLLME – dispersive liquid-liquid microextraction;

EF – enrichment factor;

ETA-AAS – atomic absorption spectrometry with electrothermal atomization;

FIA – flow injection analysis;

GC-AED – gas chromatography with atomic emission detector;

GC-FID – gas chromatography with flame ionization detector;

GC-FPD – gas chromatography with flame photometric detector;

GC-MS – gas chromatography with mass spectrometric detection;

GC-NPD – gas chromatography with nitrogen-phosphorus detector;

HLME – homogeneous liquid microextraction;

HPLC – high performance liquid chromatography;

HPLC-FLD – high performance liquid chromatography with fluorimetric detection;

HPLC-MS/MS – high performance liquid chromatography with tandem mass spectrometry;

HPLC-UV – high performance liquid chromatography with ultraviolet photometric detection;

HSA – headspace analysis;

HSME - headspace microextraction;

IL – ionic liquid;

K – distribution coefficient;

LLME – liquid-liquid microextraction;

LOD – limit of detection;

NP – nanoparticle;

QuEChERS - quick, easy, cheap, effective, rugged, safe sample preparation technique;

R – relative bias (%);

RSD – relative standard deviation;

SDME – single drop microextraction;

SHS – switchable hydrophilicity solvent;

SIA – sequential injection analysis;

SMS – supramolecular solvent;

SPE – solid phase extraction;

SPME – solid phase microextraction;

SWIA – stepwise injection analysis;

US – ultrasonic field.

INTRODUCTION

The relevance of the study

Food quality control plays an important role in protecting the health of consumers and providing them with safe products. The ever-increasing need for such control necessitates the development of highly efficient and affordable methods for chemical analysis of food products. An important step in chemical analysis is preliminary sample preparation, which most often involves separation and concentration of target analytes from complex food matrices. Typically, sample preparation is the most time-consuming and often limiting step in the analysis, which affects the accuracy of the results obtained. In turn, separation and concentration methods are subject to a number of requirements related to increasing their productivity, miniaturization, environmental friendliness, and the possibility of combining them with physical and chemical methods of analysis. New opportunities for chemical analysis of food products are opened by microextraction methods, providing fast mass transfer and phase separation at minimum consumption of solvents. In this direction the actual task is the development of selective and reliable methods of microextraction, including the use of new safe extraction systems and materials (sorbents). At the same time, there is a need to solve the problem of automation of microextraction methods, which allows to increase the productivity of analysis and reduce labor costs, to ensure high reproducibility of the results.

The relevance of the performed research is confirmed by the awarding by the Scientific Council of the Russian Academy of Sciences on Analytical Chemistry of the Prize for young scientists for the development of automated microextraction methods of separation and concentration, 2017; the Medal of the Japanese Association for Flow Injection Analysis (JAFIA) for the development of automated methods of chemical analysis, 2019; the Presidential Prize in Science and Innovation for Young Scientists for the development of materials and methods for instrumental chemical analysis of complex samples, 2022; Youth Award of the Government of St. Petersburg in the nomination “In the field of science and technology”, 2023; as well as the support of research in this area

by the Russian Foundation for Basic Research (*grants №16-33-00037, №16-33-60126*) and the Russian Science Foundation (*grants №19-73-00121, №24-23-00052*).

The extent of prior research of the issue under study

The main principles of modern “green” analytical chemistry are: miniaturization, automation and avoidance of toxic solvents. Thus, new opportunities for the analysis of food products are opened by microextraction methods, which have recently received special attention [1]. One of the ways to improve liquid microextraction methods is the search and study of new environmentally safe materials and solvents for their realization. Thus, for the first time we have proposed natural terpenoids as extractants for efficient extraction of xenobiotics from foodstuffs. New extraction systems include switchable hydrophilicity solvents (SHSs) (e.g., medium-chain fatty acids and tertiary amines) as well as supramolecular solvents. Prior to our studies, no work had been published on the use of SHS for food quality control tasks, including the first time we showed the possibility of using medium-chain fatty acids in membrane microextraction, providing efficient extraction of target analytes. In turn, primary amines as precursors of supramolecular extraction systems used in the analysis of food products have not been presented in the literature. In recent years, deep eutectic solvents (DES) have attracted great interest and have wide potential for application in analytical practice, including as extraction solvents. However, the possibility of *in situ* DES formation by heating its precursors directly in a mixture with a solid-phase food sample has not been shown before.

The literature analysis has shown that among the liquid extraction methods much attention is paid to dispersive liquid-liquid microextraction (DLLME) [2,3], which has high expressiveness and efficiency due to the rapid mass transfer of analytes into the microdroplets of extraction solvent. However, its conventional variant requires replacement or complete rejection of the dispersive solvent (usually polar organic solvent) that reduces the extraction efficiency. For the first time we have shown the possibility of using a volatile solvent as a dispersive solvent, the removal of which from the system was due to heating. Also, SHSs have not been previously studied as dispersive solvents.

Headspace microextraction (HSME) offers great opportunities for the determination of volatile analytes in complex samples [4]. The main advantage of HSME is the absence of contact between the sample and the acceptor phase, which eliminates the interference effect of matrix, and, in addition, makes it possible to analyze solid samples, including food products. Currently, the attention of analysts is directed to the search for new materials for performing headspace microextraction that would meet the requirements of “green” analytical chemistry. Moving in this direction, we have shown for the first time the possibility of using magnetic sorbents for static and dynamic HSME of volatile analytes from liquid and solid-phase food samples.

Currently, various flow methods are proposed for automation of the main stages of chemical analysis (including sample preparation), which are characterized by high sample throughput. Moreover, depending on the specificity of a particular flow method, they allow to minimize the consumption of sample and reagent solutions in comparison with stationary counterparts of the same methods [5]. In the analysis of complex samples, including food products, there are problems limiting the use of flow methods: low selectivity and sensitivity of analysis, complexity of automation of sample preparation. We have realized new approaches of automated microextraction for extraction and concentration of xenobiotics from liquid and solid-phase food samples for their subsequent determination by chromatographic methods.

The analysis by the author of the thesis of the current state of research in the field of automation and miniaturization of chemical analysis of food products resulted in five review articles: Crit. Rev. Anal. Chem. 46 (2016) 374 - 388; Talanta 179 (2018) 246 - 270; J. of Anal. Chem. 74 (2019) 846-855; ChemTexts (2021) 7:24; TrAC 178 (2024) 11783.

Aims and objectives of the study

The aim of the study was to develop new approaches to microextraction of xenobiotics using nanomaterials and last generation solvents for determination of target analytes in liquid and solid-phase food samples by spectral and chromatographic methods.

In order to achieve the goal, it was necessary to solve the following **tasks**:

- to study the possibility of selective extraction of volatile substances from liquid and solid-phase samples by methods of static and dynamic headspace microextraction on ferromagnetic nanoparticles;

- to reveal the possibility of using medium-chain fatty acids as switchable hydrophilicity solvents for membrane microextraction of organic substances (capable of ionization) from suspended samples;

- to propose a mechanism of microextraction of polar organic analytes from solid-phase food matrices into deep eutectic solvent based on quaternary ammonium salt and alcohol, formed *in situ* during sample preparation;

- to propose a mechanism of microextraction of analytes (capable of ionization) into deep eutectic solvent based on medium-chain fatty acids and natural terpenoids;

- to develop a new extraction system based on primary amine and medium-chain fatty acid for micellar microextraction of polar organic analytes from suspended samples;

- to propose a new extraction system based on hydrophobic medium-chain fatty acid and its salt for microextraction of nonpolar organic analytes;

- to substantiate the possibility of using natural terpenoid (menthol) as a “green” extraction solvent to perform dispersive liquid-liquid microextraction from liquid samples without introduction of dispersive solvent;

- to propose an approach of dispersive (organic) solvent removal from the extraction system during dispersive liquid-liquid microextraction, based on the phase transition of dispersive solvent (liquid - gas) at heating;

- to develop an automated microextraction approaches based on the principles of flow methods;

- to confirm analytical capabilities of the developed approaches on examples of chemical analysis of real objects (products of plant and animal origin, biologically active additives, alcoholic and non-alcoholic beverages).

Scientific novelty

The scientific novelty of the thesis lies in the development of fundamentally new approaches for miniaturization and automation of extraction methods based on the use of new extraction systems and materials and designed for chemical food analysis.

The possibility of using ferromagnetic nanoparticles in headspace microextraction of volatile substances from liquid and solid-phase food samples has been studied and substantiated for the first time. New methods of static and dynamic headspace microextraction for pre-concentration of volatile analytes from food products on magnetic nanoparticles have been developed. The use of magnetic nanoparticles in the schemes of headspace solid-phase microextraction provided high extraction efficiency.

“Green” extraction systems based on natural terpenoids for the realization of express DLLME of organic substances are proposed. In order to eliminate the use of dispersive solvent in DLLME, approach based on phase transitions of the organic solvent (terpenoid) have been proposed. The techniques provide the ability to increase enrichment factors.

To remove the dispersive (organic) solvent from the extraction system during DLLME, a method based on the phase transition of the dispersive solvent – liquid - gas at heating is proposed. The developed approach provides effective dispersion of the extraction solvent phase and simultaneous elimination of the interfering effect of dispersive solvent on mass transfer.

The possibility of using medium-chain fatty acid (C5-C9) as SHSs for the extraction of organic analytes capable of ionization was studied. A new approach of membrane microextraction based on the extraction of target analytes from the aqueous phase (including suspensions) into hydrophobic membranes impregnated with fatty acids was developed. The membrane allows to reliably retain microvolumes of extraction solvent in the process of mass transfer, and also prevents macrocomponents of the suspended sample from entering the extract, which eliminates the need for additional procedures during sample preparation.

The efficiency of mass transfer of analytes capable of ionization in DESs based on hydrophobic medium-chain fatty acids and terpenoids has been proved. A new approach of DLLME based on dispersion of the DES phase by carbon dioxide, formed *in-situ* as a result of a chemical reaction, has been proposed. The developed approach provides fast phase separation without centrifugation, which allowed to automate it on the principles of flow methods and thereby increase the productivity of analysis, reduce labor costs, and ensure high reproducibility of results.

A microextraction method based on *in-situ* DES formation by heating its precursors (quaternary ammonium salt and medium-chain alcohol) directly in the mixture with the sample was proposed for the extraction of polar organic analytes from the solid-phase samples of animal origin. The developed method allowed to increase the extraction recovery of target analytes from solid-phase samples.

A new extraction system based on primary amine and fatty acid was proposed; a method of micellar microextraction of polar organic analytes from solid-phase samples was developed. The proposed extraction system provides the formation of phases (extracts) with low viscosity, which allows their chromatographic analysis without additional dilution.

A new extraction system based on hydrophobic medium-chain fatty acid and its salt for DLLME of nonpolar organic analytes from aqueous food samples was proposed.

For automated extraction and pre-concentration of xenobiotics by dispersive liquid-liquid and homogeneous liquid-liquid microextraction, flow-based schemes providing the possibility of increasing the precision and productivity of chemical food analysis have been developed.

Theoretical and practical value of the study

Theoretical and practical significance of the thesis is determined by the development of microextraction and flow methods: a set of new methods and approaches based on the principles of liquid-liquid and headspace microextraction and flow methods that provide miniaturization and automation of chemical analysis of liquid and solid-phase food products is proposed. New extraction systems for realization of different

variants of liquid-liquid microextraction (membrane, micellar and DLLME), which meet the principles of “green” analytical chemistry, have been proposed and studied. Ferromagnetic nanomaterials were proposed as efficient sorbents for static and dynamic headspace solid-phase microextraction of volatile analytes. Combined methods for express and highly sensitive determination of organic and inorganic xenobiotics (antibiotics, pesticides, preservatives, polycyclic aromatic hydrocarbons (PAHs), phenols, Se (IV)) in liquid and solid-phase food products, including microextraction of analytes and their detection by chromatographic and spectral methods, have been developed. The list of food products is given in the experimental part.

The significance of the work is confirmed by the citation indicators of the main publications on the research topic. According to “Scopus” database, the author’s h-index for 2024 is 15.

Research methods and techniques

The modern instrumental base of St. Petersburg State University was used. In particular, chromatographic (HPLC-UV, HPLC-FLD, HPLC-MS/MS, GC-MS, GC-FID), spectral (AAS) and flow methods of analysis were used. The obtained organic phases were studied by Cryo-scanning electron microscopy (Cryo-SEM), coulometric Karl Fischer titration and infrared spectroscopy. For statistical processing of the results, modern software tools “Excel” and “Origin” were used. The correctness of the obtained results was confirmed by the “add-found” method and/or reference methods. The bibliographic databases “Elibrary” and “Scopus” were used to analyze the literature data.

Measure of reliability and evaluation of research work

The work was carried out at the modern scientific and methodological level in the volume sufficient for the generalizations and justification of conclusions given in the work. The reliability of the obtained results is confirmed by their reproducibility and the use of a set of modern physicochemical methods (HPLC-UV, HPLC-FLD, HPLC-MS/MS, GC-MS, GC-FID, AAS), as well as provided by the use of mathematical statistics in the processing of experimental data. Scientific statements, conclusions and

recommendations formulated in the work are argued, reliable and logically follow from the experimental data obtained by the author and their analysis, as well as comparison with the literature data.

The results of the work and the main statements of the thesis were personally presented by the author in the form of oral, invited and plenary reports at the following scientific conferences: All-Russian Conference “Ecoanalytics-2016” (Uglich, 2016), XX International Conference “Flow Injection Analysis and Related Techniques” (Palma de Mallorca, Spain, 2016), X International Conference of Young Scientists in Chemistry “Mendeleev-2017” (St. Petersburg, Russia, 2017), International Congress “International Congress on Analytical Sciences 2017” (ICAS2017) (Haikou, China, 2017), Annual international analytical conference “BIT’s 6th Annual Conference of AnalytiX 2018” (AnalytiX-2018) (Miami, USA), V All-Russian Symposium with international participation “Separation and Concentration in Analytical Chemistry and Radiochemistry” (Krasnodar, 2018), XIV International Conference “Flow Analysis” (FIA-2018) (Bangkok, Thailand, 2018), XX International Conference “Euroanalysis XX” (Istanbul, Turkey, 2019), IV International Conference “International Caparica Christmas Conference on Sample Treatment” (online, Costa da Caparica, Portugal, 2020), VI All-Russian Symposium “Separation and Concentration in Analytical Chemistry and Radiochemistry” (Krasnodar, 2021), V International Conference “International Caparica Christmas Conference on Sample Treatment” (online, Costa da Caparica, Portugal, 2021), IV Congress of Analysts of Russia (Moscow, 2022), XVI International Conference “16th Asian Conference on Analytical Sciences” (ASIANALYSIS XVI) (Kuala Lumpur, Malaysia, 2023), Scientific School “Chemistry of the Future” (Irkutsk, Russia, 2023), School-Conference of Young Scientists of the Perm Region (Perm, Russia, 2023), XI All-Russian Congress of Councils of Young Scientists and Student Scientific Societies (Nizhny Novgorod, 2023), VIII All-Russian Youth Scientific Forum “Science of the Future - Science of the Youth” (Orel, 2023), XXII Mendeleev Congress on General and Applied Chemistry (Sirius, Sochi, Russia, 2024).

Publications on the thesis

The results of the research were published in 19 articles (including 5 reviews on the topic of the thesis) in international peer-reviewed journals mainly in the first quartile (Q1), indexed by Scopus and Web of Science databases (abstracts are not taken into account):

1. Vakh C., Falkova M., **Timofeeva I.** et al. Flow analysis: A novel approach for classification // Critical Reviews in Analytical Chemistry. 2016. Vol. 46, № 5. P. 374–388. URL: https://doi.org/10.1080/10408347.2015.1087301. Q1 (IF 4,0 – 2016) – [6].

2. **Timofeeva I.**, Timofeev S., Moskvin L. et al. A dispersive liquid-liquid microextraction using a switchable polarity dispersive solvent. Automated HPLC-FLD determination of ofloxacin in chicken meat // Analytica Chimica Acta. 2017. Vol. 949. P. 35–42. URL: https://doi.org/10.1016/j.aca.2016.11.018. Q1 (IF 5,1 – 2017) – [7].

3. **Timofeeva I.**, Shishov A., Kanashina D. et al. On-line in-syringe sugaring-out liquid-liquid extraction coupled with HPLC-MS/MS for the determination of pesticides in fruit and berry juices // Talanta. 2017. Vol. 167. P. 761–767. URL: https://doi.org/10.1016/j.talanta.2017.01.008. Q1 (IF 4,2 – 2017) – [8].

4. Pochivalov A., **Timofeeva I.**, Vakh C. et al. Switchable hydrophilicity solvent membrane-based microextraction: HPLC-FLD determination of fluoroquinolones in shrimps // Analytica Chimica Acta. 2017. Vol. 976. P. 35–44. URL: https://doi.org/10.1016/j.aca.2017.04.054. Q1 (IF 5,1 – 2017) – [9].

5. **Timofeeva I.**, Kanashina D., Moskvin L. et al. An evaporation-assisted dispersive liquid–liquid microextraction technique as a simple tool for high performance liquid chromatography tandem–mass spectrometry determination of insecticides in wine // Journal of Chromatography A. 2017. Vol. 1512. P. 107–114. URL: https://doi.org/10.1016/j.chroma.2017.07.034. Q1 (IF 3,7 – 2017) – [10].

6. **Timofeeva I.I.**, Vakh C.S, Bulatov A.V. et al. Flow analysis with chemiluminescence detection: Recent advances and applications // Talanta. 2018. Vol. 179. P. 246–270. URL: https://doi.org/10.1016/j.talanta.2017.11.007. Q1 (IF 4,9 – 2018) – [11].

7. **Timofeeva I.**, Kanashina D., Kirsanov D. et al. A heating-assisted liquid-liquid microextraction approach using menthol: Separation of benzoic acid in juice samples followed by HPLC-UV determination // Journal of Molecular Liquids. 2018. Vol. 261. P. 265–270. P. 1187−1197. URL: https://doi.org/10.1016/j.molliq.2018.04.040. Q1 (IF 4,6 – 2018) – [12].

8. **Timofeeva I.**, Kanashina D., Stepanova K. et al. A simple and highly-available microextraction of benzoic and sorbic acids in beverages and soy sauce samples for high performance liquid chromatography with ultraviolet detection // Journal of Chromatography A. 2019. Vol. 1588. P. 1–7. URL: https://doi.org/10.1016/j.chroma.2018.12.030. Q1 (IF 4,05 – 2019) – [13].

9. **Timofeeva I.**, Alikina M., Vlasova A. et al. Fe_3O_4-based composite magnetic nanoparticles for volatile compound sorption in the gas phase: determination of selenium (IV) // Analyst. 2019. Vol. 144, № 1. P. 152−156. URL: https://doi.org/10.1039/C8AN01894D. Q1 (IF 4,0 – 2019) – [14].

10. Vakh C.S., **Timofeeva I.I.**, Bulatov A.V. Automation of microextraction preconcentration methods based on stepwise injection analysis // Journal of Analytical Chemistry. 2019. V. 74. № 11. P. 846−855. URL: https://doi.org/10.1134/S0044450219110112. Q4 (IF 0,5 – 2019) – [15].

11. Kanashina D., Pochivalov A., **Timofeeva I.** et al. Mixed surfactant systems based on primary amine and medium-chain fatty acid: Micelle-mediated microextraction of pesticides followed by the GC–MS determination // Journal of Molecular Liquids. 2020. Vol. 306. P. 112906. URL: https://doi.org/10.1016/j.molliq.2020.112906. Q1 (IF 6,2 – 2020) – [16].

12. **Timofeeva I.**, Alikina M., Osmolowsky M. et al. Magnetic headspace adsorptive microextraction using Fe_3O_4@$Cr(OH)_3$ nanoparticles for effective determination of volatile phenols // New Journal of Chemistry. 2020. Vol. 44, № 21. P. 8778−8783. URL: https://doi.org/10.1039/D0NJ00854K. Q1 (IF 3,6 – 2020) – [17]

13. **Timofeeva I.**, Stepanova K., Shishov A. et al. Fluoroquinolones extraction from meat samples based on deep eutectic solvent formation // Journal of Food

Composition and Analysis. 2020. Vol. 93. P. 103589. URL: https://doi.org/10.1016/j.jfca.2020.103589. Q1 (IF 4,6 – 2020) – [18].

14. **Timofeeva I.**, Stepanova K., Bulatov A. In-a-syringe surfactant-assisted dispersive liquid-liquid microextraction of polycyclic aromatic hydrocarbons in supramolecular solvent from tea infusion // Talanta. 2021. Vol. 224. P. 121888. URL: https://doi.org/10.1016/j.talanta.2020.121888. Q1 (IF 6,6 – 2021) – [19].

15. **Timofeeva I**., Nugbienyo L., Pochivalov A. et al. Flow-based methods and their applications in chemical analysis // ChemTexts. 2021. Vol. 7, № 4. URL: https://doi.org/10.1007/s40828-021-00149-8. Q2 – [20].

16. Barbayanov K., **Timofeeva I.**, Bulatov A. An effervescence-assisted dispersive liquid-liquid microextraction based on three-component deep eutectic solvent for the determination of fluoroquinolones in foods // Talanta. 2022. Vol. 250. P. 123709. URL: https://doi.org/10.1016/j.talanta.2022.123709. Q1 (IF 6,1 – 2022) – [21].

17. **Timofeeva I.I.**, Barbayanov K.A., Bulatov A.V. Automated liquid–liquid microextraction of fluoroquinolones for their subsequent chromatographic determination // Journal of Analytical Chemistry. 2023. V. 78, № 2. P. 159–165. URL: https://doi.org/10.31857/S0044450223020135. Q3 (IF 1,0 – 2023) – [22].

18. Kochetkova M.A., **Timofeeva I.I.**, Bulatov A.V. Dispersive liquid–liquid microextraction of preservatives for their chromatographic determination in beverages // Journal of Analytical Chemistry. 2023. V. 78. № 7. P. 630–636. URL: https://doi.org/10.31857/S0044450223070095. Q3 (IF 1,0 – 2023) – [23].

19. **Timofeeva I.**, Barbayanov K., Kochetkova M. et al. Recent developments in sample pretreatment techniques for the determination of fluoroquinolones in foods // TrAC - Trends in Analytical Chemistry. Elsevier B.V., 2024. Vol. 178. P. 117831. URL: https://doi.org/10.1016/j.trac.2024.117831. Q1 (IF 13,1 – 2023) – [24].

Personal contribution of the author

The author independently carried out the choice of scientific direction. In all research papers the author has a leading role in setting the aim and objectives, selecting research objects, planning and conducting experiments, interpreting and generalizing the

data. In 17 out of 19 publications on the topic of the thesis, the author is corresponding author (except two review papers). Under the supervision of the author 16 course and graduate qualification works were prepared and defended on the topic of this work, including 1 specialist's, 5 bachelor's and 3 master's theses. The main part of the work was carried out with the financial support of the Russian Foundation for Basic Research (grants № 16-33-00037, № 16-33-60126) and the Russian Science Foundation (grants № 19-73-00121, № 24-23-00052).

Structure of the work

Thesis is presented on 189 pages of printed text and includes such sections as Content, List of abbreviations and symbols, Introduction, Main part of 6 chapters, Conclusion, Acknowledgements and List of references including 207 sources. It is illustrated with 26 tables and 60 figures.

Main scientific results

New approaches to microextraction and pre-concentration of xenobiotics from liquid and solid-phase samples of food products have been developed based on the use of new extraction systems and materials, namely:

1. The adsorption ability of ferromagnetic nanoparticles towards volatile substances under dynamic and static headspace microextraction conditions has been proved [14] – personal contribution 60 %, [17] – 55 %.
2. The efficiency of hydrophobic medium-chain fatty acid for the extraction of organic analytes capable of ionization is shown; a new method of membrane microextraction based on the extraction of target analytes from suspended samples into medium-chain fatty acid located in the pores of the hydrophobic membrane is developed [9] – 50 %.
3. The extraction efficiency of organic analytes (capable of ionization) into deep eutectic solvent based on carboxylic acids and terpenoids was proved [21] – 60 %; an automated DLLME of organic analytes capable of ionization into deep eutectic solvent was developed [22] – 50 %.

4. The extraction efficiency of fluoroquinolones from meat samples into deep eutectic solvent based on quaternary ammonium salt and alcohol has been proved; a method of liquid microextraction based on *in-situ* DES formation directly in the mixture with solid-phase sample has been developed [18] – 60 %.
5. The extraction efficiency of polar pesticides into supramolecular solvent based on primary amine and medium-chain fatty acid was proved; the method of micellar microextraction using a new extraction system for the analysis of suspended samples was developed [16] – 60 %.
6. The possibility of using a natural terpenoid (menthol) as a "green" extractant to perform DLLME without dispersive solvent was studied and substantiated [12] – 60 %, [13] – 90 %.
7. To remove the dispersive (organic) solvent from the extraction system during the DLLME, an approach based on the solvent's phase transition (liquid – gas) was proposed [23] – 40 %, [10] – 80 %.
8. An automated method of DLLME, which allows to eliminate the interfering influence of proteins in solid-phase food samples and to perform extraction of xenobiotics under flow analysis conditions, was realized [7] – 50 %.
9. An automated method for micellar microextraction of nonpolar analytes into a phase based on a medium-chain fatty acid and its salt is proposed; the efficiency of analyte extraction into the proposed phase was demonstrated [19] – 70 %.
10. The automation of homogeneous liquid microextraction based on flow analysis was shown; a combined method of determination of pesticides in foods was developed [8] – 70 %.
11. The information on automated analysis techniques is summarized, their diversity, capabilities and features are shown on a variety of examples, including microextraction approaches developed within the thesis work, the results are presented in five reviews [6] – 15 %, [11] – 50 %, [15] – 35 %, [20] – 30 %, [24] – 40 %.

Scientific statements for defense

1. New methods of static and dynamic headspace microextraction on ferromagnetic nanoparticles for selective separation and pre-concentration of volatile substances from liquid and solid-phase food samples.
2. Method of microextraction of organic substances (capable of ionization) directly from solid-phase samples using a membrane impregnated with a switchable hydrophilicity solvent.
3. New approaches to microextraction of polar organic analytes from liquid and solid-phase samples into deep eutectic solvents based on medium-chain fatty acids and terpenoids, quaternary ammonium salt and alcohols.
4. New extraction systems based on primary amines and medium-chain fatty acids for micellar microextraction of polar and non-polar organic analytes from liquid and solid-phase samples.
5. Justification of the possibility of using a natural terpenoid (menthol) as a “green” extraction solvent to perform dispersive liquid-liquid microextraction without a dispersive solvent.
6. Approach for removal of dispersive (organic) solvent from the extraction system during the dispersive liquid-liquid microextraction, based on the solvent’s phase transition (liquid – gas).
7. Hydraulic schemes for automation of micellar, homogeneous and dispersive liquid-liquid microextraction of xenobiotics, providing an opportunity to improve the precision and productivity of chemical food analysis.

MAIN PART

CHAPTER 1. LITERATURE REVIEW

1.1. Chemical analysis of food products

Food products are a source of nutritional (proteins, fats, carbohydrates, vitamins, mineral salts, water) and non-nutritional (food additives, colorants, etc.) components, among which there are many components of natural or anthropogenic origin. Alien, potentially dangerous compounds of organic and inorganic nature, according to the accepted terminology, are called xenobiotics, contaminants, foreign chemical substances. According to the Food and Agriculture Organization and the World Health Organization, 80-95% of harmful and alien substances for humans come with food [25]. To date, more than 9 million xenobiotics of different nature are known in the world: heavy metals, pesticides, fertilizers, antibiotics, preservatives, polycyclic aromatic and chlorine-containing hydrocarbons, hormonal preparations, and others. Systematic consumption of foods with residual amounts of xenobiotics leads to human health problems and the emergence of resistant forms of microorganisms.

Food quality control is the key to the health of the nation, in this regard, within the framework of the implementation of the state policy in the field of healthy nutrition, more than two dozen regulatory and legal acts have been developed [25], including the approval of the first Russian document formalizing the requirements for expert evaluation and supervision of the turnover of biologically active food additives (BAA); the approval of the Technical Regulations of the Customs Union “Safety requirements for food additives, flavorings and technological aids” (TR TS - 029 - 2012); sanitary and epidemiological rules and regulations 2.3.1078-01 for the residual content of antibiotics in meat and dairy products have been established.

Taking into account the complexity of food matrices, sample preparation is an important and integral step in any chemical analysis. When preparing a sample for analysis, three main stages can be distinguished: drying, decomposition (opening) of the sample, separation and pre-concentration.

Analysis of solid food products is often a more complex task compared to the analysis of liquid samples. Most methods include a decomposition stage – sample conversion to a liquid state. Decomposition methods have long been divided into “dry” and “wet” methods. The former include thermal decomposition, fusion and sintering with various substances (salts, oxides, alkalis and their mixtures); the latter include dissolution of the analyzed sample in various solvents [26]. Particular attention should be paid to the choice of solvent, which can have an interfering effect on the further determination of target analytes. In addition, components of the sample itself may also have an interfering effect. In this case, a separation step or masking technique is used, in some cases both.

Sample decomposition can be carried out in two variants: open and closed systems. A common in the world practice express and reliable way of decomposition of complex samples is mineralization in hermetically sealed vessels (autoclaves) at high pressure and temperature. Autoclaves are used both in systems with resistive and microwave heating method. The principle of the microwave decomposition method is based on the oxidative effect of acids under high pressure due to heating on the organic matrix of the sample, which leads to its destruction [27]. The choice of the sample decomposition method depends on the nature and chemical composition of the object matrix, as well as the chemical properties of the determined component itself.

The analysis of animal products is complicated by the presence of large amounts of proteins, fats and fibrous structure. To remove proteins, acids (acetic, trichloroacetic or hydrochloric), alkalis or organic solvents (ethanol, acetonitrile, acetone) are used, which destroy the spatial structure of the protein [24]. Electrolyte solutions reduce the solubility of proteins due to the “salting out” effect [28]. As a result of dissociation of electrolytes (e.g., sodium sulfate) and their electrostatic interaction with proteins, the charge of the latter is neutralized, which leads to protein coagulation. In addition, electrolyte solutions have hygroscopic (water-removing) properties, thus reducing the hydrate shell of proteins, which eventually leads to their precipitation. Dehydration of protein molecules and their precipitation are also observed under the influence of physical factors (heating, irradiation, etc.). For the elimination of fats, *n*-hexane is most often used [29]. Centrifugation is used for phase separation, including separation of the insoluble

part of the sample, which raises doubts about the possibility of full automation of such sample preparation.

It is possible to improve the extraction efficiency of analytes from solid samples using ultrasound (US), microwave radiation and pressurized extraction [30-33].

In order to achieve maximum selectivity and lower detection limits (if necessary), liquid-liquid extraction (LLE) or solid-phase extraction (SPE), as well as QuEChERS (quick, easy, cheap, effective, rugged, safe) method, combining LLE and solid-phase purification, are used. The QuEChERS method was initially introduced for monitoring pesticide residues, but its modifications were later used for other xenobiotics [34,35]. Researchers recognize that this method is in line with green chemistry because of its low solvent consumption, resulting in minimal waste generation, and the use of non-chlorinated solvents [36]. The main disadvantages of the method are the labor intensity and the duration of the procedure (up to 3 h).

Traditional LLE and SPE variants have significant drawbacks: low extraction efficiency, difficulty in automation, use of large volumes of expensive solvents and materials, which in turn leads to the formation of a large volume of chemical waste. Thus, their miniaturized variants have recently been preferred. Moreover, modern analytical methods, primarily gas and liquid chromatography, allow for highly sensitive determination of impurities in microliter volumes of samples of analyzed substances [37].

1.2. Microextraction techniques used in food analysis

In the literature, special attention is paid to microextraction techniques as effective tools of preparation of various samples, including food products. Among them, liquid-liquid microextraction (LLME) and solid-phase microextraction (SPME) are particularly popular.

1.2.1. Liquid-liquid microextraction

Dispersive liquid-liquid microextraction

Among other microextraction techniques, dispersive liquid-liquid microextraction is highly expressive [2,3]. The conventional DLLME involves the introduction of a

mixture of an organic extractant (chloroform, tetrachloromethane, hexane, *m*-xylene, etc.) and a polar dispersive solvent (methanol, acetone, acetonitrile, etc.) into the aqueous phase of the sample, resulting in the formation of a finely dispersed emulsion that provides a large contact area between the phases, and, consequently, a high extraction rate (Figure 1). After centrifugation, the extract may be at the bottom of the vessel or on its surface, depending on the density of the extractant used. It should be noted that the conventional DLLME has a number of disadvantages. Thus, the formation of stable emulsions requires the inclusion of centrifugation stage in the analysis scheme, which significantly complicates the automation of technique. In addition, the introduction of polar solvents (dispersive solvents) into the extraction system increases the solubility of target analytes in the aqueous phase and, as a consequence, leads to a decrease in their distribution coefficients [38].

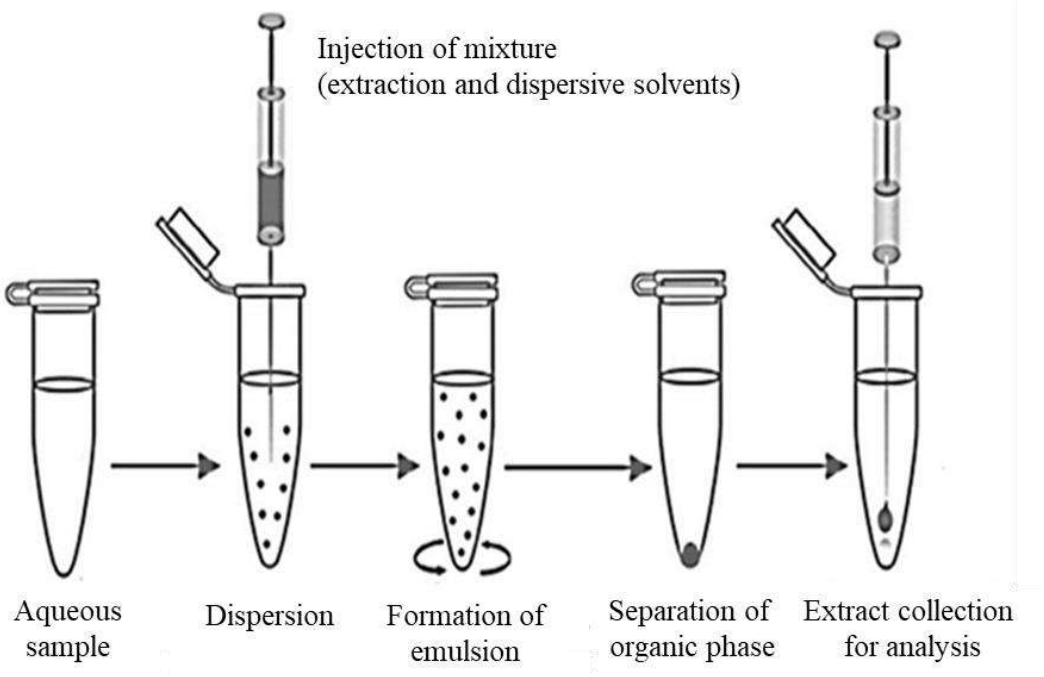

Figure 1. The scheme of DLLME.

To eliminate the above-mentioned disadvantages, different variants of DLLME have been proposed in the literature. The ultrasound-assisted DLLME proved to be cost-effective and suitable for analyzing various matrices [39,40]. Its main feature is the use of ultrasound to increase the rate of emulsion formation and decrease the equilibration time. The emulsion formed during the dispersion process is often very stable, resulting in

the need for a long centrifugation. In addition, partial loss of extraction solvent and volatile analytes may occur during the dispersion process due to heating of the emulsion. Thus, the extraction time is tried to minimize (2 - 5 min).

Also, for the analysis of solid-phase food samples, microwave-assisted DLLME was proposed [41]. However, this option is applicable only to thermally stable compounds due to the temperature increase during extraction and involves the use of only polar solvents, since nonpolar solvents practically do not absorb microwave energy.

Another variant that does not require the use of a dispersive solvents is vortex-assisted DLLME [42]. In this case, dispersion of the extraction solvent is achieved by mechanical stirring, and phase separation is obtained by stopping the stirring device. In addition, there is a combined variant of DLLME using both ultrasonic irradiation and vortex agitation [43].

For the analysis of liquid media, an air-assisted DLLME was proposed [44]. According to the scheme, the aqueous sample and extraction solvent were mixed by air bubbles using a glass syringe for aspiration. The method does not require additional equipment and is easy to implement.

In 2014, the authors [45] proposed a variant of effervescence assisted DLLME using carbon dioxide obtained *in situ* as a result of acidification of a solution containing hydrogen carbonate ions. Thus, this approach eliminated the need to use a polar organic solvent, and in addition, the salt formed as a result of the reaction can serve as a salting-out agent.

The main advantages of DLLME are small volumes of extractant (50 - 500 μL), rapidity and high (up to several thousand) enrichment factors, which allow to achieve low detection limits of impurities – 10^{-7} - 10^{-4} mg/L when combined with chromatographic analysis [46].

With the development of green analytical chemistry, much attention is paid to the search and application of new safe extraction solvents. Thus, ionic liquids (ILs) [47,48] and deep eutectic solvents (DESs) [49] have been used as extractants in DLLME.

Ionic liquids are molten organic salts at temperatures below 100 °C [50,51]. A separate category includes ILs molten at room temperature [52]. ILs conduct electric

current, have a low melting point, low saturated vapor pressure, high polarity, ability to dissolve various classes of organic compounds, which makes them attractive for use in separation and pre-concentration techniques [53]. Features of ILs include the ability to produce a variety of solvents with different extraction characteristics by varying the cation and anion of the liquid salt; and the ability to change their physical properties due to the length of the cationic, anionic, and alkyl chain [54]. There are many examples of applications of ILs in DLLME: for the determination of pesticides [55-57], antibiotics [58,59], PAHs [60], preservatives [61], and other xenobiotics in food products.

Deep eutectic solvents are considered as a separate class of solvents similar in properties to ionic liquids [62]. DES is a mixture of components, one of which is a hydrogen bond acceptor and the other is a hydrogen bond donor. The interaction between precursors results in a eutectic mixture with a lower melting point than the initial compounds [63]. Quaternary ammonium salts (choline chloride, tetrabutylammonium bromide, etc.) are most often used as hydrogen bond acceptors, and organic acids, alcohols, urea, terpenoids, and glycols are used as donors. Compared to ionic liquids, DESs have such advantages as ease of synthesis, availability, relative inexpensiveness of initial components, safety and biodegradability. The great variety due to the possibility of combining precursors and the environmental friendliness of these solvents have ensured their wide application in chemical analysis, including for food products [64-66].

In 2008, a new variant of DLLME based on solidification of the extraction solvent was proposed [67]. Organic solvents in this method have a number of requirements: they should be lighter than water, have a melting point close to room temperature (10 - 25 °C), solidify in an ice bath, have low volatility and solubility in water, and efficiently extract organic compounds [3]. Some medium-chain alcohols (1-undecanol, 1-dodecanol), saturated hydrocarbons (*n*-hexadecane) and halogenoalkanes (1-bromohexadecane, 1-chlorooctadecane) have such properties. The advantages of the method include the simplicity of extract collection; however, it should be noted that this approach involves the use of a dispersive solvent, the negative consequences of which have been discussed above.

Single-drop microextraction

Single-drop microextraction (SDME) is characterized by using record low volumes of extraction solvents (1 - 5 μL) and, as a consequence, high enrichment factors. The conventional SDME involves extraction of analytes from aqueous sample into organic solvent drop held at the tip of a syringe needle (Figure 2 A).

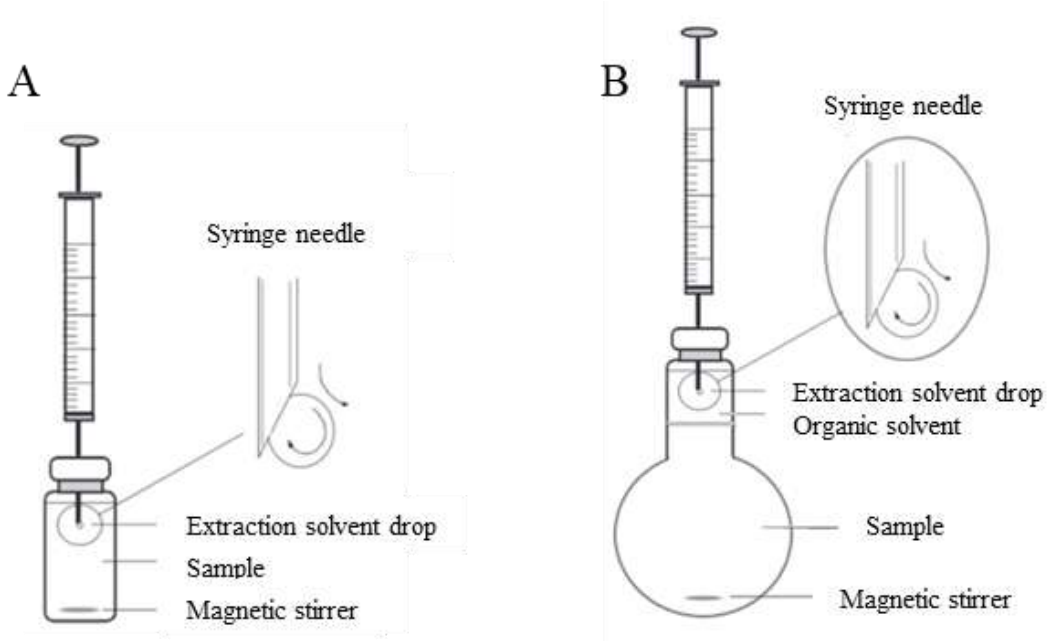

Figure 2. The scheme of two-phase (**A**) and three-phase (**B**) SDME.

A "syringe-free" version of the method is also possible: in this case, a drop of low-density extractant is applied to the surface of the liquid sample, and after achieving the required degree of concentration, the upper phase is withdrawn carefully with a microsyringe and analyzed [68]. Taking into account the complexity of the drop collection, often the crystallization of organic phase is carried out under cooling. However, this approach can be realized with a very short list of extraction solvents [69].

Target analytes are extracted by passive or convective diffusion, which leads to a long process of equilibrium establishment [70]. To accelerate the onset of phase equilibrium and increase the efficiency of SDME, vortex agitation [71] or ultrasonic irradiation [72] can be used. Vortex agitation, on the one hand, accelerates mass transfer, thereby reducing the extraction time, and on the other hand, increases the instability of droplet. In this case, it is convenient to use the dynamic mode: the sample solution is repeatedly withdrawn into the microsyringe and discharged, the sample solution and the

extraction solvent film are periodically renewed, and analyte is periodically transported from the sample solution in the sample vial to the extraction solvent in the microsyringe [73]. This mode allows reducing the extraction time of target analytes to several minutes, however, it has lower reproducibility (RSD > 10 %) [74].

In the literature, a three-phase SDME of substances exhibiting either acidic or basic properties is known [75]. In this case, an aqueous solution (acceptor solution) serves as the extraction solvent. Due to the large pH difference between the donor solution and the acceptor solution (drop) and the ionization ability of the substance, it is possible to concentrate the target analyte. In this case, the organic solvent actually acts as a “liquid” membrane, and its volume should be minimal to accelerate re-extraction into the droplet (Figure 2 B).

The main problem of SDME is the instability of the droplet. This problem can be overcome by using high-boiling, viscous solvents; increasing the adhesion of the syringe surface to the droplet; and introducing electrolytes that reduce the solubility of the extraction solvent in the aqueous sample phase and additionally provide a salting-out effect for polar analytes [37]. Thus, when selecting an extraction solvent in the SDME, its density, water solubility, boiling point, and compatibility with the method of determination are taken into account.

Homogeneous liquid microextraction

Another effective pretreatment technique in the analysis of liquid samples is homogeneous liquid microextraction (HLME), which is able to ensure rapid achievement of phase separation [76]. The method involves the injection of a polar organic solvent (acetonitrile, acetone, etc.) into a liquid-phase sample to form a homogeneous solution with subsequent phase separation due to the salting-out [77] or sugaring-out [78] effects (Figure 3). The salting-out extraction is based on phase separation by addition of salt into the solution. The main disadvantage of this approach is the use of high concentrations of the salting-out agent (usually an inorganic salt) required to achieve separation of phases, which may react with the target analytes and interfere with their subsequent

determination. This disadvantage is overcome in case of sugaring-out agent (glucose, fructose, etc.).

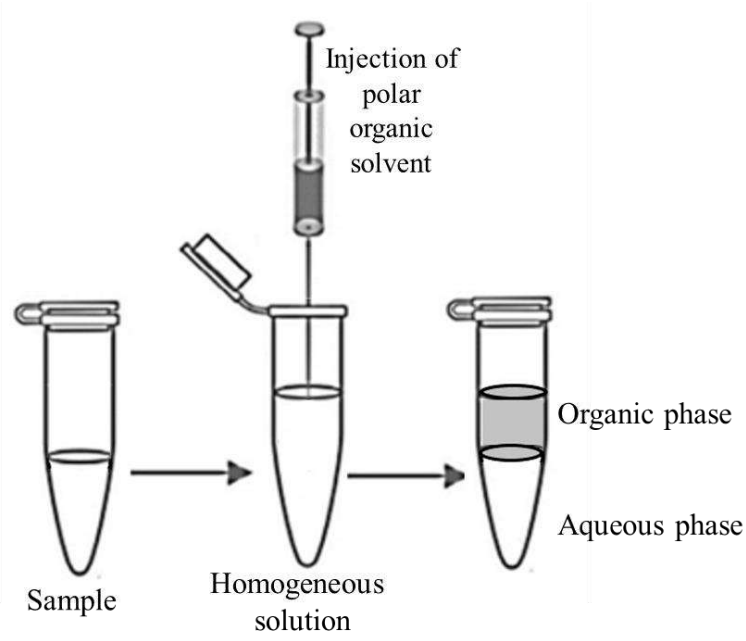

Figure 3. The scheme of HLME.

Phase separation can also be initiated by adding water [79], changing the temperature [80] or the pH [81] of the solution. The HLME method is simple and versatile, reduces the consumption of reagents and expensive solvents, the extraction time and the cost of analysis, and can be easily automated on the principles of flow methods [15]. The HLME method is widely used for the extraction of polar organic compounds from various objects, including food products, and their subsequent determination directly in the extracts by HPLC and capillary electrophoresis methods [82].

The use of extractants with switchable hydrophilicity solvents has become a new trend in HLME. Tertiary amines [83] and medium-chain fatty acids [84] were proposed as such solvents. The basic principle of the method is to switch between hydrophilic (miscible) and hydrophobic (immiscible) forms of the extraction solvent depending on the composition of the solution or its pH value. This results in a large surface area between the hydrophilic form of the extractant and the aqueous phase, rapid establishment of phase equilibrium and subsequent phase separation. In addition, this feature can be used to dissolve the organic phase (extract) containing analytes by changing the pH value

(difference between pH and pKa of a few units) in the aqueous phase, compatible with various analytical instruments (e.g. HPLC and CE).

To obtain the hydrophilic form of the medium-chain fatty acid (salt), an alkaline solution, most often sodium carbonate, is used. Acidification results in the formation of acid microdroplets (hydrophobic form) and intensive release of carbon dioxide bubbles, which promotes mixing of phases and their effective separation. Such a method, involving microextraction of the analyte into medium-chain fatty acid followed by chromatographic analysis of the separated phase, was applied in 2014 for the determination of alkylphenols in wastewater [85] and in 2016 for the determination of ofloxacin in urine [86]. Later, in 2018, the method was also modified for food analysis. Thus, for the determination of fluoroquinolones and tetracyclines in seafood, the authors [84] proposed the use of nonanoic acid as SHS to realize homogeneous microextraction with the only difference that phase separation was performed by solidification of the extract. The authors noted the simplicity, rapidity and environmental safety of the technique, providing low LOD and high extraction efficiency.

However, earlier in 2017, we proposed the use of nonanoic acid as SHS in Membrane-based liquid-phase microextraction for the determination of fluoroquinolone antibiotics in shrimp samples [9].

Membrane-based liquid-phase microextraction

Membrane-based liquid-phase microextraction involves the separation and concentration of analytes from complex matrices, including suspensions, without the formation of emulsions [87]. In the simplest case, the scheme of microextraction involves placing an inert membrane (polymer membranes, glass fiber) impregnated with an organic extraction solvent in the sample phase until phase equilibrium is established.

To obtain high selectivity and high extraction recovery, the extraction solvent must be properly selected. It should provide high solubility of the analyte, similar polarity and not react with accompanying compounds in the sample solution. High-boiling organic solvents (medium-chain alcohols, saturated hydrocarbons, etc.) are used as extraction solvents in this method. The membranes with concentrate are washed with organic

solvents for analysis. The main limitations of the method are kinetically slow processes of membrane extraction and subsequent re-extraction, which significantly increase the time of sample preparation, as well as the need to use relatively large volumes of organic solvents for re-extraction, which leads to decrease in enrichment factors. To eliminate partially the mentioned disadvantages impregnation of membranes with organic SHSs is carried out. When the acidity of the medium changes, SHSs ionize and, as a consequence, provide rapid re-extraction of analytes into the aqueous phase for subsequent instrumental detection, which has not been shown in the literature before us.

Micellar microextraction

The micellar microextraction is based on the use of surfactants, which are amphiphilic molecules with hydrophobic and hydrophilic fragments [88] (Figure 4).

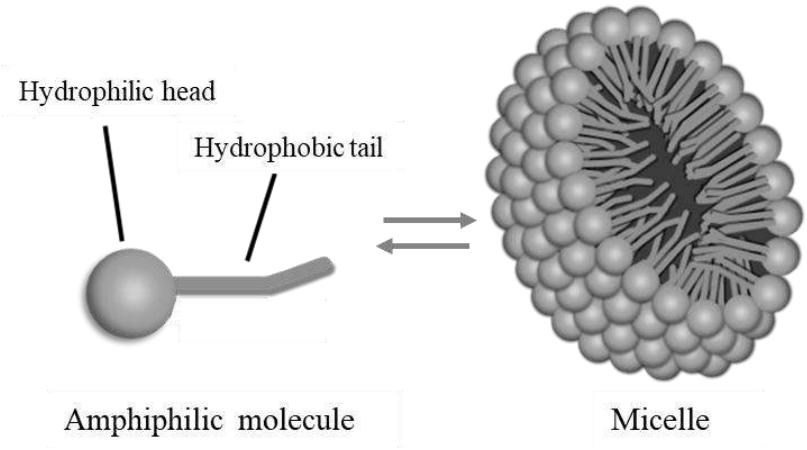

Figure 4. Schematic representation of an amphiphilic surfactant molecule and micelle formation from monomers.

Chemical classification of surfactants is based on the nature of the hydrophilic group. There are four main classes of surfactants: non-ionic (Triton X-100), cationic (cetyl trimethyl ammonium bromide), anionic (sodium dodecyl sulfate) and zwitterionic (lauryl hydroxysultaine). Nevertheless, surfactants have disadvantages: they are not considered to be environmentally friendly and their high viscosity limits their use in chemical analysis. The radical of a surfactant molecule can be represented by a linear or branched carbon chain, the length of which varies from 8 to 18 carbon atoms. The main physicochemical properties of surfactants are determined by the degree of branching and the length of carbon chain [89]. Coacervation can be caused by temperature change,

addition of electrolytes or substances competing with surfactants for solvent, pH change, or addition of surfactants with opposite sign of the headgroup charge [90].

Currently, micellar extraction is emphasized as one of the approaches in liquid-liquid extraction, and supramolecular solvents are considered to be new promising "green" extraction solvents. Supramolecular solvents (SMS) are nanostructured systems formed in colloidal solutions of amphiphilic compounds as a result of self-organization phenomena: micelle formation and coacervation [91]. Initially, in a homogeneous solution of an amphiphilic substance, a process of self-organization occurs in which isolated amphiphile molecules with a critical micelle concentration (CMC) combine into three-dimensional aggregates, such as micelles or vesicles. Further, the process of coacervation takes place and the aggregates, joining into larger particles, separate from the colloidal solution and form a new liquid phase immiscible with water (micellar phase) enriched with amphiphilic compounds [92]. Such solvents provide high recovery rates, are suitable for handling both solid [93] and liquid samples [94], and are combined with various instrumental analytical techniques [95]. Similar to ILs and DESs, the properties of SMSs can be modified by combining different amphiphiles and varying the phase separation triggers (temperature, pH, salt).

1.2.2. Solid-phase microextraction

Another widely used separation and pre-concentration method for food analysis is solid-phase microextraction (SPME). The method involves the extraction of target analytes by adsorption on the surface of solid-phase carriers (sorbents). For the subsequent determination of analytes, elution with organic solvents is performed or thermos desorption in the evaporator of a gas chromatograph is used, which allows to completely eliminate the use of organic solvents. SPME is easily combined with chromatographic systems and capillary electrophoresis, provides high sensitivity of determination (at the level of picograms per gram) and allows to create fully automated analytical systems [96].

The sorbent nature, primarily the presence of functional groups (polar or nonpolar), as well as its specific surface area significantly affects the SPME efficiency. The

efficiency of analyte extraction is higher the greater the specific surface area of the sorbent.

The following sorption mechanisms are distinguished: molecular adsorption (due to van der Waals forces, formation of hydrogen bonds), ion exchange, complexation sorption, stereospecific sorption (separation of analytes on polymeric sorbents with molecular imprints).

Sorption processes are realized in static or dynamic modes. Dynamic sorption is realized by the scheme of passing the sample flow (donor phase) through a column filled with sorbent. In static sorption, the absorbed substance is in contact with a stationary sorbent. In static sorption mode, the establishment of interfacial equilibrium occurs rather slowly, due to which this method has not been widely used until recently. However, since the 1990s, when the authors of [97] presented the SPME method, which involves sorption of the analyte on a quartz fiber coated outside with the corresponding stationary phase, this method has regained popularity. The successful combination of the method with HPLC has been illustrated by the determination of weakly volatile or thermally unstable compounds, the determination of which is not possible by GC method [98]. Currently, SPME is used to extract target analytes from gas and liquid phases into the sorbent phase based on organic, organosilicon polymers or nanomaterials.

For concentration of nonpolar and weakly polar analytes, polymeric phases based on polydimethylsiloxane, polyacrylate, polydivinylbenzene, polyethylene glycol, etc. are used. For polar analytes, phases based on modified silica gels, as well as polymer coatings such as Carboxen and Carbowax are used [99].

Recently, magnetic nanoparticles have been widely used for the separation and preconcentration of various compounds from complex matrices, including food products [100,101]. It is well known that magnetic materials as sorbents have a number of advantages over conventional sorbents for sorption in the aqueous phase. Separation and concentration processes can be carried out directly in an aqueous solution containing magnetic nanoparticles, and after sorption, the particles can be easily collected and separated from the liquid phase using an external magnetic field [102] (Figure 5). The large surface area and small volumes of the sorbent allow for highly efficient pre-

concentration. Compounds adsorbed on magnetic nanoparticles can be easily eluted with a suitable solvent for subsequent detection.

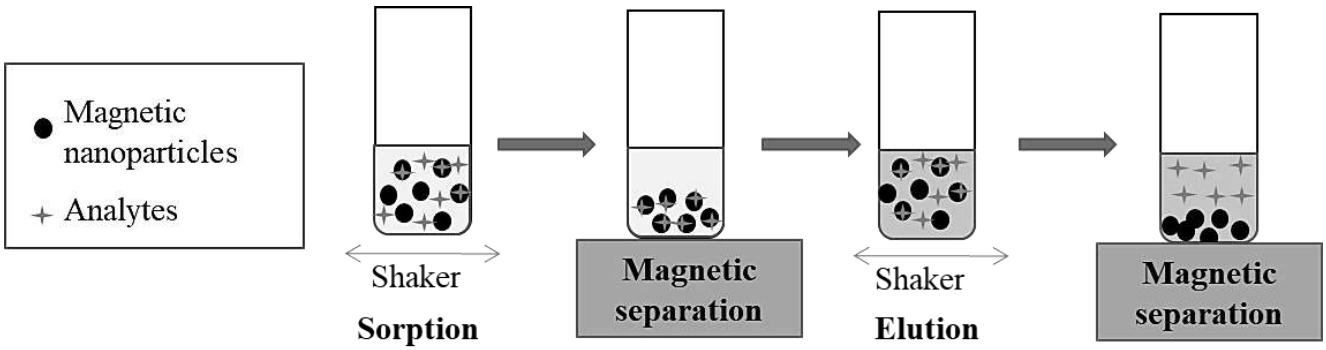

Figure 5. Solid-phase microextraction on magnetic nanoparticles.

However, the use of magnetic nanoparticles as sorbents for headspace SPME has not been previously reported in the literature.

1.2.3. Headspace microextraction

For the determination of volatile analytes in liquid and solid-phase food products, headspace analysis (HSA) based on gas-extraction of analytes with their subsequent detection is widely used. HSA has a number of advantages, such as eliminating the influence of the sample matrices and the possibility of analyzing solid and highly viscous media, simplification of the procedure of identification and quantification of analytes [4].

There are three variants of HSA according to the method of gas extraction process realization: static, dynamic and flow-through [4]. In the *static* variant, fixed volumes of sample and extractant gas are in contact in a closed system within a specified time. In *dynamic* HSA, the extractant gas flow passes over or through a fixed sample volume for a certain period of time. In the *flow-through* variant, the extractant gas and liquid sample flows simultaneously through a mass transfer device.

In the case of headspace analysis of solid-phase objects, equilibrium is established very slowly; therefore, not the equilibrium, but the stationary state in the system is established [4].

HSA also includes various options for additional concentration of analytes. Three-phase systems are widespread, in which, in addition to distribution between the gas phase

and the sample, analytes are extracted into the phase above the sample: a liquid drop or solid sorbent (Figure 6).

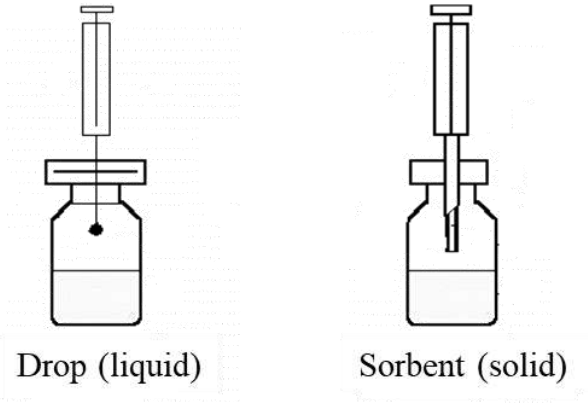

Figure 6. The scheme of headspace single-drop microextraction (left) and headspace solid phase microextraction (right).

In this case, two phase equilibria are established, which can be described by the corresponding partition coefficients of analyte:

$$K_{LG} = C_L/C_G,$$

where C_L and C_G – concentrations of analyte in liquid and gas phases, respectively.

$$K_{SG} = C_S/C_G,$$

where C_S and C_G – concentrations of analyte in sorbent and gas phases, respectively [4].

Headspace single-drop microextraction is a variation of SDME. In this case, a drop of extractant is placed over the sample surface and analytes are concentrated from the vapor phase into it. Headspace SDME is applicable to both polar and non-polar volatile compounds, he choice of extractant is a determining factor. Recently, the last generation of solvents, such as DES [103], have gained wide popularity as extractants. Compared to the conventional SDME, the advantage of the headspace variant is the absence of interfering influence of the sample matrices, since the contact of phases is excluded.

The headspace SDME has the same advantage over the conventional SPME; moreover, the absence of contact between the sample and the sorbent leads to an increase in the lifetime of the latter. It is also worth noting the possibility of increasing the enrichment factors of nonpolar and weakly polar analytes, for which K_{LG} values in the case of aqueous solutions are significantly lower than K_{SG}. Compared to headspace

SDME, the headspace SPME variant provides greater reproducibility. This is due to the instability of the extractant droplet in the first variant, as well as partial evaporation of the solvent during the long concentration process.

Hydrophobic sorbents [104,105], which retain volatile organic compounds of different polarity well, as well as nonpolar polymeric sorbents such as Tenax and various carbon sorbents, including sorbents based on nanocarbon materials [106], are most often used as sorbents for the headspace SPME. It should be noted that sorption on magnetic nanoparticles in case of headspace microextraction has not been previously realized.

1.3. Automation of chemical food analysis

When it is necessary to perform mass analyses, the most important criteria for selecting analytical methods are: expressiveness, minimization of labor costs, as well as reduction of reagent and sample consumption, and, consequently, generated waste. One of the common solutions to all these problems is automation based on the principles of flow methods.

Currently, various flow methods have been proposed for automation of the main stages of chemical analysis including sample preparation. Flow systems are characterized by high sample throughput and, depending on the specifics of a particular flow method, allow to minimize the consumption of sample and reagent solutions compared to the stationary counterparts of the same methods [5].

To automate chemical analysis, flow systems typically include pumps, valves, switch tubes, mixing/reaction devices, and detectors. The presence of microextraction in process implies the inclusion of special devices such as extraction columns, extraction chambers, membrane and chromatomembrane cells, and hollow fiber tubes [15]. The sequence of all analytical procedures is often controlled by a computer or microprocessor [6].

The possibility of combining flow analysis with efficient hyphenated methods such as capillary electrophoresis, chromatography and others is presented in a review article by Prof. Cerda [107]. The main advantage of combined methods is the possibility of

automated highly sensitive multicomponent analysis using small volumes of samples and reagent solutions.

In the analysis of complex samples, including food products, there are problems limiting the use of flow methods: low selectivity and sensitivity of analysis, and difficulty in automating sample preparation.

DLLME is a more convenient method for automation than SPME, which in most cases can cause difficulties associated with the appearance of excess pressure in the flow system.

Based on the literature data of Scopus database, it was concluded that the most popular flow methods used in food analysis are: flow injection analysis, sequential injection analysis and lab-in-syringe analysis.

Flow injection analysis (FIA), proposed by Ruzicka and Hansen in the mid-1970s, involves the periodic injection of a small volume of sample into a continuous laminar flow of media. The joint flow of carrier and sample is combined with the flow of reagent solution and mixed in the mixing coil and then enters the detector, where continuous recording of the analytical signal occurs (Figure 7).

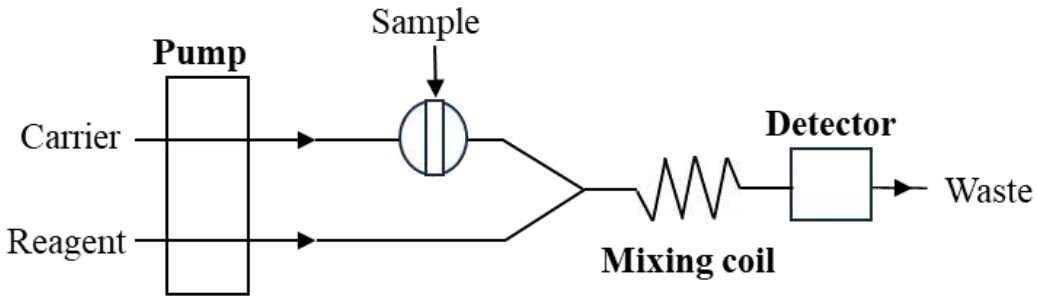

Figure 7. The scheme of FIA.

Reviews [11, 108-110] present various examples of the use of FIA for food analysis. The main advantage of the method is its rapidity and low sample consumption. However, as noted by the authors [111], the method has not been widely used in laboratory control of food products due to the difficulty of automation of sample preparation.

In contrast to FIA in **sequential injection analysis** (SIA), proposed in the 1990s by Ruzicka and Marshall, the flow of solutions moves alternately in two different directions provided by syringe or peristaltic reversible pump. Using a multi-way solenoid

valve and pump, discrete portions of sample and reagents are withdrawn into a holding coil. By reversing the direction of flow, the contents of the coil are fed into the reaction/mixing coil, where an analytical form is formed, which is further directed to the detector (Figure 8). Significant progress has been made with SIA in terms of universality of manifolds [112]. The introduction of the *mixing chamber* in the SIA scheme has expanded the capabilities of the method, however, it is also possible to analyze only liquid food samples [111].

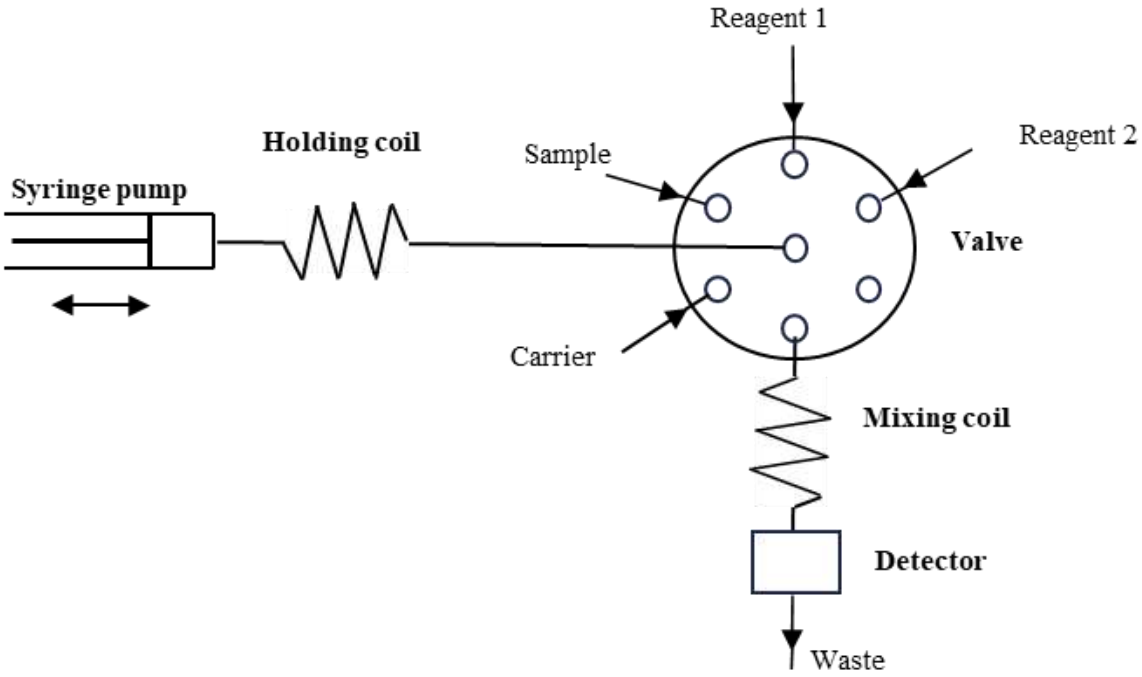

Figure 8. The scheme of SIA.

Lab-in-syringe, proposed by Prof. Cerda's group in 2012, presents the possibility of integrating different extraction methods inside a syringe [113]. The device consists of a computer-controlled syringe pump equipped with a three-way solenoid valve connected to a detector. Stirring is provided by a magnetic stirrer insert driven by an adapter placed on the glass syringe cylinder (Figure 9). This setup allows thorough mixing of the sample with reagents and/or extraction solvents, facilitating efficient extraction of analytes [20]. For the first time, a fully automated DLLME based on the “in-a-syringe” concept was used for the determination of rhodamine B dye in water and soft drink samples [113]. Later, various approaches of DLLME [114-116] were automated for the analysis of food

samples. The possibility of using a wide range of extraction solvents allows to combine the “in-a-syringe” analysis with various methods of determination.

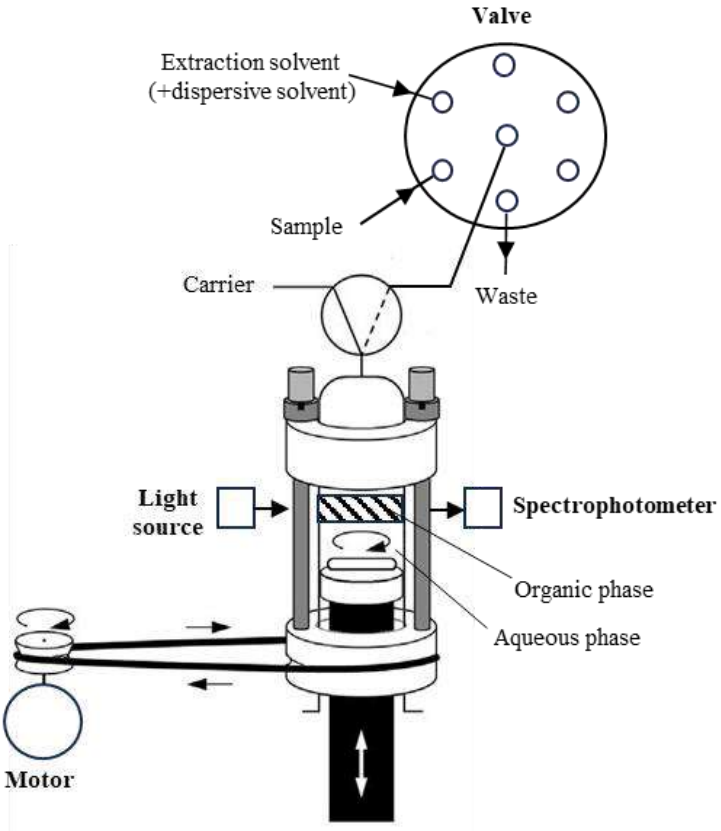

Figure 9. The scheme of lab-in-syringe with DLLME.

The above listed flow methods (except for SIA with mixing chamber) belong to the group of dispersion-convection flow methods, which assume the delivering of sample zone in the laminar flow of a carrier to a detector. The two mass transfer phenomena primarily responsible for the transportation of samples through dispersion-convection flow methods systems are convection and diffusion. The degree of dispersion depends on various parameters: flow rate, sample volume, length and diameter of tubes, configuration and length of mixing coil, and detector design.

Stepwise injection analysis (SWIA), proposed by A.V. Mozzhukhin, A.L. Moskvin and L.N. Moskvin (Figure 10), is a forced-convection flow method promising for automation of chemical food analysis. The concept of SWIA is based on strict execution of all the stages of routine analysis; including sampling, sample preparation, analyte preconcentration (when necessary) or conversion into detectable form, analyte absorption into solution (for gas analysis), analyte dissolution (for solid sample analysis),

addition of reagent solutions to sample solution, mixing solutions (by air-bubbling), thermostating (when necessary), allowing a time period for the formation of product of reaction, and finally, the measurement of analytical signal [112]. Each of the cycles (registration of the "base" line, measurement of the analytical signal at different analyte concentrations for the construction of the calibration graph and measurement of the analytical signal of the sample) can be repeated many times to reduce random errors of analysis. A distinctive feature of this method is the inclusion of a "gas" line in the scheme, which provides intensive and effective mixing of sample and reagent solutions in the reaction vessel. However, SWIA has not been previously used to automate the analysis of solid-phase food products.

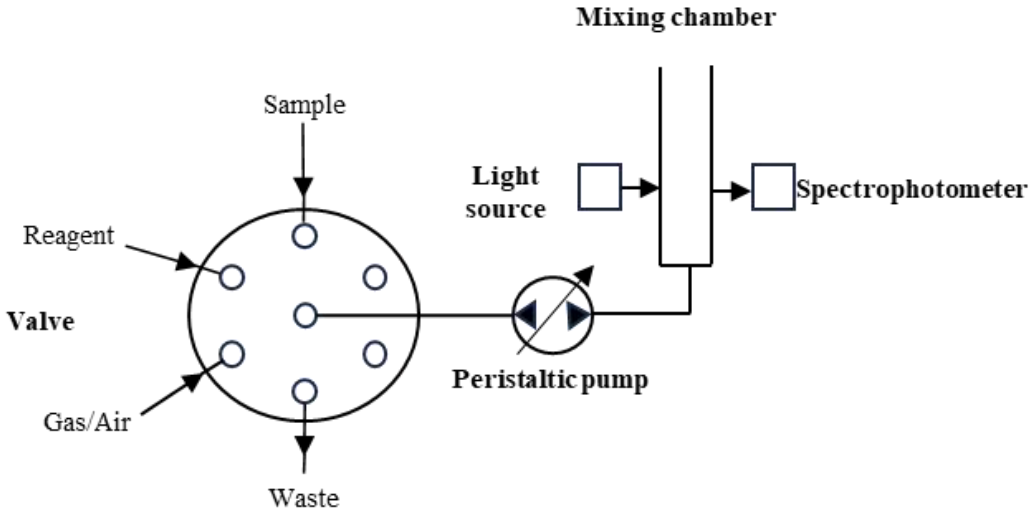

Figure 10. The scheme of SWIA.

1.4. Conclusions of the literature review

In modern analytical chemistry, special attention is paid to the development of new methods for rapid and highly efficient chemical analysis of samples with complex compositions, including food products. Modern instrumental analysis of such objects is practically impossible without sample preparation, which in most cases includes preliminary separation and concentration of analytes in order to increase sensitivity and selectivity. One of the traditional methods of separation and pre-concentration, widely used in the practice of chemical food analysis, is extraction (liquid and solid phase), which

involves long and labor-intensive procedures and requires large consumption of toxic solvents.

To improve the efficiency of chemical analysis of food products, microextraction methods of separation and pre-concentration are actively introduced into analytical practice. A large number of works are devoted to the development of various variants of liquid-liquid, solid-phase and headspace microextraction (HSME) (Figure 11), as well as the possibility of their combination with instrumental methods. It should be noted that the development of green analytical chemistry dictates the need to search for new environmentally friendly and selective extraction systems and sorbents that provide effective extraction of target analytes from liquid and solid-phase food matrices. In this direction, special attention is paid to the study of the possibility of using DES, natural solvents and micellar phases as extractants. Nanomaterials offer new opportunities for solid-phase microextraction.

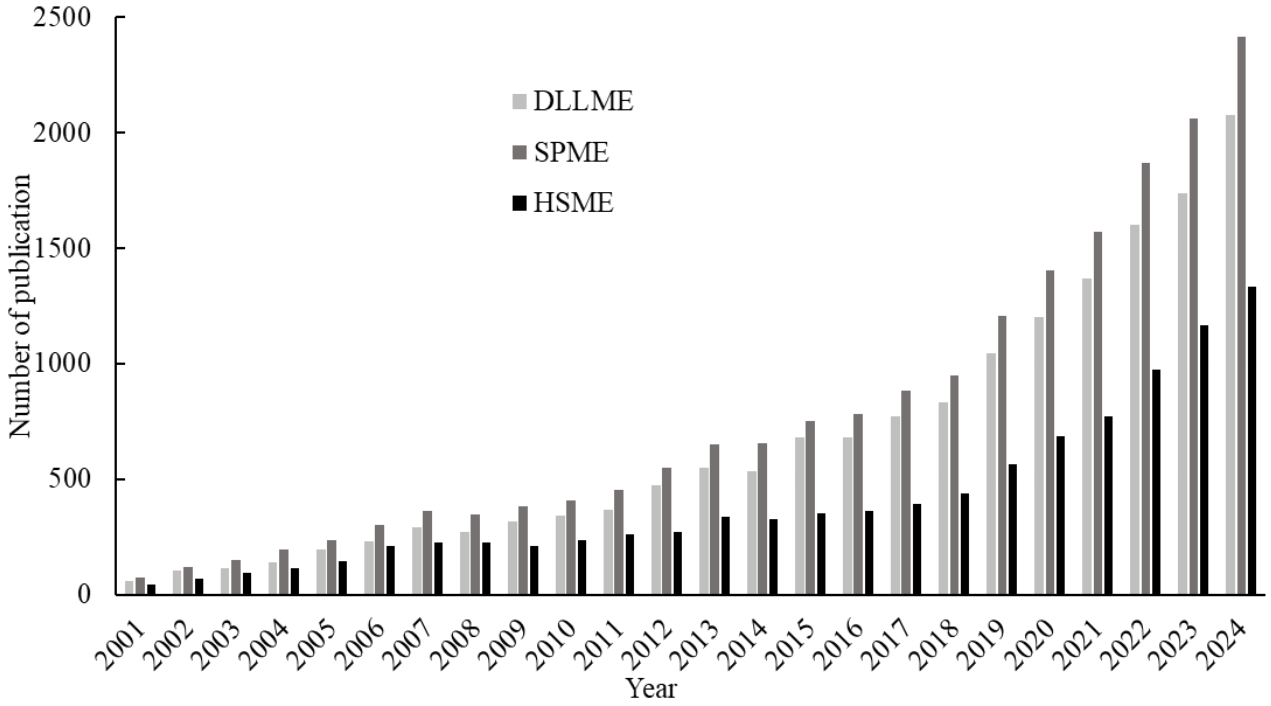

Figure 11. Distribution of the number of publications on DLLME, SPME and HSME methods in food analysis by year (original articles and reviews are taken into account, www.sciencedirect.com).

The analysis of literature data revealed a great interest of researchers in the automation of microextraction methods based on the principles of flow methods, providing high reproducibility of results and reducing labor costs. It should be noted that the automated microextraction methods proposed in the literature are mainly focused on the chemical analysis of aqueous samples and biological fluids.

Thus, within the framework of the development of modern analytical chemistry, the development of new highly effective microextraction techniques providing a high degree of pre-concentration and the rapid phase separation, the possibility of miniaturization and automation, eco-friendliness, as well as compatibility with instrumental methods of detection is of scientific importance. At the same time the actual direction is the search and study of new environmentally friendly and selective extraction systems and materials. The development of new methods to improve the efficiency of interphase distribution of separated substances with a focus on the characteristics of the chemical composition of the analyzed food samples and their aggregation state is also under consideration.

CHAPTER 2. EXPERIMENTAL PART

2.1. Measuring instruments and equipment

1. Liquid chromatograph LC-20 (Shimadzu, Japan) with photometric (diode array) and fluorimetric detectors.

2. Triple quadrupole liquid chromatograph-mass spectrometer LCMS-8030 (LC-MS/MS) (Shimadzu, Japan).

3. Gas chromatograph “Chromatec-Crystal 5000” (Chromatec, Russia) with flame ionization detection.

4. Gas chromatograph “GCMS-QP2010 SE” (Shimadzu, Japan) with mass spectrometric detection.

5. Atomic absorption spectrometer “AA-7000G” (Shimadzu Europa GmbH) with electrothermal atomizer GFA-7000, six hollow cathode lamps, double background corrector: deuterium corrector and corrector for self-reversed spectral line, having a spectral range of 185 - 900 nm and a detection limit of 1 μg/L.

6. IR Affinity-1 spectrometer with Shimadzu own software (Shimadzu, Japan).

7. Laboratory Densitometer DMA 4500 (Anton Paar, Austria).

8. Scanning electron microscope Tescan MIRA3 LMU (Tescan, Czech Republic).

9. Ubbelohde viscometer.

10. Freeze Dry System (FreeZone Plus 2,5 L, Cascade, Labconco, Kansas City, Missouri, USA).

11. Vacuum filtration system Supelco 58062-U (Germany).

12. Magnetic stirrer with heating "IKA RH digital" (Germany).

13. Magnetic stirrer "IKA topolino" (Germany).

14. Microwave oven MDS-10 Master (Sineo, China).

15. Analytical balance "Ohaus Pioneer PA214C" (Ohaus, China), 2nd accuracy class, weighing limit 210 g, error 0.1 mg.

16. Laboratory centrifuge OPN-8 (Russia).

17. Mixing device LOIP LS-120 (CJSC "Laboratory equipment and devices", Russia).

18. Six-way solenoid valve (Cole - Parmer, USA).

19. Six-way valve (Sciware Systems SL, Spain)

20. Peristaltic pump «MasterFlex L/STM» (Cole - Parmer, USA) (flow rate from 0.5 to 6 ml/min).

21. Syringe pump (Sciware Systems SL, Spain) (flow rate from 0.14 to 7.5 ml/min).

22. Ultrasonic bath 2.8 L (Sapphire, Moscow, Russia) (250 W, 35 kHz, heating temperature range 15-70 °C, temperature maintenance accuracy ± 1 °C).

23. Chromatography column Phenomenex C18 (250 x 4.6 mm, particle size 5 μm).

24. Chromatography column Luna C18 (3 μm, 250 × 3.0 mm, 100 Å).

25. Chromatography column Zorbax Bonus-RP (Agilent, 100 × 2.1 mm, 3,5 μm).

26. Capillary column "Optima-1" (25 m x 0.32 mm; 0.35 μm; Macherey-Nagel, Germany).

27. Chromatographic syringes with a volume of 10 μL and 100 μL (Hamilton, Switzerland).

2.2. Chemicals, reagents and preparation of solutions

All chemicals and reagents were of analytical grade.

Preparation of 1 mg/L selenite ion solution

0.1 ml of a selenite ion solution (Merck, Germany) with a concentration of 1 g/l was placed in a 100 ml measuring flask and the flask volume was filled up to the mark with deionized water. The solution was stored at a temperature of 4 ºC for a week. Working solutions of 50, 100, 250, 500, 750 μg/l were prepared daily by diluting 1 mg/l of a selenite ion solution with deionized water.

Preparation of 0.2 mol/L sodium borohydride solution

To prepare a 0.2 mol/L sodium borohydride solution (Merck, Germany) 0.76 g of solid sodium borohydride was placed in a 100 mL volumetric flask and the flask volume was filled up to the mark with 0.1 mol/L sodium hydroxide solution. The solution was prepared daily.

Preparation of 1 g/L solution of phenol, guaiacol, p-cresol and o-cresol

0.025 g of each phenol (Acros, USA) was placed in a 25 mL volumetric flask and filled up to the mark with methanol. The solution was stored at 4 ºC for a week.

Preparation of 1 g/L mixed fluoroquinolone solution

0.025 g of fluoroquinolone (ofloxacin, lomefloxacin hydrochloride, norfloxacin, fleroxacin (Sigma-Aldrich, USA)) was placed in a beaker, 5 mL of 0.01 mol/L sodium hydroxide solution was added and the mixture was stirred until the substances were completely dissolved. The solution was quantitatively transferred to a 25 mL measuring flask, the volume of the solution was filled up to the mark with 0.01 mol/L sodium hydroxide solution and mixed thoroughly. The stock solution was stored in the refrigerator for 1 month. Working solutions were prepared daily by sequential diluting the stock solution with deionized water.

Preparation of sodium dihydrogen phosphate solution (50 mmol/L)

3.9 g of $NaH_2PO_4 \cdot 2H_2O$ was placed in a beaker and dissolved in 20 mL of deionized water with stirring. The solution was quantitatively transferred to a 500 mL volumetric flask, the beaker was washed with three 10 mL portions of deionized water. Then the volume of the solution in the flask was filled up to the mark with deionized water and mixed thoroughly.

Preparation of sodium hydrogen phosphate solution (50 mmol/l)

3.55 g of Na_2HPO_4 was placed in a beaker, 20 mL of deionized water was added and the mixture was stirred until the salt was completely dissolved. The solution was quantitatively transferred to a 500 mL measuring flask, the beaker was rinsed with three 10 mL portions of deionized water, the volume of the solution in the flask was filled up to the mark with deionized water and mixed thoroughly.

Preparation of phosphate buffer solution (pH 6.4)

125 mL of 50 mmol/L sodium hydrogen phosphate solution and 375 mL of 50 mmol/L sodium dihydrogen phosphate were mixed and stirred in a 500 mL flask. The pH value of the solution was monitored using a pH meter.

Preparation of phosphate buffer solution (pH 6.4, 20 mmol/L triethylammonium acetate)

125 ml of 50 mmol/L sodium hydrogen phosphate solution and 375 mL of 50 mmol/L sodium dihydrogen phosphate were added to a 500 mL beaker and mixed. The pH value of the solution was monitored using a pH meter. The resulting solution was then mixed with a solution of triethylammonium acetate (1 mol/L) in a ratio of 98:2 (vol./vol.).

Preparation of 100 mg/L solutions of benzoic and sorbic acids

0.01 g of each acid (Sigma-Aldrich, USA) was placed in a beaker and dissolved in 300 μL of methanol. After the acid sample was completely dissolved, the solution was quantitatively transferred to a 100 mL volumetric flask, the beaker was rinsed with three 5 mL portions of deionized water, the volume of the solution in the flask was filled up to the mark 100 mL with deionized water and then mixed thoroughly. The stock solutions were stored at 4 °C. Working solutions were prepared daily by diluting the stock solutions with distilled water.

Preparation of 1 g/L pesticide solutions

0.01 g of each pesticide (malathion, diazinon, phosalone, triadimefon, triadimenol, bifenthrin, imidacloprid and bayleton (Ekolan, Moscow, Russia)) was placed in a beaker and 2 mL of methanol was added. After the pesticide sample was completely dissolved, the solution was quantitatively transferred to a 10 mL measuring flask, the beaker was rinsed with three 2 mL portions of methanol, the volume of the solution in the flask was filled up to the mark 10 mL with methanol and mixed thoroughly. The stock solutions were stored in the refrigerator at 4 °C. Working solutions were prepared daily by diluting the stock solutions with deionized water.

Preparation of 500 mg/L mixed PAH solution

0.005 g of PAH (benzo[a]pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, fluorene, dibenz[a,h]anthracene, benzo[ghi]perylene, naphthalene, anthracene, fluoranthene, phenanthrene and pyrene (Sigma-Aldrich, USA)) were placed in a beaker, 5 mL of acetonitrile was added and the mixture was stirred until the substances were completely dissolved. The solution was

quantitatively transferred to a 10 mL measuring flask, the beaker was rinsed with three 1 mL portions of acetonitrile, the volume of the solution was filled up to the mark 10 mL with acetonitrile and mixed thoroughly. The stock solution was stored in a refrigerator at 4 °C for 3 months. Working solutions were prepared daily by dilution of the stock solution with deionized water.

2.3. Sampling and sample preparation

Sampling and sample pretreatment of biologically active additives (for section 3.1)

Samples of biologically active additives from various manufacturers were purchased from local pharmacies in St. Petersburg. Preliminary treatment included microwave digestion in a laboratory microwave oven. For this purpose, (0.15 ± 0.05) g of the crushed homogeneous sample was weighed and placed in a vial for microwave digestion. After that 6 mL of concentrated nitric acid and 1 mL of hydrogen peroxide were also added there. The temperature program of digestion is presented in Table 1. The working power during digestion was 600 W.

Table 1. Temperature program for microwave digestion of samples of biologically active additives.

T, °C	t, min	Power, W
100	10	600
130	10	600
150	5	600
180	10	600
210	30	600

Sampling and sample pretreatment of smoked sausage samples (for section 3.2)

Smoked sausages of various brands were purchased from local supermarkets in St. Petersburg. Sausage samples were homogenized using a blender and stored in a refrigerator at 4 ºC for a week. Spiked samples were prepared as follows: (0.50 ± 0.05) g of ground sausage sample was placed in a vial, then 1 mL of methanol was added, and phenols from samples were extracted into methanol with vigorous stirring for 30 min. After that, the methanol phase was separated and the operation was repeated 3 more times.

As a result, a sample was obtained without target analytes (confirmed using the developed method), after that the additives of a standard solution with a known phenol concentration were introduced.

Sampling and sample pretreatment of shrimp samples (for section 4.1)

Frozen peeled shrimp were purchased from a local supermarket in St. Petersburg, delivered to the laboratory, and placed in a freezer at -15 °C. Thawed shrimp were homogenized using a blender. To prepare spiked samples an appropriate aliquot of the fluoroquinolone standard solution was added to the ground thawed shrimp sample, resulting in three samples with concentrations of 50, 100, and 150 μg/kg for each analyte. Spiked samples were prepared immediately before analysis.

Sampling and pretreatment of meat samples (for sections 4.2, 6.1)

Different meat samples (minced chicken, chicken fillet and ground beef) were purchased from local supermarkets, homogenized using a blender and freeze-dried using a freeze dry system. The amount of evaporated water varied within 66 - 76 % by weight. The samples were stored at 5 °C in a refrigerator. Immediately before analysis, a volume of distilled water (or a solution of fluoroquinolones with appropriate concentrations – for the “added-found” method) corresponding to the amount of evaporated water was added to 10 g of the freeze-dried meat sample, and the resulting mixture was stirred using a magnetic stirrer for 20 min. Samples without additives were analyzed without freeze-drying.

Sampling and pretreatment of milk samples (for section 4.3)

A milk sample (1.8 % fat) was purchased from a local supermarket in St. Petersburg and stored according to the instructions on the packaging. Before analysis, fats were removed. For that 5 mL of milk were placed in a 10 mL vial, 0.7 mL of hexane was added, the mixture was shaken and centrifuged for 10 min. Then the upper (organic) phase containing hexane and the separated fats was removed. To remove proteins, 0.5 mL of 4 mol/L HCl was added to the supernatant and mixed, after which the mixture was centrifuged for 10 min. Then 0.4 mL of 5 mol/L NaOH was added to 4.6 mL of the supernatant obtained. The solution was used for analysis.

Spiked milk samples were prepared as follows: 15 mL of milk were placed in a 25 mL flask, then 50 or 100 μL (for 50 μg/L and 100 μg/L, respectively) of a mixed solution of fluoroquinolones with a concentration of each analyte 25 mg/L were added, after that the volume of the solution was filled up to the mark with milk sample. Before analysis, fats and proteins were removed according to the above-described scheme.

Sampling and pretreatment of shrimp samples (for section 4.3)

Peeled shrimp were purchased from a local supermarket in St. Petersburg, delivered to the laboratory and placed in a freezer (-15 °C). To prepare spiked samples, thawed shrimps were completely immersed in working solutions of analytes (100 μg/L or 200 μg/L) for 14 h at 4 °C. After that, the solution was separated, and the shrimp samples were homogenized using a mixer. The residual concentration of analytes in the separated aqueous phase was monitored using HPLC-FLD. The amount of antibiotics adsorbed by shrimp tissues was calculated based on the residual amount of antibiotics in the aqueous phase using the formula:

$$n_{ads} = n_{added} - n_{found},$$

where n_{ads} – amount of fluoroquinolones adsorbed by shrimp tissue; n_{added} – the added amount of fluoroquinolones (in the working solution); n_{found} – residual amount of fluoroquinolones in solution.

To precipitate proteins 0.5 g of homogenized spiked shrimp sample was mixed with 6 mL of 0.01 mol/L NaOH in a 10 mL vial. The mixture was centrifuged (10 min, 5000×g), the obtained supernatant was placed in another vial, and the solution was neutralized by adding 2 mol/L HCl, the pH was controlled using a pH meter. Figure 12 shows the chromatograms of the extracts of shrimp samples without additives (A) and spiked samples (immersed in a working solution of 200 μg/L each analyte) (B).

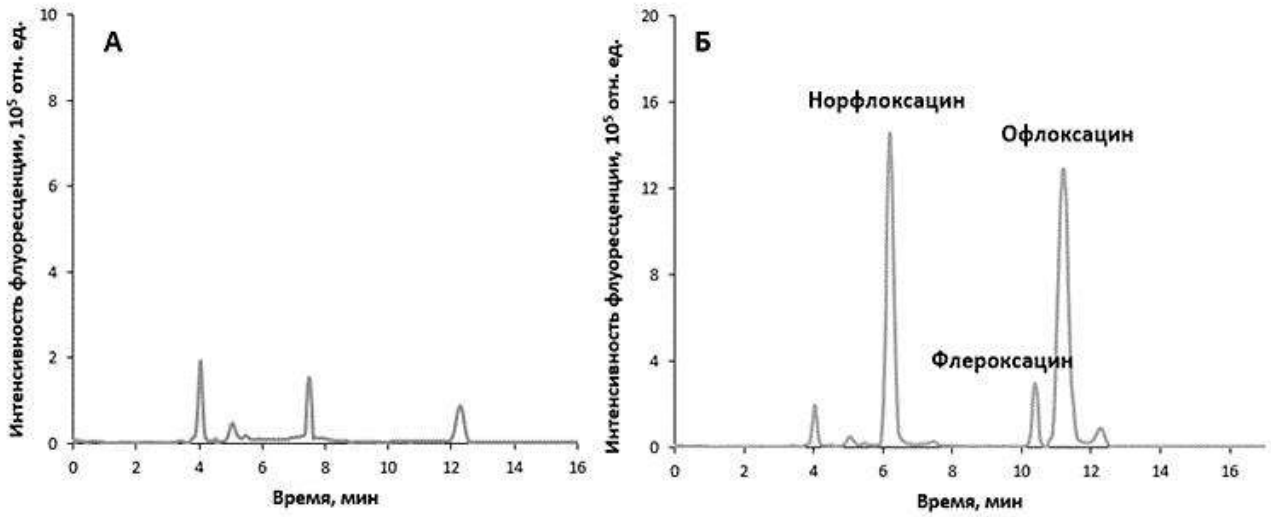

Figure 12. Chromatograms of the extracts of shrimp samples without additives (A) and spiked samples (B).

Sampling and pretreatment of shrimp cucumber samples and baby vegetable food (for section 4.4)

Cucumbers (short-fruited prickly and long smooth) and baby vegetable food made from broccoli (puree) were purchased from a local supermarket in St. Petersburg. Cucumber samples were homogenized using a mixer. Baby food did not require homogenization.

Spiked samples were prepared as follows: 10 μL of an aqueous solution of a mixture of pesticides with appropriate concentrations were injected into 800 mg of the homogenized sample and then thoroughly mixed.

Sampling and pretreatment of berry and fruit juices, carbonated beverages and soy sauce (for sections 5.1, 5.2)

Juices, carbonated beverages and soy sauce were purchased in local supermarkets in St. Petersburg. All juices were of different brands and flavors. Despite the fact that some juices contained pulp and carbonated drinks contained dyes, no sample pretreatment was carried out before analysis. It is worth noting that all samples had a pH below 7, however, in accordance with the developed technique, they were acidified to pH 2 by adding a small volume of 1 mol/L sulfuric acid insignificant for dilution.

To get the spiked samples (100 mg/L) 0.001 g of acid was placed in a beaker and dissolved in 300 μL of methanol. After the acid sample was completely dissolved, the solution was quantitatively transferred to a 10 mL flask, the beaker was rinsed with three 1.5 mL portions of the sample, acidified till pH 2, then the volume of the solution in the flask was filled up to the mark 10 mL with the sample and mixed thoroughly. If it was necessary to obtain a lower analyte addition, the samples were diluted with the corresponding food product.

Samples spiked with mixture of benzoic and sorbic acids were prepared in a similar way.

Sampling and pretreatment of wine samples (for section 5.2)

Five samples of semi-sweet wine (four white and one red) of different brands were purchased from local supermarkets in St. Petersburg. Before analysis, the wine samples were diluted with deionized water in a ratio of 1:3 (vol./vol.), respectively.

Sampling and pretreatment of tea samples (for section 6.3)

Black, green, white and jasmine teas of various brands were purchased from local supermarkets in St. Petersburg, Russia. Samples were stored in their original packaging and at the recommended conditions (room temperature) until analysis. Samples were homogenized with a blender to a powder before analysis.

The tea infusion preparation scheme was chosen based on literature data [117]: 1 g of homogenized tea was brewed in 10 mL of hot deionized water (90 ºC) for 5 min in a glass beaker. The tea was then cooled to room temperature and the solid sediment was removed by decantation.

To prepare spiked samples (with 0.5 μg/L and 5.0 μg/L PAH), 45 mL of freshly brewed tea sample was placed in a 50 mL measuring flask and 10 μL or 100 μL of a PAH solution with a concentration of each analyte of 2.5 mg/L, respectively, were added to it. The solution was then filled up to the mark with the sample.

2.4. Methods and conditions for determining xenobiotics

ETA-AAS determination of selenium (IV)

Selenium (IV) was determined using a spectrometer Shimadzu AA-7000 with a GFA-7000 graphite furnace; the instrument settings are presented in Table 2.

Table 2. Parameters of spectrometer Shimadzu AA-7000 with GFA-7000 graphite furnace for the determination of selenium (IV).

Parameter	Value
Current of hollow cathode lamp, mA	23
Resonance wavelength, nm	196.0
Slit width, nm	0.5
Graphite furnace	With pyrolytic coating
Chemical modifier	2 μL of 1000 mg/L palladium solution
Matrix	0.1 mol/L HNO_3

HPLC-FLD determination of phenols

Determination of phenols was carried out on a Shimadzu LC-20 chromatograph with a Luna 3 μm C18 column (250 mm × 3.0 mm, 100 Å) and the following separation and detection conditions: excitation wavelength – 270 nm, emission wavelength – 310 nm, column temperature 30 °C. The mobile phase consisted of acetonitrile and 0.1 % formic acid in a volume ratio of 40 : 60, respectively, the analysis time was 11 min (Figure 13).

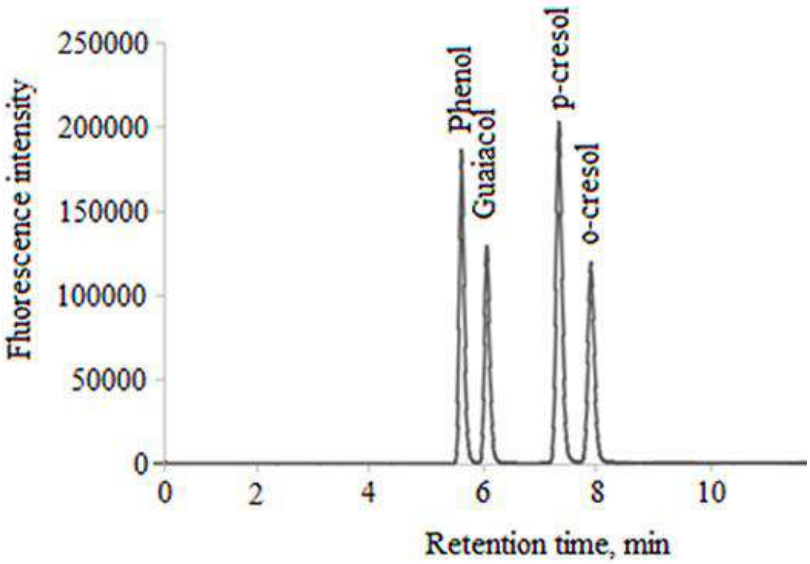

Figure 13. Chromatogram of solution of phenols ($C_{analyte}$ = 1 mg/L).

HPLC-FLD determination of fluoroquinolones

Chromatographic measurements were performed using a LC-20 Prominence HPLC-FLD system (Shimadzu, Japan). A chromatographic column Phenomenex C18 column (250 × 4.6 mm, particle size 5 μm) and mobile phase of methanol (A) and phosphate buffer (pH = 6.4) containing 20 mmol/L triethylammonium acetate (B) (45:55, v/v) at flow-rate of 0.7 mL min-1 ensured the chromatographic separation. The system was operated at 30 °C. The fluorescence excitation and emission wavelengths were 278 and 466 nm, respectively. The total time of chromatographic analysis is 14 min (Figure 14).

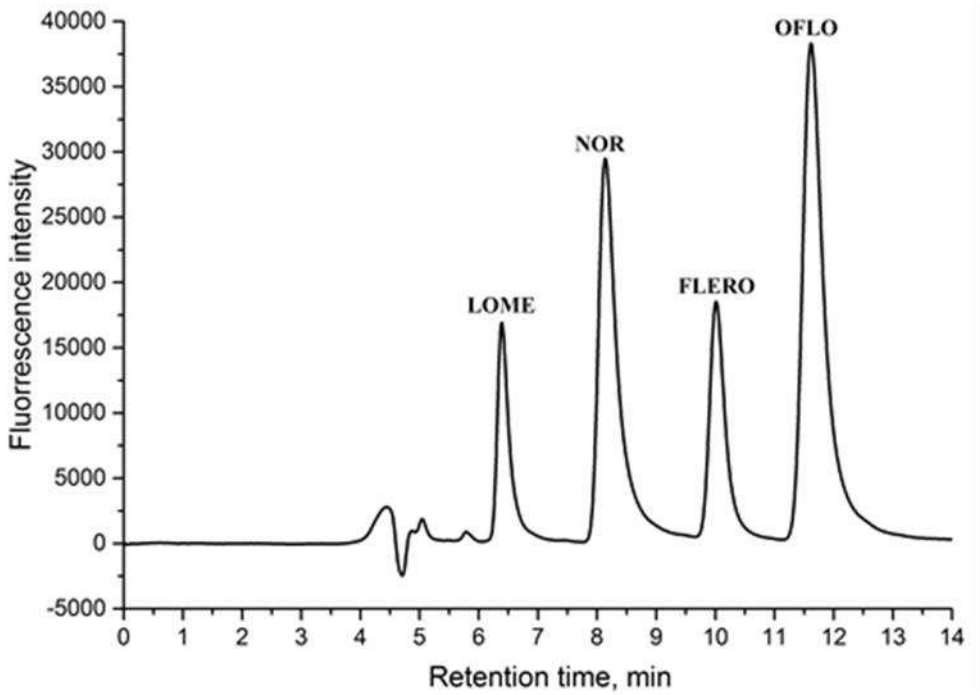

Figure 14. Chromatogram of the extract of a mixture of fluoroquinolones: lomefloxacin (LOME), norfloxacin (NOR), fleroxacin (FLERO) and ofloxacin (OFLO) ($C_{analyte}$ = 100 μg/L).

HPLC-UV determination of benzoic and sorbic acids

Chromatographic separation was carried out using a LC-20 Prominence HPLC system (Shimadzu, Japan) with Supelco C18 column (250 mm × 4.6 mm, 5 μm) at 45 °C. UV-vis spectrophotometer set at 230 nm was used as the detector, and the volume of the injected sample was 20 μL. The mobile phase was methanol – ammonium acetate buffer solution (0.05 mol/L) (30:70, vol./vol.); the flow rate was 0.5 mL/min. Retention times for benzoic and sorbic acids were 8.4 min and 10.4 min, respectively (Figure 15).

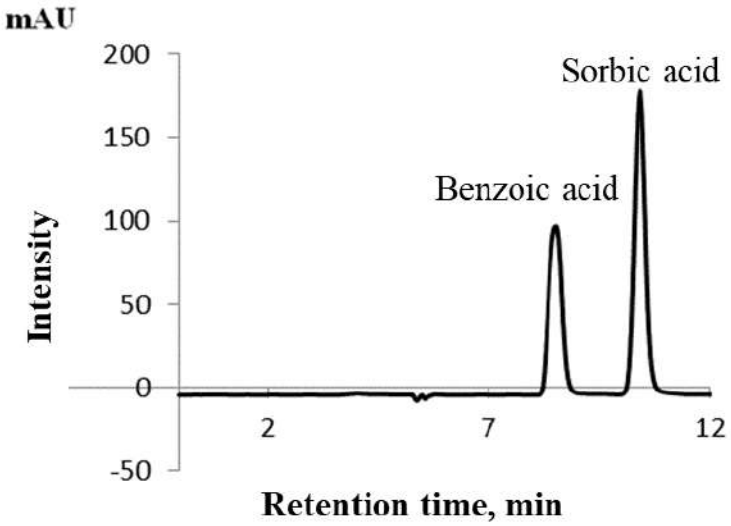

Figure 15. Chromatogram of the extract of a mixture of benzoic and sorbic acids ($C_{analyte}$ = 25 mg/L).

GC-MS determination of pesticides

A gas chromatograph (GCMS-QP2010 SE, Shimadzu, Japan) was used for the determination of pesticides. Separation was performed on an Optima 1 capillary column (25 m × 0.32 mm × 0.35 μm; Macherey-Nagel, Germany). Helium (99.999 %) was used as a carrier gas at a constant flow rate of 1.0 mL/min. The temperature of injection port was 200 °C. The temperature was programmed as follows: initial 150 °C (held for 1 min), from 150 °C to 280 °C at a rate of 15 °C/min and held at 280 °C for 7 min.

A mass spectrometer was operated with an electron impact source in multiple reaction monitoring (MRM) mode. The energy of electron impact ionization was 70 eV, ion source and MS quadrupole temperatures were set at 250 °C. The total running time was 17 min, also it was set a solvent delay at 3.0 min to prevent mass spectrometer damage. The instrument data system held an EI-MS library specially created for target analytes under our conditions. Each pesticide residue was quantified based on peak area of qualifier ions (diazinon – 137, triadimefon – 208, triadimenol – 112, bifenthrin – 181). The selection of characteristic ratios of the ion mass and its charge (m/z) was carried out on the basis of data from the electronic database of the National Institute of Standards and Technology (NIST). The volume of the injected sample was 1 μl, the total analysis time was 12 min (Figure 16).

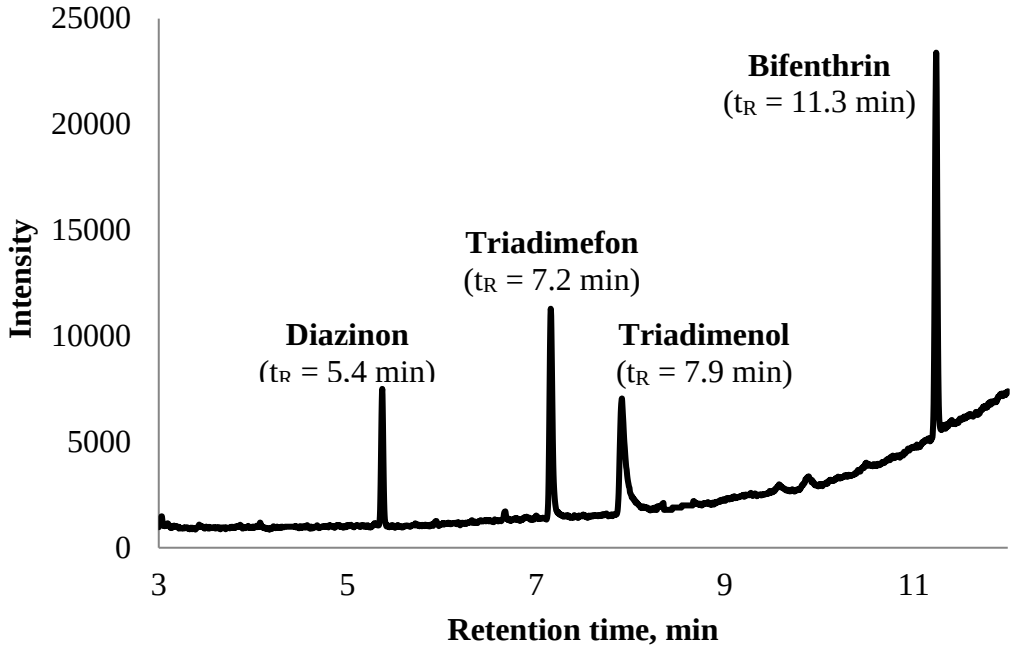

Figure 16. Chromatogram of a standard solution of a mixture of pesticides ($C_{analyte}$ = 100 μg/L).

HPLC-MS/MS determination of pesticides

To determine pesticides, a liquid triple-quadrupole chromatograph mass spectrometer LCMS-8030 (LC-/MS/MS) (Shimadzu, Japan) was used. Chromatographic separation was performed using a Zorbax Bonus-RP column (Agilent, 100 × 2.1 mm, 3.5 μm). Mobile phase A and B were deionized water and methanol containing 0.1 % (vol./vol.) formic acid, respectively. The analytes were eluted with a flow rate of 0.3 mL/min using a solvent gradient as followed: 0 – 8 min, 20 – 80 % B; 8 – 11 min, 80 % B. After each injection, there was 2 min post time for the mobile phase to revert to the initial 20 % B. The source temperature, drying gas (N_2) flow rate, DL temperature and nebulizer gas flow rates were set at 400 °C, 15 L/min, 250 °C and 3 L/min, respectively. Collision-induced dissociation gas was argon (230 kPa). MRM transitions and the collision energies were optimized during the experiments. The final settings are collected in Table 3.

Table 3. Collision energies, MRM transitions and retention times of pesticides.

Analyte	Collision energy (eV)	Molecular ion (m/z)	Fragment ion (m/z)	Retention time (min)
Imidacloprid	-15	256.05	209.10	3.8
	-19	256.05	175.20	
	-10	256.05	210.10	
Phosalon	-23	368.00	182.05	6.0
	-16	368.00	111.15	
	-14	368.00	138.15	
Malathion	-15	331.00	127.10	7.5
	-25	331.00	99.15	
	-10	331.00	285.00	
Triadimefon	-23	294.05	69.20	7.8
	-16	294.05	197.10	
	-14	294.05	225.00	
Diazinon	-20	305.10	169.00	8.5
	-20	305.10	153.10	
	-35	305.10	97.05	

HPLC-FLD determination of PAHs

Chromatographic separation were performed using a LC-20 Prominence HPLC-FLD system (Shimadzu, Japan). The chromatographic separation was achieved with a Zorbax Eclipse PAH column (100 mm × 2.1 mm, 3.5 μm; Agilent Technologies) at 40 °C. The gradient elution program and FLD conditions are shown in Table 4. The flow rate of the mobile phase was 0.4 mL/min.

A typical chromatogram of solution of a mixture of 13 PAHs is presented on Figure 17.

Table 4. The gradient elution program and FLD conditions for the PAHs determination.

Time, min	Mobile phase H_2O/acetonitrile	FLD wavelength, nm		Detected compounds
		Excitation	Emission	
0.0	45/55	215	330	
1.5	45/55	215	330	Nap
3.6		220	325	Flu
5.2		250	380	Phe, Ant
7.7	0/100	270	450	Flt
8.9		270	390	Pyr
10.0	0/100	270	390	BaA, Chr
12.5		260	420	
13.0	45/55	260	420	BbF, BkF, BaP
		290	415	DahA, BghiP
18.0	45/55	215	330	

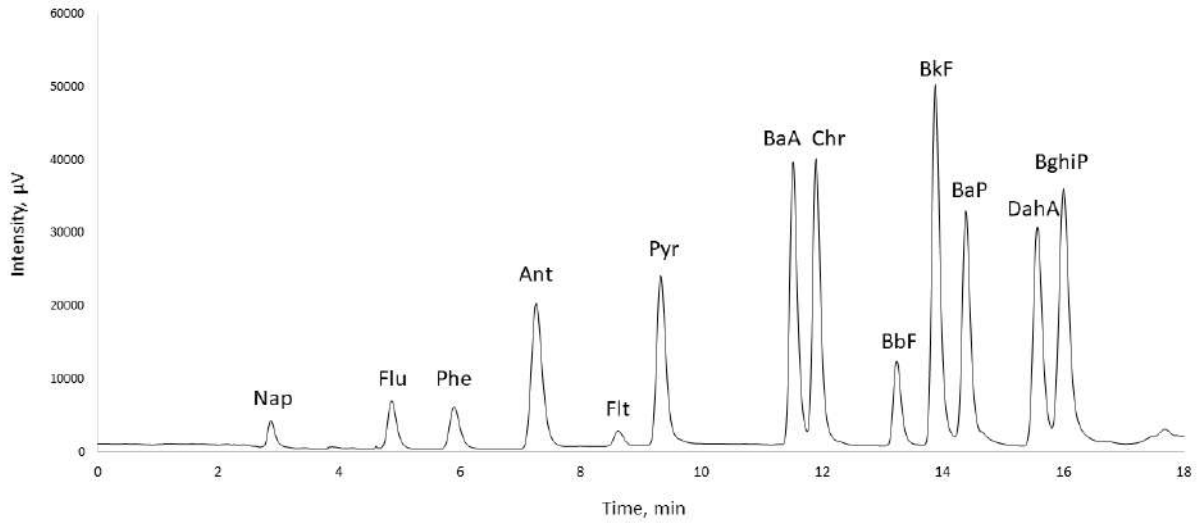

Figure 17. Chromatogram of an aqueous solution of a mixture of PAHs: BaA – benzo[a]anthracene, Chr – chrysene, BbF – benzo[b]fluoranthene, BaP – benzo[a]pyrene, BkF – benzo[k]fluoranthene, DahA – dibenz[a,h]anthracene, BghiP – benzo[ghi]perylene, Nap – naphthalene, Flu – fluorene, Phe – phenanthrene, Ant – anthracene, Flt – fluoranthene, Pyr – pyrene ($C_{analyte}$ = 5 µg/L).

2.5. Methods of analysis of obtained organic phases

GC-FID determination of 1-nonylamine and 2,2-dimethylpropanoic acid

A gas chromatography with flame ionization detection (GC-FID) system (Chromatek-Crystal 5000 system, Chromatek, Russia) was applied for the determination of 1-nonylamine and 2,2-dimethylpropanoic acid. The separation was carried out on an Optima capillary column (25 m × 0.32 mm × 0.35 μm).

The detection conditions for the determination of 1-nonylamine were as follows: the oven temperature was held at 100 °C for 5 min, the injection volume was 0.5 μL, the flow rate of nitrogen was 2.2 mL/min, and temperature of injector was 250 °C.

The detection conditions for the determination of 2,2-dimethylpropanoic acid were as follows: the oven temperature was held at 65 °C for 2 min, increased from 65 to 225 °C at 20 °C min-1 and held at 225 °C for 5 min. The injection volume was 1.0 μL, and the flow rate of helium was 2.2 mL/min.

GC-FID determination of heptanoic acid and thymol

A gas chromatography Chromatek-Crystal 5000 system (Chromatek, Russia) with flame ionization detection (GC-FID) was fitted with a HP-5 capillary column (5 % cyanopropyl phenyl and 95 % methylpolysiloxane, 10 m × 0.53 mm i.d., 2.65 μm coating) was utilized for the determination of DES precursors. The oven and column temperatures were kept at 250 °C and 180 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 30 mL/min, a sample volume was 1.0 μL. The obtained chromatogram of the ER phase is shown in Figure 18.

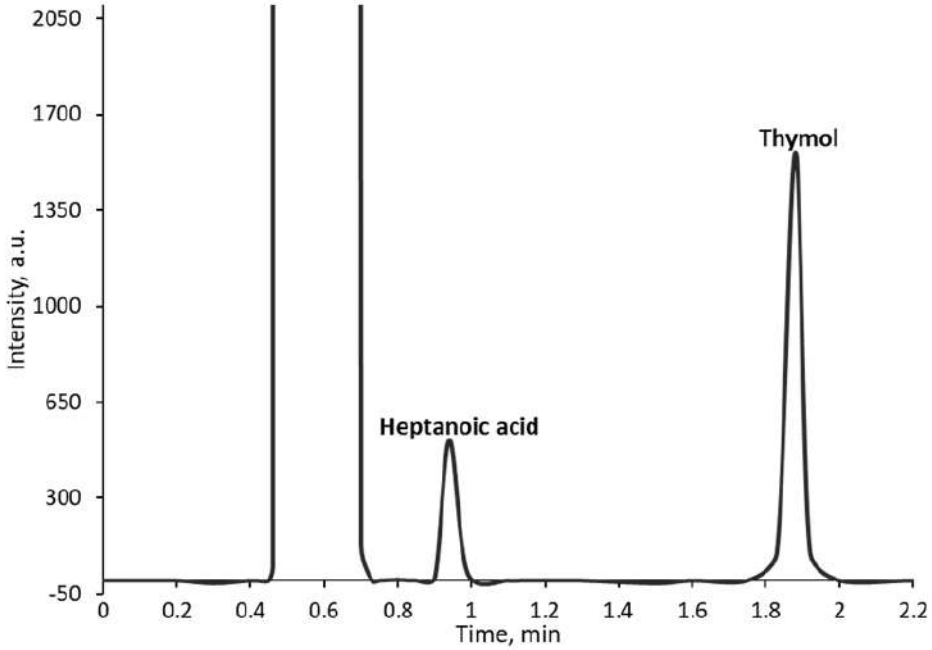

Figure 18. Chromatogram of the DES phase.

2.6. Evaluation of extraction efficiency

In the process of liquid-liquid extraction, under equilibrium conditions the distribution of substance A in a system consisting of two liquid phases (organic (o) and aqueous (a)), limitedly miscible with each other, can be represented in the following form [118]:

$$A_{(a)} \leftrightarrow A_{(o)}$$

In practice, when choosing an extractant, the *distribution coefficient (K)* is used, which takes into account the ratio of the total concentrations of all forms of the substance in two phases and shows how the substance is distributed between equal volumes of the aqueous and organic phases and is expressed by the formula:

$$K = \frac{C_o}{C_a},$$

where C_o – concentration of the substance in the organic phase; C_a – concentration of the substance in the aqueous phase.

Another important characteristic of the extraction process is the *extraction recovery*, which characterizes the extraction efficiency of substance A from one phase to another. The degree of extraction is calculated in % using the formula:

$$Extraction\ recovery,\ \% = \frac{\mathrm{Co}Vo \cdot 100}{\mathrm{C}aVa + \mathrm{Co}Vo} = \frac{K \cdot 100}{K + Va/Vo},$$

where V_o – the volume of the organic phase; V_a – the volume of the aqueous phase.

The *enrichment factor (EF)* also serves to assess the extraction efficiency and shows how many times the ratio of the concentration of a substance in the extract (C_o) changes in relation to its initial concentration in the sample (C_{sample}) and is expressed by the formula:

$$EF = \frac{Co}{\mathrm{C}sample}$$

If there was no loss of substance during its extraction, it is assumed that the enrichment factor is approximately equal to the ratio of the sample volume to the volume of the organic phase.

2.7. Evaluation of results of analyte determination

Statistical data analysis was carried out in accordance with [119].

Calibration curves for determination of target analytes were obtained by analyzing series of solutions with known concentrations of analytes and processing the obtained data using the least squares method. The limit of detection (LOD) was calculated as three times the standard deviation of the background signal (3σ), expressed in concentration units based on the equation of the calibration curve, the limit of quantification was calculated similarly as 10σ. Repeatability was characterized using the relative standard deviation (RSD, %) of the measurement results under the corresponding conditions.

Rounding the results was carried out according to three general rules [120]:

1. The results of intermediate measurements were obtained using 3-4 significant figures (digits).

2. The absolute and relative error of the analysis result was rounded to two significant digits if the first of them was equal to 1 or 2, and to one digit if the first was equal to 3 or more.

3. The analysis result was rounded to the same decimal place that ends the rounded value of the total error.

The correctness of the obtained results was confirmed by the "add-found" method and/or the reference method.

The "add-found" method

The *relative bias (R, %)* was calculated using the following formula:

$$R,\% = 100 \cdot |1 - \frac{C_1 - C_0}{C_{add}}|,$$

where C_1 – concentration of analyte in spiked sample; C_0 – concentration of analyte in sample without additive; C_{add} – concentration of the introduced additive.

The reference method

Accuracy and reliability of the proposed method were verified by analyzing the samples using reference method. The Fisher's test (F-test) and the Student's test (t-test) were applied. F-values $\leq F_{cr}$ indicated insignificant difference in precision between both methods at the 95% confidence level. And t-values $\leq t_{cr}$ indicated insignificant difference between the results obtained using these methods.

CHAPTER 3. HEADSPACE MICROEXTRACTION OF VOLATILE ANALYTES FROM FOOD SAMPLES

Headspace microextraction of volatile analytes using sorbents is of particular interest for the analysis of complex food samples. In this case, the interfering effect of matrix components of the sample is excluded. The selection of a suitable sorbent for the target analyte is the main task of headspace microextraction. For these purposes, we proposed to use ferromagnetic nanoparticles (NPs). Ferromagnetic NPs is high-tech materials that have a number of advantages, such as high sorption capacity due to the developed surface; high magnetic properties, which eliminates the centrifugation stage; as well as low susceptibility to oxidation and external influences. Compounds sorbed on magnetic NPs can be easily eluted with a suitable solvent for subsequent determination. Previously, their use was reported only in the analysis of liquid samples [121–123].

3.1. Dynamic headspace microextraction

As a general approach to sample preparation of liquid food products, a scheme of on-line extraction of inorganic substances capable of forming volatile compounds. It includes reactive gas extraction of analytes and their subsequent headspace microextraction on ferromagnetic NPs. Further analysis involves a combination of the proposed method with various detection methods, which is ensured by choosing a suitable eluent.

The capabilities of the proposed approach were demonstrated using the example of selenium determination in mineralized biologically active food additives. Selenium plays an important role in many processes occurring in the human and animal organisms (the required daily dose of selenium for humans is 50-100 μg). Despite the beneficial properties of selenium, its excess can cause poisoning, as well as the development of various diseases, including cancer [124]. Often, food manufacturers intentionally add selenium to increase their beneficial properties. Thus, there is a task to control the selenium content in food products and biologically active additives (BAA) to food.

The developed sample preparation scheme (Figure 19) involves converting selenium (IV) into its volatile form, hydrogen selenide, by injecting a sodium borohydride solution into an acidified sample solution using a peristaltic pump, followed by passing the resulting gas phase through a column with magnetic nanoparticles equipped with external magnet. Hydrogen selenide was adsorbed on the surface of the ferromagnetic NPs in the column. Elution of the analyte into the aqueous phase in an ultrasonic field and separation of the eluate from the particles in an external magnetic field provided a solution for subsequent analysis by electro thermal atomization-atomic absorption spectroscopy (ETA-AAS).

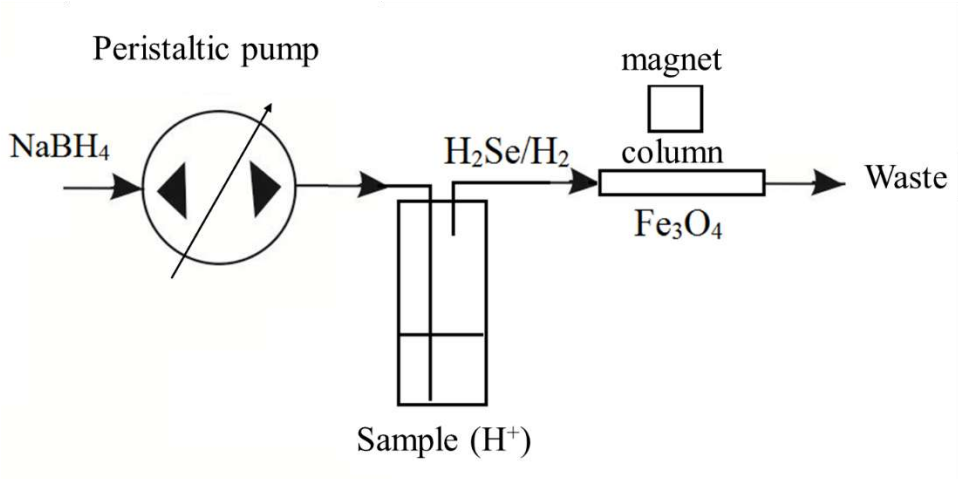

Figure 19. Scheme of dynamic headspace microextraction of volatile analyte on magnetic nanoparticles.

Seven types of MNPs (Fe_3O_4, Fe_3O_4@5AlOOH, Fe_3O_4@10AlOOH, Fe_3O_4@20AlOOH, Fe_3O_4@33AlOOH, Fe_3O_4@50AlOOH, Fe_3O_4@66AlOOH) were investigated for H_2Se sorption. Fe_3O_4@AlOOH was chosen because it is known as an effective sorbent for ions separation [125]. It should be noted that the presence of a hydroxide shell did not affect the magnetic properties of the sorbents. The nanomaterials were provided by the staff of the Department of general and inorganic chemistry of the Institute of chemistry of St. Petersburg State University. The NPs were characterized by various methods (X-ray diffraction analysis, Fourier transform infrared spectroscopy, transmission electron microscopy, scanning electron microscopy) [17]. Figure 20 shows the distribution of sizes of synthesized magnetic NPs.

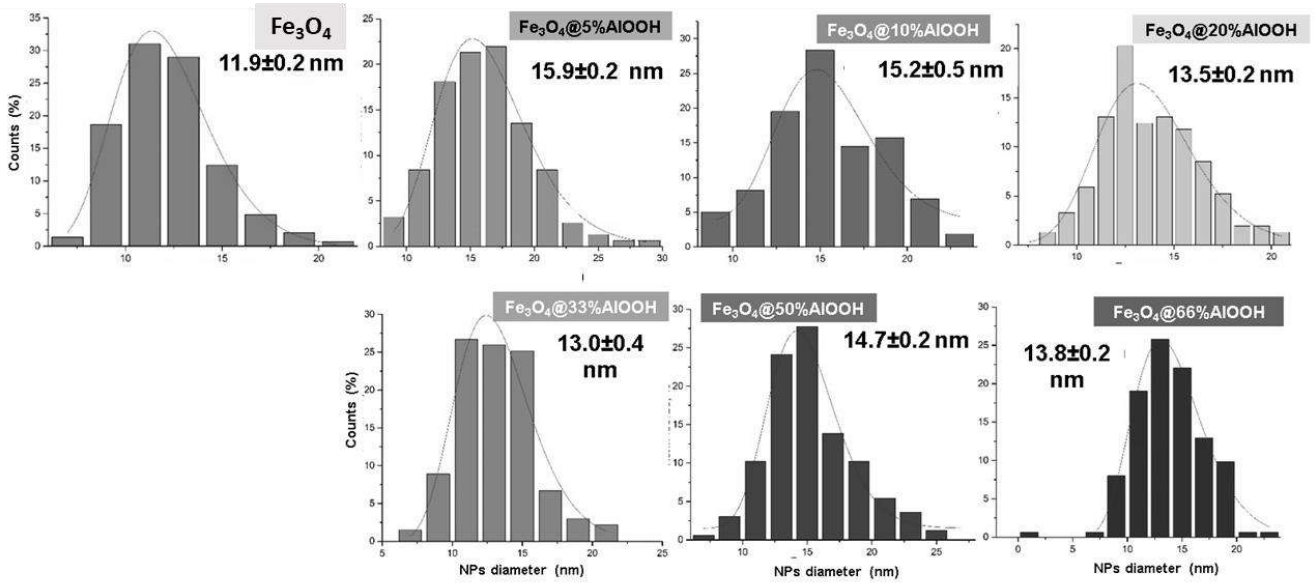

Figure 20. Size distribution of synthesized magnetic NPs.

It was found that all synthesized NPs ensure the extraction of hydrogen selenide. Coating Fe_3O_4 particles with a shell, on the one hand, led to an increase in their stability in the ultrasound field during the elution process, on the other hand, a decrease in the efficiency of hydrogen selenide sorption was observed (up to 65 ± 5%), since the area of the active surface available for interaction with gas molecules decreased (Figure 21). A higher energy of interaction of particles with analytes in an external magnetic field, calculated according to the reference book [126], was observed in the case of uncoated magnetic NPs.

The sorption efficiency was calculated as using the following equation:

$$\text{Sorption efficiency (\%)} = \frac{Q_{ads}}{Q_{st}} \times 100,$$

where Q_{st} – initial amount of analyte in standard solution of Se (IV); Q_{ads} – amount of analyte found in eluate.

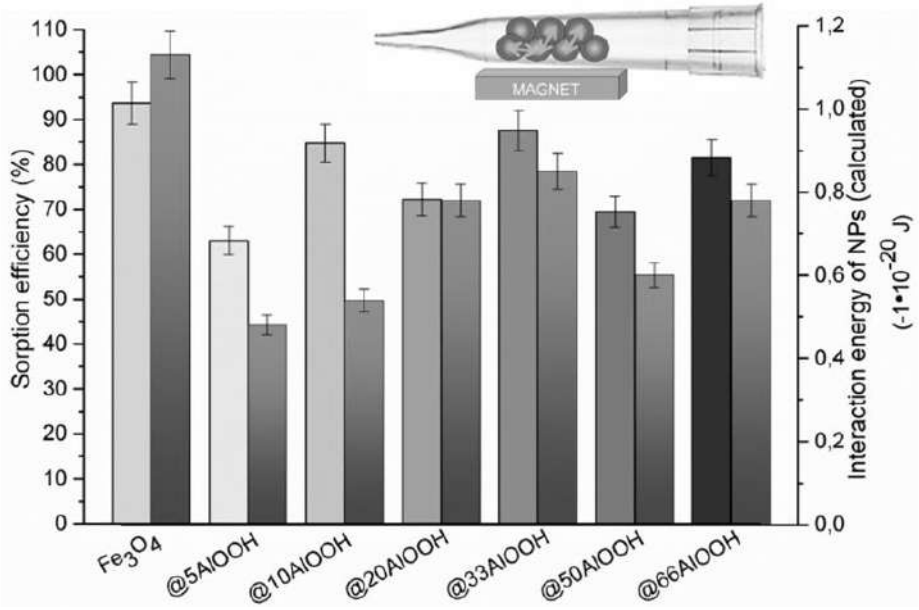

Figure 21. Sorption efficiency (left) and interaction energy values (right) in gas phase under the external magnetic field for MNPs synthesized (5 mg of MNPs) (n = 3).

The maximum sorption efficiency of hydrogen selenide was observed for Fe_3O_4 type particles (90 ± 5 %). Therefore, Fe_3O_4 type particles were selected for the extraction of hydrogen selenide according to the proposed scheme. NPs assume their single use, due to the fact that after exposure to an ultrasonic field, their sorption properties worsened.

To understand sorption mechanisms of H_2Se on Fe_3O_4 magnetic NPs surface the adsorption isotherm was plotted. Two widely used models based on Langmuir and Freundlich isotherm equations were applied. The Langmuir isotherm describes the process of monomolecular absorption on a homogeneous surface with identical adsorption sites and can be written in the following linear form:

$$\frac{1}{Qe} = \frac{1}{Q_M} + \frac{1}{KLQ_M}\frac{1}{C},$$

where Q_e and Q_M – equilibrium and maximum adsorption capacity, respectively, K_L – Langmuir constant, C – concentration.

The linear form of the Freundlich isotherm describes the polymolecular absorption process of a heterogeneous surface and can be represented as follows:

$$\lg Q_p = \lg K_f + \mathbf{n}\lg C,$$

where K_f – Freundlich's constant, related to the adsorption capacity of the sorbent, n – empirical parameter related to the intensity of adsorption.

The linearization results indicated that in our case the adsorption process was polymolecular, Freundlich model represented a good agreement with data obtained (adjusted R-square value for linear least squares fitting was equal to 0.998 (Figure 22)). Thus, it can be concluded, that H_2Se adsorption on Fe_3O_4 NPs was characterized by the sorbent surface heterogeneity because Freundlich isotherm model represents heterogeneity of solid surfaces. The n value (0.947) obtained from the Freundlich model was smaller than 1. Thus, nonlinear sorption process takes place.

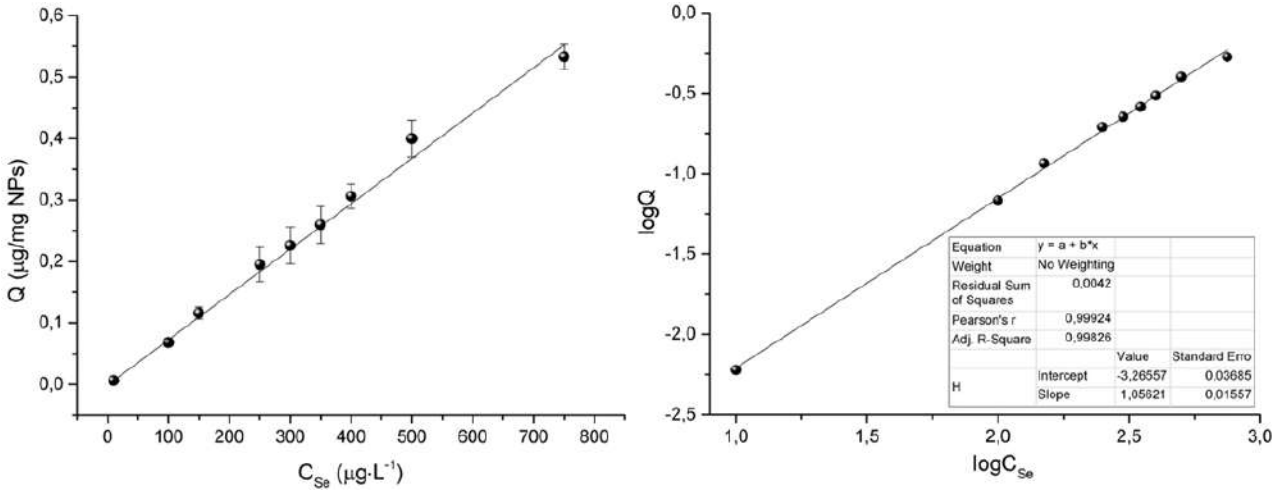

Figure 22. H_2Se adsorption isotherm for bare Fe_3O_4 nanoparticles (left) (5 mg of MNPs) and Freundlich model linearization (right).

The influence of the sorbent mass (Fe_3O_4) in the range from 2.5 to 10 mg on the sorption efficiency was also studied, and it was found that the NP mass of 5 mg with a sample volume of 5 mL provided the highest degree of sorption efficiency – (90 ± 5) %.

The flow rate of $NaBH_4$ solution injected in a sample solution influenced on a flow rate of gas phase generated. The flow rate of $NaBH_4$ solution (3 %) in the range of 0.2 – 1 mL/min was varied, and the gas flow rate was measured using a gas flow meter. It was found, that $NaBH_4$ solution flow rate of 0.5 mL/min provided optimal conditions for gas phase generation (gas flow rate 10 mL/min) and H_2Se sorption (sorption efficiency 95±5 %). At higher reagent flow rate the excessive hydrogen generation was observed, which caused solution spraying and getting droplets of aqueous phase into a microcolumn. In this case, the magnetic NPs were wetted and H_2Se sorption was not observed.

Taking into account the need to convert H_2Se to a water-soluble form for its elution from magnetic NPs as well as the features of pyrolytic graphite tubes and the properties of NPs, such oxidants as $KMnO_4$ (10^{-7} - 10^{-5} mol/L) and H_2O_2 (5 - 30 %) were studied as components of elution solution. Such concentrations of $KMnO_4$ were chosen to eliminate background interferences during ETA-AAS analysis and to exclude its negative effect on the pyrolytic graphite tube. The results obtained showed low elution efficiency (30 %) in case of $KMnO_4$. It was established that the maximum absorbance peak area was observed at using 30 % H_2O_2, thus, this eluent was chosen for further experiments. Elution efficiency was higher in 2.5 times compared to one with $KMnO_4$ solution.

It is well known that sonication is widely used to activate MNPs surface [127]. Ultrasonic irradiation can improve the mass transfer between two immiscible phases and reduce the equilibrium time, and thus can be helpful for elution process. It was established that the use of ultrasonic irradiation enhanced analytical signal in two times. To choose the optimal sonication time, the elution process was implemented at 1, 5, 10, and 15 min of sonication (325 W, 35 kHz). The maximal absorbance peak area was observed at 10 min of sonication and no improvement was found at 15 min. Thus, 10 min of sonication was chosen for analyte elution from magnetic NPs.

The effect of potentially interfering volatile compounds (AsH3 and H2S) on H_2Se sorption and selenium determination was also investigated. It was performed by addition of known concentration of $KAsO_2$ and Na_2S to 50 μg/L selenium (IV) solution. The tolerable concentration of each taken foreign compound is considered to be less than 5 % of relative error in the signal. It was shown that AsH_3 and H_2S generated did not interfere on H_2Se sorption and selenium determination up to the highest studied concentration of As (III) and S^{2-} (1 mg/L).

The developed scheme of selenium extraction in combination with ETA-AAS allowed to provide the linear calibration range of 1 - 20 μg/L, the LOD of selenium (IV) (3σ) was 0.3 μg/L. Repeatability was characterized by the relative standard deviation (RSD), which was calculated based on the data obtained over 5 days. Thus, the RSD values calculated for 1 and 20 μg/L were 6 - 9 % and 4 - 8 %, respectively. The sample volume was chosen to be 5 mL.

The developed procedure was applied for the determination of Se (IV) in three types of dietary supplements. The dietary supplements were complex matrices, which included selenoxanthen (sample № 1), sodium selenite (samples № 2, 3) as biologically active substances, and different other components (cellulose, dextrose, starch), anti-caking agents (magnesium stearate, silicon dioxide, talc), some vitamins ((2R)-2,7,8-Trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol (vitamin E) and L-ascorbic acid (vitamin C)), and salts/oxides of zinc, iron, and calcium. Sampling and preliminary treatment of samples is described in section 2.3. Generally, the obtained results corresponded to the contents indicated on each package (Table 5). Accuracy and reliability of the resulting information were further studied by "add-found" method.

When analyzing real samples using the developed method, the relative bias did not exceed 13% (Table 5), which demonstrates the correctness of the results obtained. In accordance with [128], for samples with an analyte content of 100 μg/kg, a relative bias of up to 20 % is allowed.

Table 5. Determination of selenium (IV) content in dietary supplement samples (P = 0.95, n = 3).

Sample №	Labeled value, μg	Added, μg	Found, μg	R, %
1	50	0	45 ± 8	-
		50	90 ± 12	10
		100	147 ± 21	2
2	50	0	54 ± 7	-
		50	101 ± 10	6
		100	146 ± 17	8
3	40 - 60	0	52 ± 8	-
		50	99 ± 11	6
		100	139 ± 19	13

In addition, the developed method was tested using the standard sample SRM 7340-96 (Se (IV) solution, Ecokhim, Russia). In this case, the t-test was used to establish the statistical significance of the results. The results showed no differences, which were

detected at a confidence level of 95 %, between the Se (IV) value indicated for the standard sample as (500 ± 5) μg/L and the obtained Se (IV) value of (500 ± 5) μg/L (n = 3). Thus, the obtained result is consistent with the standard sample value ($t_{exp} < t_{cr}$): t critical and expected values were 2.78 and 1.34, respectively.

The proposed automated method of dynamic headspace microextraction using ferromagnetic nanoparticles as a sorbent has a number of *advantages*. Firstly, Fe_3O_4-based NPs are easy to obtain, cheap and have readily available precursors, which allows them to be used once, thereby eliminating the stages of washing and conditioning, as well as cross-contamination of samples. Secondly, magnetic NPs can be easily retained by an external magnetic field in the gas phase, which leads to the sorption of the analyte in the gas phase flow and eliminates the interfering effect of the sample matrices. Thirdly, compared to conventional sorbents, magnetic NPs allow the elution of the analyte without centrifugation and filtration. In addition, the proposed dynamic mode of hydrogen selenide concentration on a ferromagnetic sorbent in combination with the sensitive ETA-AAS method provides low selenium (IV) concentrations, on average an order of magnitude lower than in previously proposed methods (Table 6).

The *limitation* of the developed method is the high cost of the reagent (sodium borohydride).

The results obtained were published in the journal Analyst [14].

Table 6. Comparison of headspace SPME methods for selenium (IV) determination.

Detection method	Sample	Sorbent	Reagent	LOD, ng/mL	Reference
GC-AED[1]	Water samples	Divinylbenzene/ Carboxene/ PDMS[2]	Sodium tetraethylborate	7.3	[129]
GC-AED	Water samples	Divinylbenzene/ Carboxene/ PDMS	4,5-Dichloro-1,2-phenylenediamine	3	[129]
GC-MS	Urine	PDMS	Sodium tetraphenylborate, sodium tetraethylborate	0.03	[130]
IMS[3]	Blood serum, natural waters	Polypyrrole	1,2-phenylenediamine	12	[131]
OES[4]	Beer, wort, brewer's yeast	PDMS/Carboxene	Sodium borohydride	0.8	[132]
ETA-AAS	BAA	Magnetic NPs	Sodium borohydride	0.3	This work

GC-AED[1] – Gas chromatography with atomic emission detection;
PDMS[2] – Polydimethylsiloxane;
IMS[3] – Ion mobility spectrometry;
OES[4] – Optical emission spectrometry.

3.2. Static headspace microextraction

To extract volatile organic analytes from solid-phase food matrices, a method of static headspace microextraction was developed, including sorption of analytes on magnetic NPs with their subsequent elution. Figure 23 shows a combined version of the developed approach with a chromatographic system.

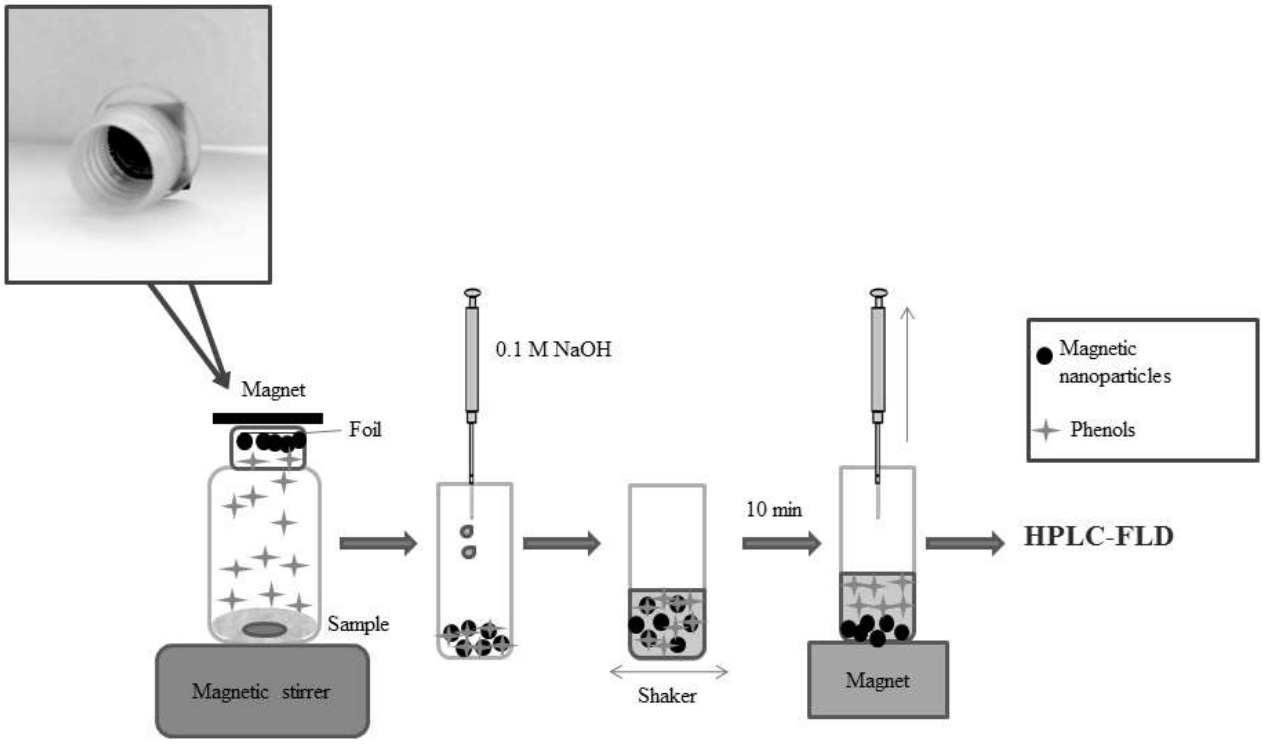

Figure 23. A combined method for the determination of volatile analytes, including static headspace microextraction on a magnetic sorbent, elution and subsequent chromatographic analysis.

The capabilities of the proposed approach were illustrated using the example of determining volatile phenols in smoked meat samples. The four most common phenols in smoked food products were selected as analytes: phenol, *o*-cresol, *p*-cresol, and guaiacol [133,134]. A study of semi-smoked sausages [133] showed that during the smoking process, the bulk of phenols enters the casing (0.1 mg %). Subsequently, the concentration of phenols decreases due to their penetration into the sausage, as well as the evaporation of some of them from the surface of the product. Phenols dissolve well in fat, so there are more of them in adipose tissue than in muscle tissue.

According to the proposed method, the extraction of phenols from a solid-phase sample was carried out in a hermetic system: a glass vessel (10 mL volume) with a tight cap, on the inner surface of which a thin layer of magnetic nanoparticles was held by an

external magnet (commercially available adhesive magnetic tape). It was found that the most effective extraction of phenols from homogenized solid-phase food samples occurs with uniform stirring and heating, so the system was placed on a heated magnetic stirrer.

After the phenols had been isolated, the lid of the hermetic system was removed and the particles were transferred to a clean vial by disconnecting the external magnet. At the next stage, the analytes were eluted with vigorous stirring; then the magnetic NPs were held at the bottom of the container using a magnet, and obtained supernatant with the phenols was collected and analyzed using HPLC-FLD. The conditions were selected and the calibration curve was plotted using a sample prepared according to section 2.3 with the introduction of the corresponding analyte additives.

The most frequently used magnetic materials are based on Fe_3O_4. Eight types of NPs (Fe_3O_4, Fe_3O_4@$Cr(OH)_3$, Fe_3O_4@$Ni(OH)_2$, Fe_3O_4@$Cu(OH)_2$, Fe_3O_4@$Co(OH)_2$, Fe_3O_4@NiO, Fe_3O_4@Fe_2O_3, Fe_3O_4@CuO) were prepared by the staff of the Department of general and inorganic chemistry of the Institute of chemistry of St. Petersburg State University and characterized by different methods (X-ray diffraction analysis, Fourier transform infrared spectroscopy, transmission electron microscopy, scanning electron microscopy) [19]. Figure 24 shows the distribution of sizes of synthesized magnetic NPs.

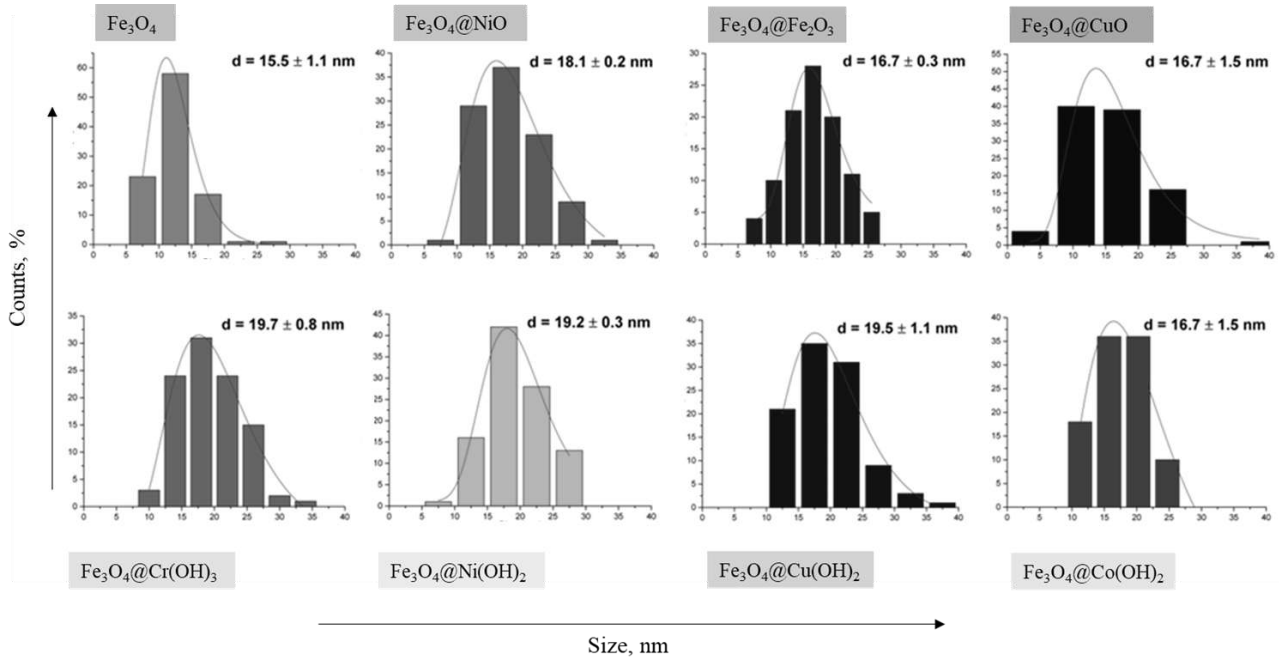

Figure 24. Size distribution of synthesized magnetic NPs.

It was experimentally established that nanoparticles Fe_3O_4@$Cr(OH)_3$ provided greater efficiency of sorption of target analytes compared to other NPs (Figure 25), so they were selected for further studies. Presumably, the sorption process is determined by two key factors: the boiling point of the analyte and the interaction of the sorbent surface with the analyte.

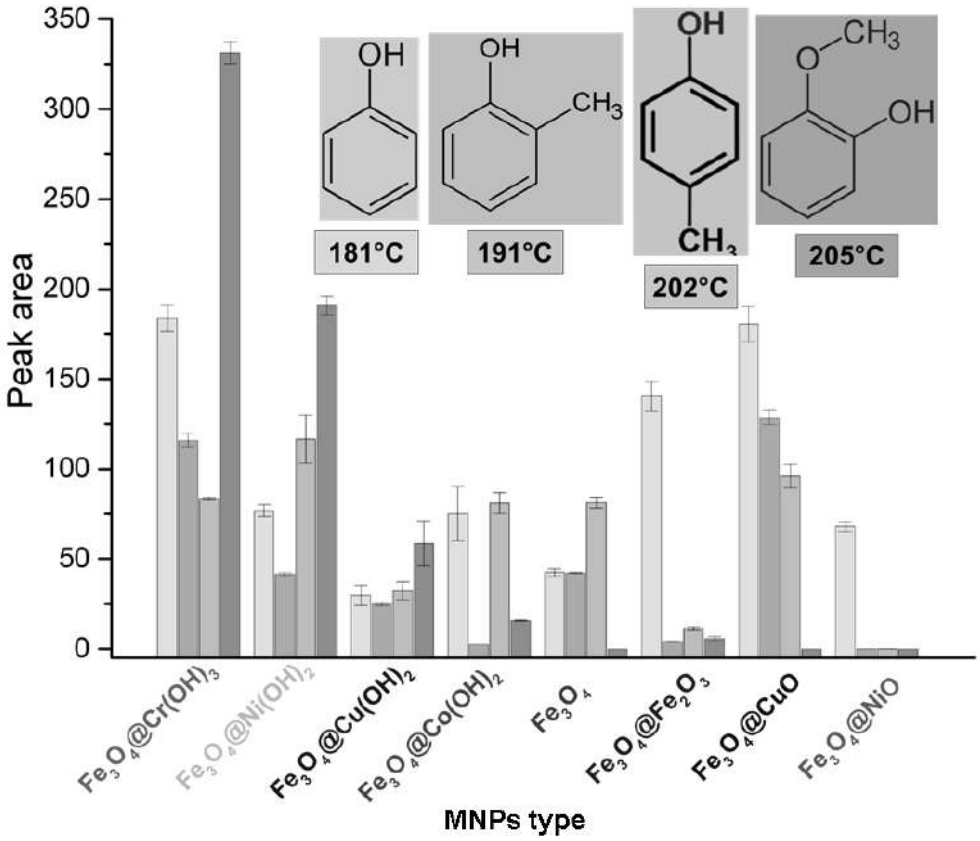

Figure 25. Effect of magnetic sorbent composition on the efficiency of headspace microextraction of phenol, *o*-cresol, *p*-cresol and guaiacol ($C_{analyte}$ = 0.5 mg/kg, sample weight – 0.5 g, n = 3).

In the case of oxide shell, only the interactions between surface metal atom and analytes took place. The obtained row (copper, iron, and nickel oxides) is in a good agreement with 3d metal complexes stability constants, which confirms assumption of metal-analytes interactions. The highest analytical signal was obtained for phenol characterized by the lowest boiling point of 181°C. As noted above it could be due to higher phenol concentration in gaseous phase in comparison with other analytes.

For hydroxide shell, the additional interaction could occur between analytes and hydroxyl groups of magnetic NPs. It should be pointed out, that the sorption efficiency

becomes higher with increasing metal atom valence and electronegativity, since there is a maximal probability of two types of interactions. Using Fe_3O_4@hydroxide NPs the highest sorption efficiency was obtained for guaiacol characterized by the highest boiling point of 205 °C. Unlike other analytes guaiacol contains hydroxyl and methoxyl groups, which can increase the sorption efficiency due to two binding points.

The amount of magnetic NPs is an important factor affecting the sorption efficiency and reproducibility. The effect of NPs (Fe_3O_4@$Cr(OH)_3$) mass was investigated in the range from 1 to 20 mg. The optimal sorbent mass was determined as 10 mg. The smaller sorbent mass did not ensure complete and uniform surface cap filling, what resulted in the lower sorption efficiency, while sorbent mass more than 10 mg did not lead to higher analytical signal.

Temperature is a significant parameter for volatile analytes' excretion. The temperature was studied in the range of 40 - 100 °C. It was found that the higher the temperature, the higher the analytical signal, however, at temperatures above 90 °C lead to a condensation of water micro-droplets from samples on NPs, which negatively affected the results. Under the optimal temperature (90 °C) time of phenols' separation was investigated. It was established that after 10 min the analytical signal was not changed, thus, the procedure should be carried out at 90 °C for 10 min.

An ultrasonic irradiation is widely used tool during extraction process due to ultrasonic irradiation can speed up the mass transfer from sample into headspace and reduce the equilibrium time [136]. However, it was found that the use of ultrasonic irradiation did not influence on the analytical signal; therefore, it was not used for further studies.

It was necessary to elute the analytes for their determination by HPLC-FLD. The elution process was performed by placing the NPs containing analytes into a 5 mL beaker with 0.5 mL of different eluents and mixing during 30 min with a shaker. Ultra pure water, 0.01 mol/L NaOH, methanol and acetonitrile were investigated as eluents in this research. The sorption of phenols on magnetic NPs surface can occur both by interaction through hydroxyl groups with the formation of hydrogen bonds, and by the formation of bonds between the surface metal atoms and the hydroxyl groups of the sorbent. This assumption

was supported by the fact that less polar solvent (acetonitrile) provided minimal elution efficiency. The highest analytical signals were observed with using 0.5 mL of sodium hydroxide solution (Figure 26). The increase of NaOH concentration did not affect the analytical signals within the standard deviation in the range of 0.01 – 0.5 mol/L, therefore, 0.01 mol/L was chosen as optimal.

The reduction of eluent volume can increase the concentration of analytes in final solution and as result can improve the sensitivity. Thus, different volumes of 0.01 mol/L NaOH solution were studied. It was found that the low volume was used the higher analytical signal was achieved, however, the eluent volume low than 0.1 mL was not considered due to the necessity of complete NPs wetting.

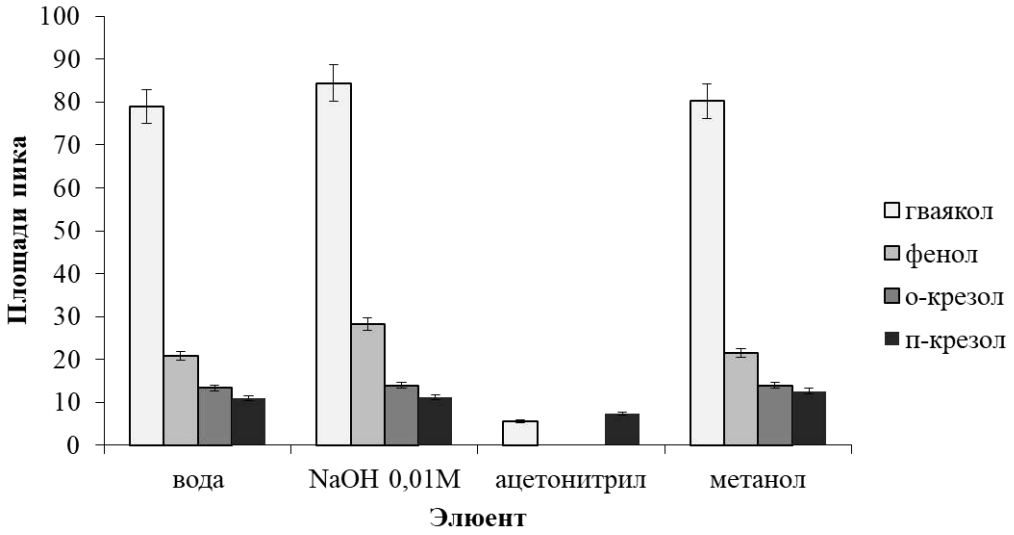

Figure 26. Efficiency of the elution process of phenols from magnetic nanoparticles Fe_3O_4@$Cr(OH)_3$ using different eluents ($C_{analyte}$ = 0.1 mg/kg, n = 3).

Elution time is a parameter affecting the elution efficiency of analytes. To choose the optimal time, the elution process was implemented in a range of 1-30 min (n=3). The maximal peak areas were observed at 10 min and no improvement was found during the next 20 min. Thus, elution time of 10 min was chosen for analytes elution from NPs.

Under the optimal conditions, the analytical performance of the proposed procedure was evaluated. The linear calibration ranges of 0.5 - 2500 μg/kg. The LODs, calculated from a blank test, based on 3σ, were 0.2 μg/kg. The extraction recovery,

calculated as the ratio of the amount of analyte found in the eluate to the amount of analyte introduced into the sample, was (82 ± 5) %. Time of the procedure developed was 20 min.

The repeatability, characterized by the value of the RSD value, did not exceed 8 %. The specificity of the determination of phenol, guaiacol, *o*-cresol and *p*-cresol in the analysis of real samples was estimated by comparing the retention time of each analyte in the analysis of the real sample and the standard solution; the discrepancy in retention times did not exceed 2 %. The time of the proposed sample preparation method was 20 min.

The proposed method was used for the determination of phenol, guaiacol, *p*-cresol, and *o*-cresol in three types of smoked sausage (Table 7). Sampling and sample treatment are described in Section 2.3. All four analytes were found in all sausage samples, and the phenol content, as expected, was higher in smoked sausages than in the semi-smoked sample. The correctness of the results was confirmed by the “add-found” method.

Among the huge variety of methods that allow quantitative extraction of phenols from food products, the most common are LLME [39] and SPME [137]. Headspace SPME is used much less frequently, although it has a number of *advantages* such as no need for preliminary sample preparation (except for homogenization), elimination of the interfering effect of multicomponent sample matrices, and disposable sorbents.

Compared to the literature-described approaches of conventional and vacuum headspace microextraction of phenol using commercial fibers [138] and in combination with GC-FID analysis, the developed method has lower LODs, as well as wider linear calibration ranges. In addition, a vacuum introduces additional complications into the sample preparation procedure. The advantages of using magnetic NPs as sorbents were noted in Section 3.1.

The research results were published in the New Journal of Chemistry [17].

Table 7. Determination of volatile phenols in smoked sausage samples (P = 0.95, n = 3).

Sample	Analyte	Added, mg/kg	Found, mg/kg	R, %
Sausage №1 (smoked)	phenol	0	1.142 ± 0.014	-
		0.5	1.68 ± 0.07	8
		1	2.32 ± 0.05	18
	guaiacol	0	3.69 ± 0.07	-
		0.5	4.24 ± 0.04	10
		1	4.83 ± 0.14	14
	o-cresol	0	1.05 ± 0.04	-
		0.5	1.63 ± 0.04	16
		1	2.11 ± 0.06	6
	p-cresol	0	1.25 ± 0.06	-
		0.5	1.72 ± 0.09	6
		1	2.26 ± 0.05	1
Sausage №2 (semi-smoked)	phenol	0	0.114 ± 0.013	-
		0.5	0.65 ± 0.04	8
		1	1.01 ± 0.18	10
	guaiacol	0	0.96 ± 0.08	-
		0.5	1.55 ± 0.15	18
		1	2.11 ± 0.14	15
	o-cresol	0	0.16 ± 0.03	-
		0.5	0.61 ± 0.04	10
		1	1.16 ± 0.03	90
	p-cresol	0	0.18 ± 0.04	-
		0.5	0.69 ± 0.06	2
		1	1.31 ± 0.05	13
Sausage №3 (smoked)	phenol	0	1.22 ± 0.04	-
		0.5	1.71 ± 0.08	2
		1	2.19 ± 0.05	3
	guaiacol	0	5.72 ± 0.14	-

		0.5	6.21 ± 0.13	2
		1	6.63 ± 0.11	7
	o-cresol	0	1.06 ± 0.06	-
		0.5	1.63 ± 0.05	14
		1	2.19 ± 0.11	13
	p-cresol	0	2.05 ± 0.11	-
		0.5	2.62 ± 0.08	14
		1	3.12 ± 0.11	7

CHAPTER 4. LIQUID-LIQUID MICROEXTRACTION FOR THE EXTRACTION OF ANALYTES FROM SOLID-PHASE FOOD SAMPLES

An important aspect of modern chemical analysis is its environmental friendliness, which manifests itself in concern for both the chemist performing the analysis and the environment. This has led to the development of an entire scientific field – green analytical chemistry, the main principles of which are a radical reduction in toxic solvents or their complete replacement with natural analogues, minimization of waste, and automation of analysis in order to reduce labor costs [139]. Environmentally friendly extractants include ionic liquids, deep eutectic solvents, switchable hydrophilicity solvents and micellar media [140,141]. Such extractants are also called the “new generation solvents” or “designer solvents” [24,83].

This thesis implements fundamentally new approaches to microextraction and pre-concentration of xenobiotics from food products using new generation solvents.

4.1. Microextraction using impregnated membrane

The analysis of solid-phase food samples is complicated by preliminary sample preparation, often including their acid or alkaline digestion for subsequent LLME. Membrane microextraction is an attractive method of separation and concentration in terms of the possibility of its implementation in suspended samples. In this case, the membrane acts as a kind of barrier and prevents the influence of sample macrocomponents on the efficiency of extraction, while the extractant itself is retained in the pores of the hydrophobic polymer membrane due to capillary forces.

Medium-chain fatty acid acids are of particular interest in terms of their use as new organic extractants, since they meet the main criteria of green analytical chemistry: low toxicity and environmental safety. In an alkaline medium, they are almost completely ionized, as a result of which the acid phase dissolves in the aqueous phase. However, when the solution is acidified, the carboxylic acid passes into a hydrophobic form, forming its own phase, which allows the use of medium-chain fatty acids as extractants.

Due to their hydrophobic properties, medium-chain fatty acids are able to wet hydrophobic polymer microporous membranes.

The first step of the analysis involved passive transport of the polar analyte in molecular form from acidified solutions/suspensions into the organic phase of the SHS located in the pores of the hydrophobic membrane (Figure 27a). In the second step (Figure 27b), after pH changing by addition of excess of KOH solution (pH > pKa), the back-extraction of analytes takes place as a result of two processes, which occur simultaneously: increasing pH induces the fast conversion of supported medium-chain fatty to water-soluble salt and rapid transfer of analytes from organic to aqueous acceptor phase. Thus, the back-extraction process proceeds only by simple pH changing without the use of organic solvents or thermal desorption.

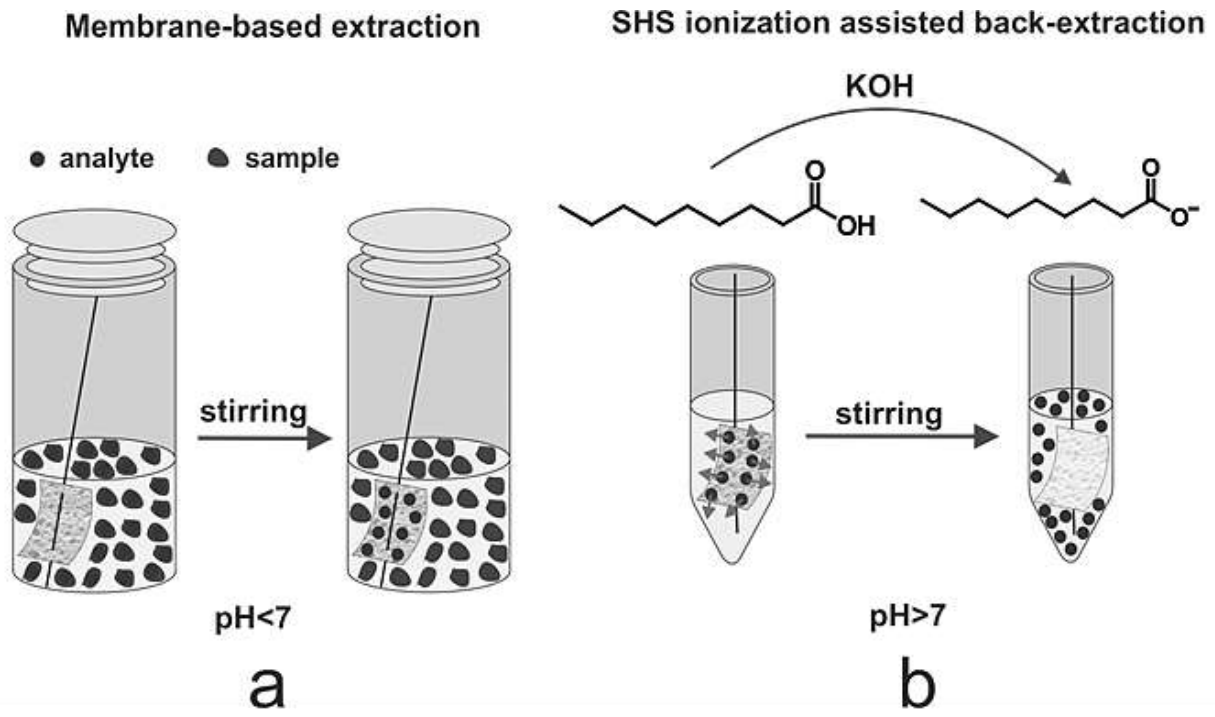

Figure 27. Scheme of microextraction of fluoroquinolones from suspended samples on membranes impregnated with medium-chain fatty acid: extraction (a) and back-extraction (b) processes.

Two types of commercially available hydrophobic membranes based on a copolymer of tetrafluoroethylene and vinylidene fluoride (MFFK-0.25, pore size 0.25 μm) and polypropylene (MNPP-0.20, pore size 0.2 μm) were studied. A 10×10 mm membrane was impregnated with switchable hydrophilicity solvents, fixed on a needle

and placed in 3 mL of an aqueous solution of fluoroquinolones for 40 min with stirring. At the next stage, analytes were eluted from the membrane using 500 μL of 0.1 mol/L potassium hydroxide solution, after which the eluate was adjusted with 10 % acetic acid to a pH of 8 and analyzed by HPLC-FLD. The values of partition coefficient *P* for fluoroquinolones differ in different literature sources, therefore, in this work, the $\log P_{o/w}$ ranges are given for some substances: norfloxacin (-1.0), lomefloxacin ((-0.86) – (-0.43)), fleroxacin ((-0.58) – (-0.1)) and ofloxacin ((-0.39) – 0.09). The obtained data showed (Figure 28) that the MFFK-0.25 membrane ensured more efficient extraction of target analytes than the MNPP-0.20 membrane. This is due to the greater hydrophobicity of the membrane material and the larger pore size, which increases the phase contact area and, consequently, the extraction efficiency. The following SHS were studied: nonanoic ($\log P_{o/w} = 3.4$), hexanoic ($\log P_{o/w} = 1.9$) and 2,2-dimethylpropanoic ($\log P_{o/w} = 1.5$) acids. It was found that nonanoic acid is retained in the membrane pores better than others (Figure 28), which can be explained by its lowest solubility in water.

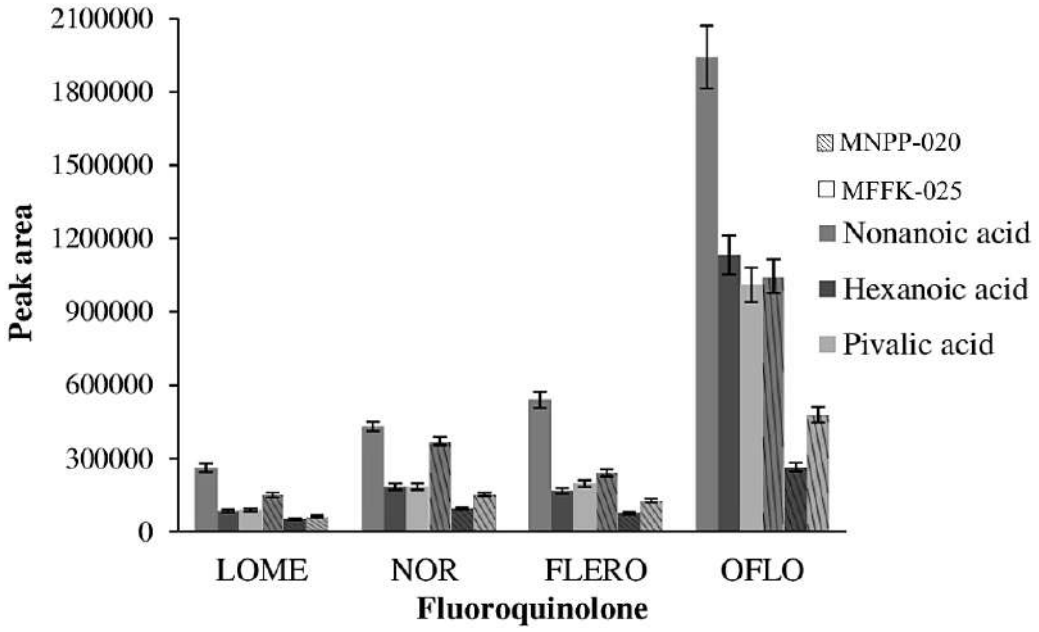

Figure 28. The influence of membrane type and extraction solvent on the chromatographic peak areas of fluoroquinolones ($C_{analyte}$ = 100 μg/L; n = 3).

Membrane surface area may play an important role in the extraction process. Thus, three different sizes of the MFFK-0.25 membrane (5×5, 10×10 and 15×15 mm) were investigated. The membranes with sizes of 10×10 mm have provided similar extraction

efficiency of FQs to the membranes with sizes of 15×15 mm. However, the membranes with sizes of 15×15 mm required larger volume of KOH solution for back-extraction of the analytes. Therefore, the membrane size of 10×10 mm was selected as optimal. The volume of extraction solvent required to completely wet a 10×10 mm membrane was 4 μl. The membrane preparation procedure consisted of cutting the membranes to the required size, after which 4 μl of nonanoic acid was applied to their surface using a chromatographic syringe. The membrane was then left in a static position for 5 min, after which it was used for analysis.

It is known that fluoroquinolones exist in different forms depending on pH. In an acidic medium, fluoroquinolones exist as positively charged ionic forms and have a low affinity for the hydrophobic phase of the extraction solvent, therefore, at low pH values, the degrees of extraction efficiency are small. With an increase in pH and approaching a neutral medium, the extraction efficiency increase, since fluoroquinolone molecules pass into uncharged (electroneutral) forms that have a higher affinity for the nonpolar phase of nonanoic acid. However, the stability of the organic phase also depends on the pH of the solution and decreases with its increase (pKa of nonanoic acid is 4.96). To study this process, a standard solution of a mixture of fluoroquinolones was mixed with a citrate-phosphate buffer solution with an appropriate pH value. A pH value of 4.9 (the studied range is 2.2 - 6.4) is a compromise between these two processes. Without using a buffer solution, a higher extraction efficiency is observed for lomefloxacin and norfloxacin, but when analyzing real samples, it is necessary to have a fixed pH value to maintain constant conditions, thus, a citrate-phosphate buffer solution with pH = 4.9 was used.

The ratios of the volumes of the contacting phases were also studied. An increase in the analytical signal was observed with an increase in the sample volume from 1 mL to 3 mL, after which the dependence reached a plateau, which may be due to partial dissolution of the extractant at large sample volumes. Thus, the volume of aqueous sample phase equal 3 mL providing high analytical signal and low RSD value was chosen as optimal.

Another important factor is the extraction time for achieving high enrichment factors, taking into account, that the passive diffusion from the aqueous sample into the

supported organic phase takes place. The time required to reach equilibrium is usually independent from analytes concentration, and its selection is always a compromise between the sample throughput, sensitivity and repeatability. The effect of extraction time on extraction efficiency was studied in the range of 5 – 40 min. It was found, that the peak area values were increased in the range of the extraction time from 5 to 30 min (Figure 29). The extraction time of 30 min provided high enrichment factors along with maximum sample throughput.

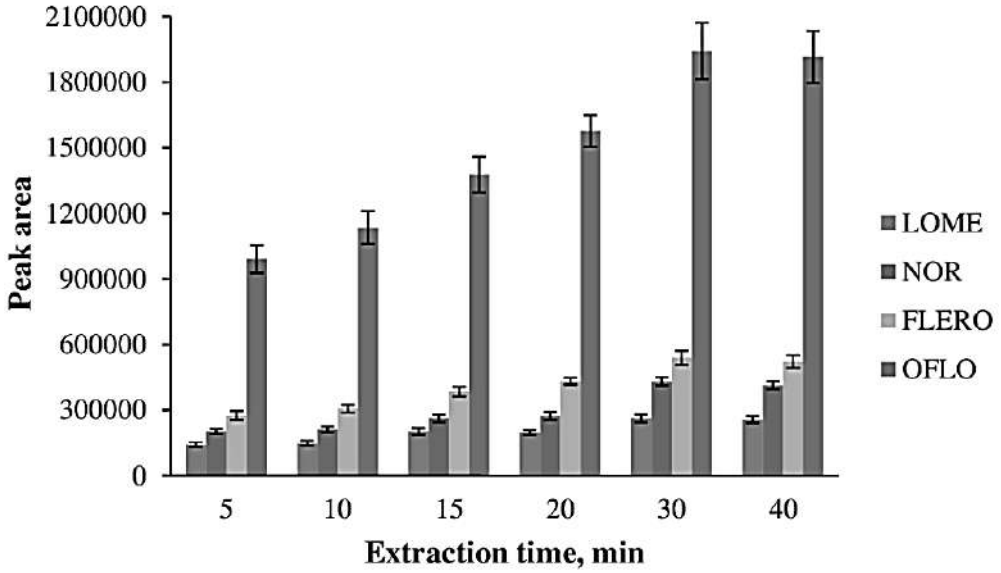

Figure 29. Effect of extraction time on the chromatographic peak areas of fluoroquinolones ($C_{analyte}$ = 100 μg/L; n = 3).

Typically, an organic solvent or a mixture of organic solvents is used to elute the extractant from the membrane. In the case of medium-chain fatty acids (SHS), elution is carried out using an aqueous solution of potassium hydroxide due to a chemical reaction during which a water-soluble salt of the carboxylic acid is formed. This approach allowed for rapid and effective elution of target analytes without the use of additional toxic organic solvents.

The following parameters were studied and established: the concentration and volume of elution solvent and back-extraction time. Based on the data obtained, presented in [142], 0.5 mL of 0.1 mol/L KOH and 1 min were required to obtain reproducible results with maximum extraction efficiency. It is worth noting that HPLC-UV analysis of the

eluate confirmed that 85 % of nonanoic acid passed into the alkaline solution as nonanoate.

High-performance liquid chromatography with fluorimetric detection was chosen as the detection method. The elution conditions of the components for their separation and detection were optimized, in particular the composition of the mobile phase and the wavelengths of fluorescence excitation and emission.

Satisfactory resolution was achieved using the mobile phase consisted of methanol and phosphate buffer solution with pH value of 6.4 (45:55, vol./vol.). However, the chromatographic determination of fluoroquinolones was frequently associated with peak tailing because of secondary interactions between free silanol groups of the stationary phase and amine basic groups of FQs. To overcome this problem competing-base reagents, such as amines or quaternary ammonium salts, are usually added. In our research, triethylammonium acetate was intended for this purpose. Triethylammonium ions compete with fluoroquinolones for silanol groups and reduce the probability of their interaction, acting as tailing suppressor. Different concentrations of triethylammonium acetate in the mobile phase were investigated. The triethylammonium acetate concentration of 0.02 mol/L in phosphate buffer solution excluded peak tailing.

Under the optimal conditions, series of experiments were performed to consider the enrichment factors (EFs), extraction recoveries, linear calibration ranges, LODs, LOQs, regression coefficients, slopes and intercepts and repeatability (intra-day and inter-days) of the proposed method. Enrichment factors for all analytes were in the range of 5.0 - 5.2. The extraction recovery values (n = 3) were equal to (84 ± 4) %, (87 ± 4) %, (83 ± 4) % and (85 ± 4) % for ofloxacin, norfloxacin, lomefloxacin and fleroxacin, respectively (calculations were carried out in accordance with the formula given in section 2.6). The calibration curves were linear over the concentration ranges of 40 – 1800 μg/kg for ofloxacin, 120 - 1200 μg/kg for norfloxacin, 180 - 1800 μg/kg for lomefloxacin and fleroxacin with a regression coefficients in the range from 0.996 to 0.999. The LODs, calculated from the blank tests based on 3σ, were 12 μg/kg for ofloxacin, 36 μg μg/kg for norfloxacin and 60 μg/kg for lomefloxacin and fleroxacin. The repeatability, characterized by RSD values, did not exceed 10 %. The specificity of the determination of ofloxacin, norfloxacin, lomefloxacin and

fleroxacin in the analysis of real samples was estimated by comparing the retention time of each analyte in the analysis of the real sample and the standard solution; the discrepancy in retention times did not exceed 2 %.

The method was applied for the determination of ofloxacin, norfloxacin, lomefloxacin and fleroxacin in shrimp samples prepared according to section 2.3. In all three samples the concentration of fluoroquinolones was below the established LOD values (Table 8). The accuracy of the obtained results was assessed using the "add-found" method and the reference method [142].

Table 8. The results of determination of fluoroquinolones in shrimp samples (n = 3, P = 0.95, F_{cr} = 19.00, t_{cr} = 2.78).

Sample (analyte content)	Analyte	Added, µg/kg	Found, µg/kg		F-test	t-test	R, %
			This method	Reference method			
1 (<LOD)	Fleroxacin	50	45 ± 6	49 ± 4	2.34	0.99	10
	Lomefloxacin		49 ± 5	46 ± 7	1.92	1.04	2
	Norfloxacin		52 ± 6	50 ± 5	2.02	0.31	4
	Ofloxacin		48 ± 5	51 ± 6	2.74	0.45	4
2 (<LOD)	Fleroxacin	100	96 ± 11	95 ± 10	1.21	0.29	4
	Lomefloxacin		98 ± 11	99 ± 10	1.44	0.28	2
	Norfloxacin		97 ± 14	103 ± 11	1.62	1.46	3
	Ofloxacin		98 ± 11	97 ± 9	1.49	0.30	2
3 (<LOD)	Fleroxacin	150	147 ± 16	144 ± 15	1.14	0.59	2
	Lomefloxacin		145 ± 15	147 ± 14	1.15	0.42	3
	Norfloxacin		151 ± 17	149 ± 16	1.13	0.37	1
	Ofloxacin		153 ± 18	155 ± 17	1.12	0.35	2

Table 9 shows examples of methods for determining fluoroquinolones in tissues of animal origin presented in the literature. Using the developed approach of membrane microextraction, it was possible to reduce the consumption of organic solvents several times, as well as to ensure economic benefits, including through the use of commercially available inexpensive membranes.

Table 9. Comparison of methods for the determination of fluoroquinolones in foods.

Detercion method	Sample	Sample preparation	Volume of extraction solvent, mL	LOD	Reference
HPLC-UV	Chicken, pork, fish	DLLME into IL	0.05	0.5 - 1.1 μg/L	[143]
HPLC-FLD	Shrimps	Liquid extraction into a mixture of 0.3 % orthophosphoric acid and acetonitrile (1:1. vol./vol.), SPME	4	3.6 - 8.4 μg/kg	[144]
HPLC-MS/MS	Chicken, pork, fish	Liquid extraction into a mixture of trichloroacetic acid and acetonitrile followed by concentration into hexane	15	0.06 - 0.1 μg/kg	[145]
HPLC-FLD	Shrimps	Liquid extraction into a mixture of methanol and acetic acid followed by SPME	5	15 - 25 μg/kg	[146]
HPLC-FLD	Chicken	Liquid extraction into acetonitrile, solvent evaporation, SPME	10	0.06 - 0.1 μg/kg	[147]
HPLC-FLD	Shrimps	Membrane liquid microextraction	0.004	12 - 60 μg/kg	This work

The *advantages* of the developed method are its environmental friendliness, no need for additional filtration and centrifugation operations when analyzing suspended samples. The membrane allows to hold reliably of microvolumes of the extractant during mass transfer, and also prevents macrocomponents of the sample from entering the phase of extraction solvent, which eliminates the need to remove proteins during sample pretreatment. The *limitations* include low productivity associated with the slow achievement of equilibrium (30 min). In addition, it was found that the developed approach is possible only for determining analytes capable of ionization (e.g., fluoroquinolone antibiotics), while the efficiency of membrane microextraction of analytes whose molecules cannot be ionized under these conditions is negligible. This was confirmed by the example of determining organophosphorus pesticides in food products.

The main results of the research are published in the journal Analytica Chimica Acta [9].

4.2. Microextraction with in-situ formation of extractant

Another promising new generation solvent, widely used for sample preparation tasks, is the class of deep eutectic solvents. For the determination of polar analytes in food products of animal origin, a new combined method was developed, which includes microextraction of target analytes due to in situ DES formation by heating its precursors directly in a mixture with a solid-phase sample and subsequent HPLC-FLD analysis of the obtained extract.

The mechanism of microextraction of analytes into DES formed *in situ* included several stages (Figure 30). In the first stage, the DES's precursor, a quaternary ammonium salt, was added to the solid-phase sample. When the mixture was heated, the quaternary ammonium salt dissolved in the aqueous phase included in the sample. In the next stage, under the same conditions, the second DES's precursor, alcohol, was added to the mixture. Upon heating, the formation of DES was observed, accompanied by mass transfer of fluoroquinolones from the sample to the organic phase. At the final stage, phase separation was performed by centrifugation.

The capabilities of the proposed approach were demonstrated using the example of extracting two fluoroquinolone antibiotics (ofloxacin and fleroxacin) frequently used in animal husbandry from solid-phase samples of animal origin.

Preliminary studies for the extraction of fluoroquinolones included a quasi-hydrophobic DES based on tetrabutylammonium bromide and heptanol (1:2, mol/mol) and hydrophilic DES based on choline chloride and glycerol (1:2, mol/mol) and choline chloride and ethylene glycol (1:2 mol/mol).

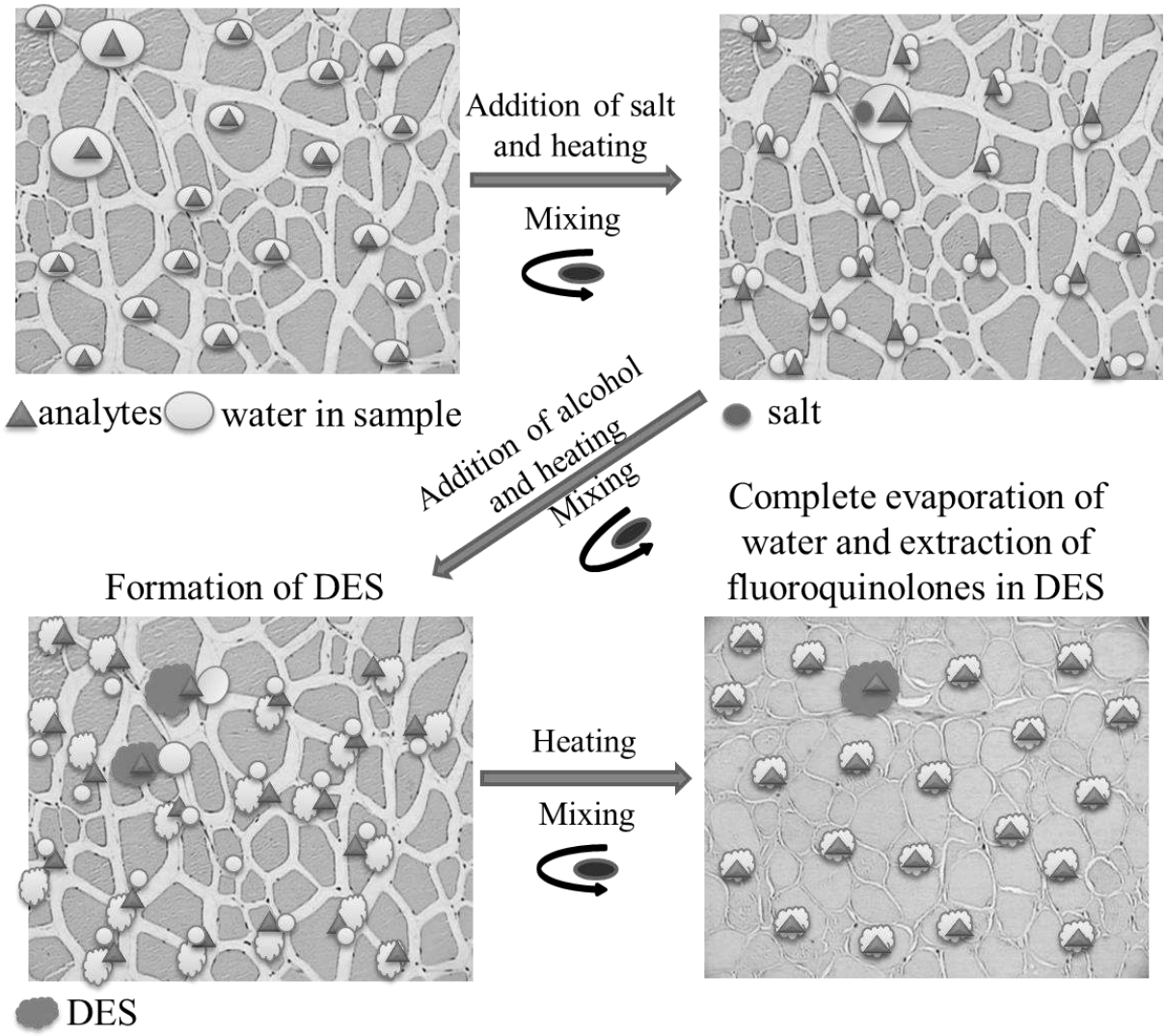

Figure 30. Mechanism of microextraction of fluoroquinolones from meat sample.

For this purpose, 0.2 g of spiked chicken meat sample, prepared according to section 2.3, was mixed with 0.3 mL of the DES for 30 min at 100 °C. After centrifugation and phase separation, 200 µL of the DES phase were collected, re-extracted in 30 µL of 0.005 mol/L NaOH, and the extract was analyzed by HPLC-FLD. Based on the obtained data (Figure 31), a hydrophobic DES based on tetrabutylammonium bromide and heptanol (1:2, mol/mol) was selected for the studies.

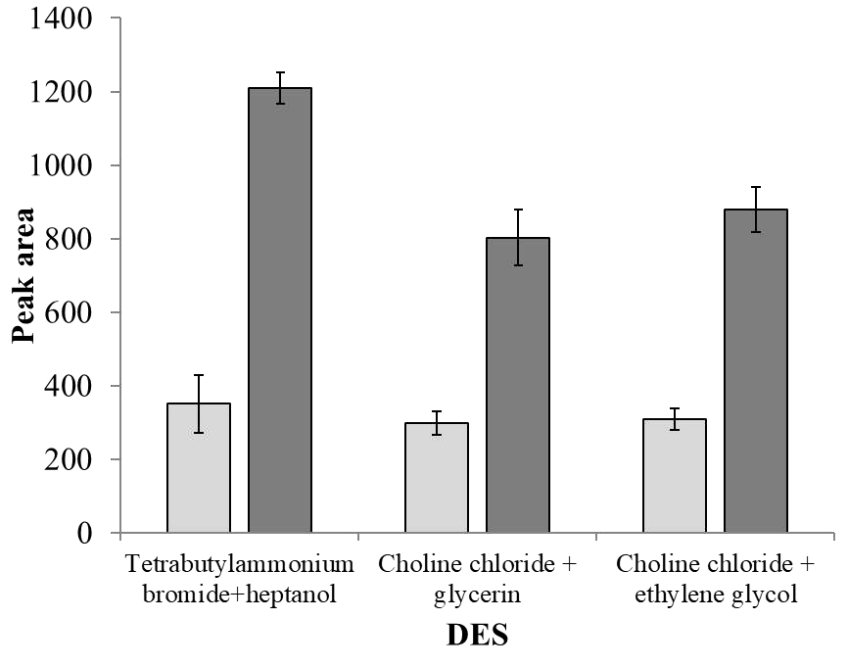

Figure 31. Effect of DES composition on the chromatographic peak areas of fleroxacin and ofloxacin ($C_{analyte}$ = 100 μg/L; n = 3).

When comparing the extraction efficiency of analytes in pre-prepared DES (classical version) and DES formed during sample preparation (*in situ*), it was found that the developed method allowed to increase the degree of target analyte extraction by 2 times compared to the classical version. This phenomenon can be explained by an increase in the solubility of the analyte in the extract phase in the presence of tetrabutylammonium bromide.

In order to optimize microextraction, various alcohols (heptanol, octanol, decanol, and dodecanol) were studied as hydrogen bond donors for DES formation. It was found that the extraction efficiency of antibiotics decreased with increasing length of the alcohol carbon chain, which is explained by the higher solubility of fluoroquinolone antibiotics in polar organic solvents [148]. The effect of the nature of the quaternary ammonium salt on the efficiency of antibiotic extraction was also studied. The following salts were studied as hydrogen bond acceptors for DES formation: tetrabutylammonium bromide, tetraethylammonium chloride, and tetramethylammonium bromide. The highest values of analytical signals (peak areas) were observed when using tetrabutylammonium bromide, while DES formation did not occur in the presence of tetramethylammonium bromide. Moreover, the molar ratio of the selected DES precursors 1:2

(tetrabutylammonium bromide and heptanol, mol/mol) ensured the maximum completeness of extraction of target analytes from tissues.

To confirm DES formation at optimal extraction conditions, differential scanning calorimetry investigations were performed. In the thermograms (Figure 32), the peaks corresponding to tetrabutylammonium bromide (melting point of 105 °C) and heptanol (melting point of -34 °C) were observed. Melting point depression of -10 °C was observed for DES. It can be concluded from the thermograms obtained that the thermal behavior of DES was different from that of the precursors.

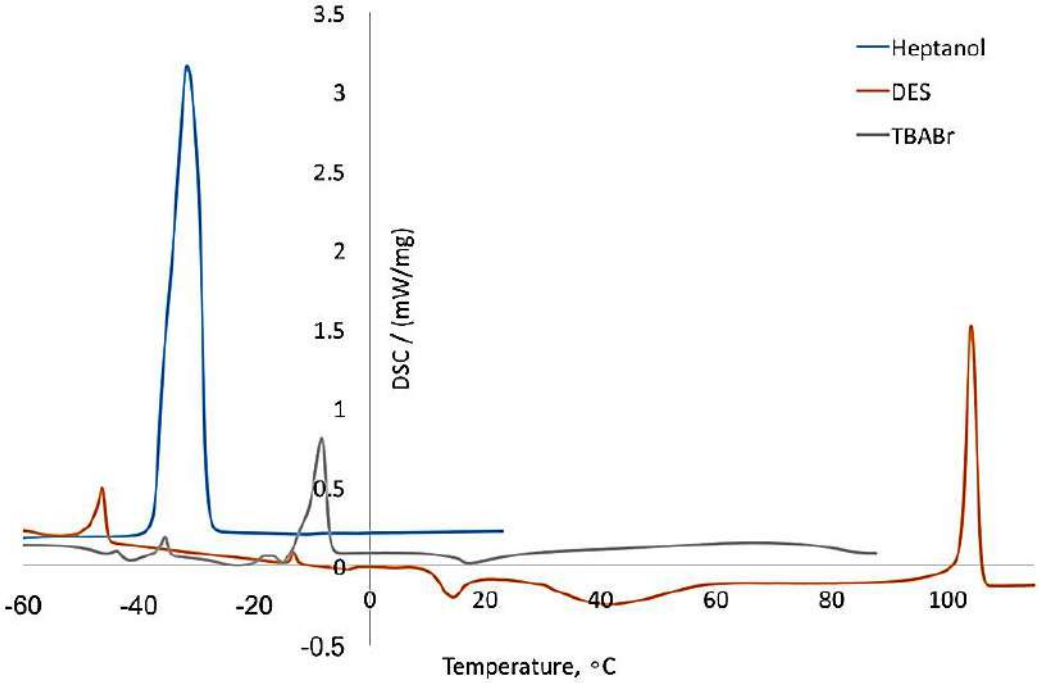

Figure 32. Differential scanning calorimetry record of DES (tetrabutylammonium bromide (TBABr) and heptanol at a 1:2 molar ratio) and its precursors.

To find optimal extraction conditions, the mass of DES had to be established. Thus, different amounts of tetrabutylammonium bromide and heptanol at 1:2 molar ratio were used for the extraction of analytes from 0.2 g of sample. It was found, that higher analytical signals were achieved with lower total DES mass. Nevertheless, DES mass lower than 0.3 g did not provide reproducible analytical signals because the sample could not get completely wet with heptanol. The influence of sample mass was also studied; and the highest analytical signals were achieved with sample and DES ratio of 2:3 (w/w). Thus, 0.2 g of sample and 0.3 g of DES were chosen for further studies.

Temperature is an important parameter for the rate of DES formation and, thus, for the extraction time in general. The effect of temperature was studied within the range of 80-130 °C. It was found, that results obtained at temperatures in the range of 80 - 130 °C were characterized by comparable analytical signals. It should also be noted that a temperature of 120 °C provided a minimum extraction time of 20 min. It was found that under these conditions, the evaporation of water from the extraction system promotes the formation of DES and the extraction of fluoroquinolones from the sample matrix.

The developed method ensured wide linear calibration ranges: 30 -3000 μg/kg for ofloxacin and 40 - 4000 μg/kg for fleroxacin, the detection limits (3σ) were 10 and 15 μg/kg, respectively. Repeatability, characterized by the RSD values, did not exceed 10 %. The specificity of ofloxacin and fleroxacin determination in the analysis of real samples was estimated by comparing the retention time of each analyte in the analysis of a real sample and a standard solution, the retention time discrepancy did not exceed 2 %.

Using the developed method, ofloxacin and fleroxacin were determined in meat products (beef and chicken mince, chicken fillet). The results of the determinations are presented in Table 10. The correctness of the results obtained was confirmed by the "add-found" method and the reference method [142].

Table 10. The results of determination of ofloxacin and fleroxacin in meat samples (n = 3, P = 0.95; F_{cr} = 19.00; t_{cr} = 2.78, two-tailed tests).

Sample	**Analyte**	**Concentration, μg/kg**			**t-test**	**F-test**	**R, %**
		Added	**This method**	**Reference method**			
Chicken mince	Fleroxacin	0	<LOD	< LOD			-
		50	51 ± 2	54 ± 4	0.25	2.53	2
		100	104 ± 6	104 ± 5	0.63	0.36	3
	Ofloxacin	0	< LOD	< LOD			
		50	52 ± 3	50 ± 4	0.64	1.44	3
		100	100 ± 5	99 ± 6	1.48	2.08	1
	Fleroxacin	0	< LOD	< LOD			-

Chicken fillet		50	52 ± 3	49 ± 3	0.80	2.74	4
		100	99 ± 6	100 ± 6	0.80	0.83	1
	Ofloxacin	0	< LOD	< LOD			-
		50	52 ± 3	55 ± 5	0.47	2.11	3
		100	107 ± 9	103 ± 7	0.74	1.56	7
Beef	Fleroxacin	0	< LOD	< LOD			-
		50	49 ± 4	53 ± 4	0.74	2.90	2
		100	108 ± 10	107 ± 9	0.69	0.42	8
	Ofloxacin	0	< LOD	< LOD			-
		50	53 ± 4	52 ± 3	1.17	0.44	6
		100	105 ± 7	109 ± 10	1.96	1.36	5

The developed method of liquid microextraction eliminates the need to use toxic classical solvents as in most previously proposed methods (Table 9), is simple and financially inexpensive, which can undoubtedly be attributed to the *advantages*. However, the *limitation* of this approach is the impossibility of its automation due to the need for a centrifugation.

The data obtained were published in the journal Food Composition and Analysis [18].

4.3. Microextraction with dispersion of extractant by gas phase

The possibility of using DES based on SHS and terpenoid for microextraction of polar organic analytes has been studied and substantiated. A new method of DLLME has been developed using a three-component extraction mixture consisting of a terpenoid, hydrophilic and hydrophobic carboxylic acids. It has been established that when the three-component mixture is introduced into an aqueous phase containing hydrocarbonate ions, intensive formation of carbon dioxide occurs, which disperses the organic phase (DES), thereby ensuring an increase in the efficiency of mass transfer of target analytes in an electroneutral form and rapid phase separation (Figure 33).

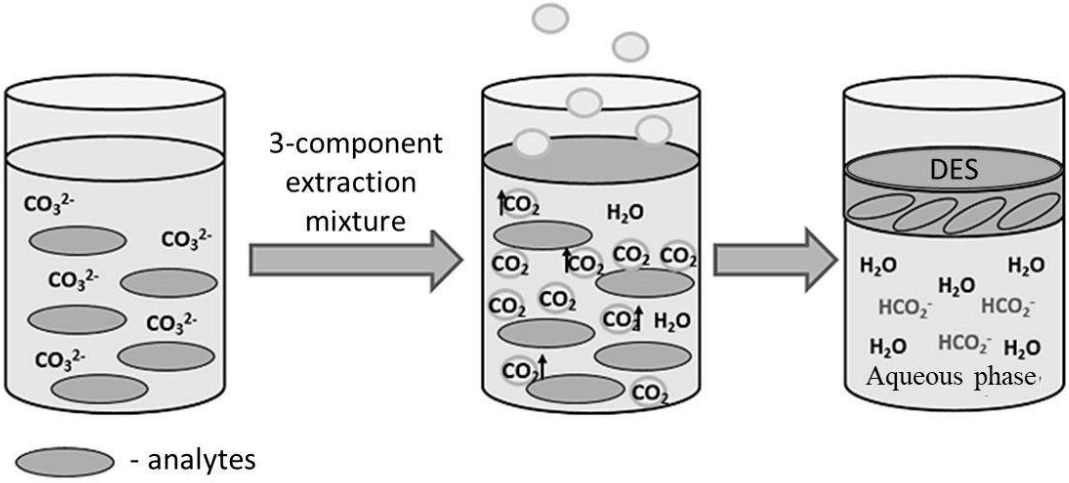

Figure 33. Dispersive liquid-liquid microextraction into a deep eutectic solvent with dispersion of the gas phase formed *in situ*.

The capabilities of the new DLLME method are demonstrated in the determination of fluoroquinolones (norfloxacin, fleroxacin and ofloxacin) in food samples (milk, shrimp) using the HPLC-FLD method.

At the preliminary stage, hexanoic ($logP_{o/w} = 1.9$), heptanoic ($logP_{o/w} = 2.3$), octanoic ($logP_{o/w} = 3.0$) and nonanoic acids ($logP_{o/w} = 3.4$) were studied as hydrogen bond donors for the formation of DES. For this purpose, microextraction was performed in DES based on menthol and carboxylic acids from aqueous solutions of antibiotics, followed by analysis of phase with HPLC-FLD. It was found that the extraction efficiency decreases with increasing length of the carbon chain of the hydrogen bond donor (Figure 34).

Fluoroquinolones are relatively polar analytes, so their affinity for more polar extractants is observed. However, the greater solubility of hexanoic acid in the aqueous phase (compared to other acids) leads to a decrease in the distribution coefficients of fluoroquinolones. Heptanoic acid, which provides maximum areas of chromatographic peaks, was chosen as the DES precursor.

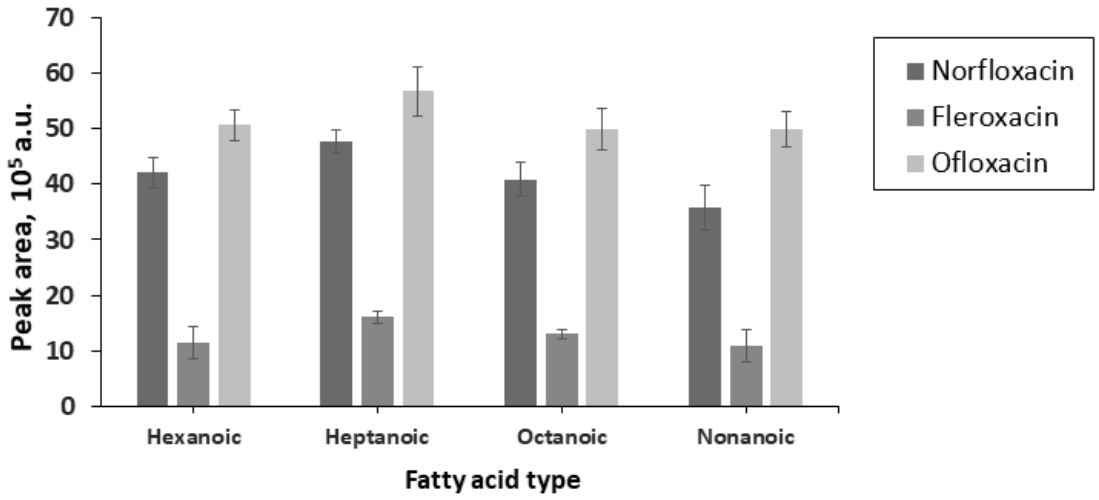

Figure 34. Effect of hydrogen bond donor in DES on chromatographic peak areas of fluoroquinolones ($C_{analyte}$ = 200 μg/L, volume of extractant 100 μL, n = 3).

Two natural terpenoids, menthol and thymol, were studied as hydrogen bond acceptors for DES formation (Figure 35).

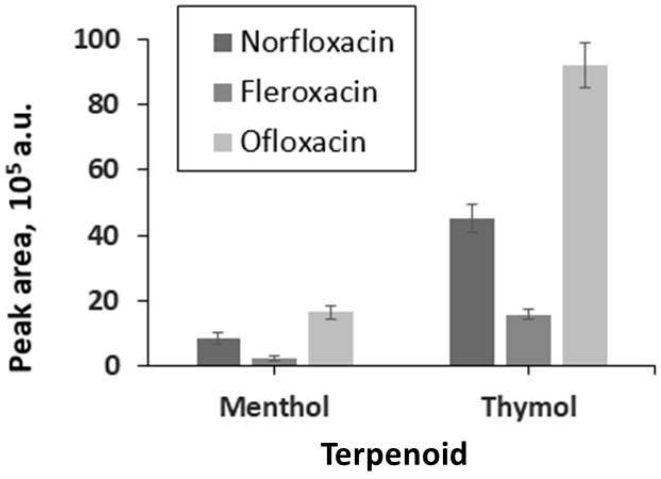

Figure 35. Effect of hydrogen bond acceptor in DES on chromatographic peak areas of fluoroquinolones ($C_{analyte}$ = 200 μg/L, volume of extractant 100 μL, n = 3).

It was shown that the presence of an aromatic ring in the terpenoid molecule plays a key role in the extraction of target analytes. The extraction efficiency in DES based on thymol was maximum, which is associated with intermolecular interactions between the aromatic rings of thymol and fluoroquinolones.

The ratio of heptanoic acid to thymol in DES was also studied in the range from 5:1 to 1:8 (mol/mol). It was found that the molar ratio of heptanoic acid to thymol of 1:2 provides better extraction of the target analytes (Figure 36). In addition, a synergistic effect was confirmed due to the greater extraction capacity of the extractant mixture than its individual components [149].

The synergy is explained by the fact that the solvents in the mixture interact with each other and form molecular complexes by establishing hydrogen bonds (which is consistent with the theory of DES formation). Such complexes are characterized by increased dissolving power in relation to the dissolved or extracted substance. As evidence of the presence of a synergistic effect, a comparison was made of the efficiency of extraction of fluoroquinolones in the selected DES (1:2, mol/mol) and its precursors (Figure 36). From the presented dependence, it is evident that the values of the analytical signal increase by 2 - 2.5 times in the case of using DES.

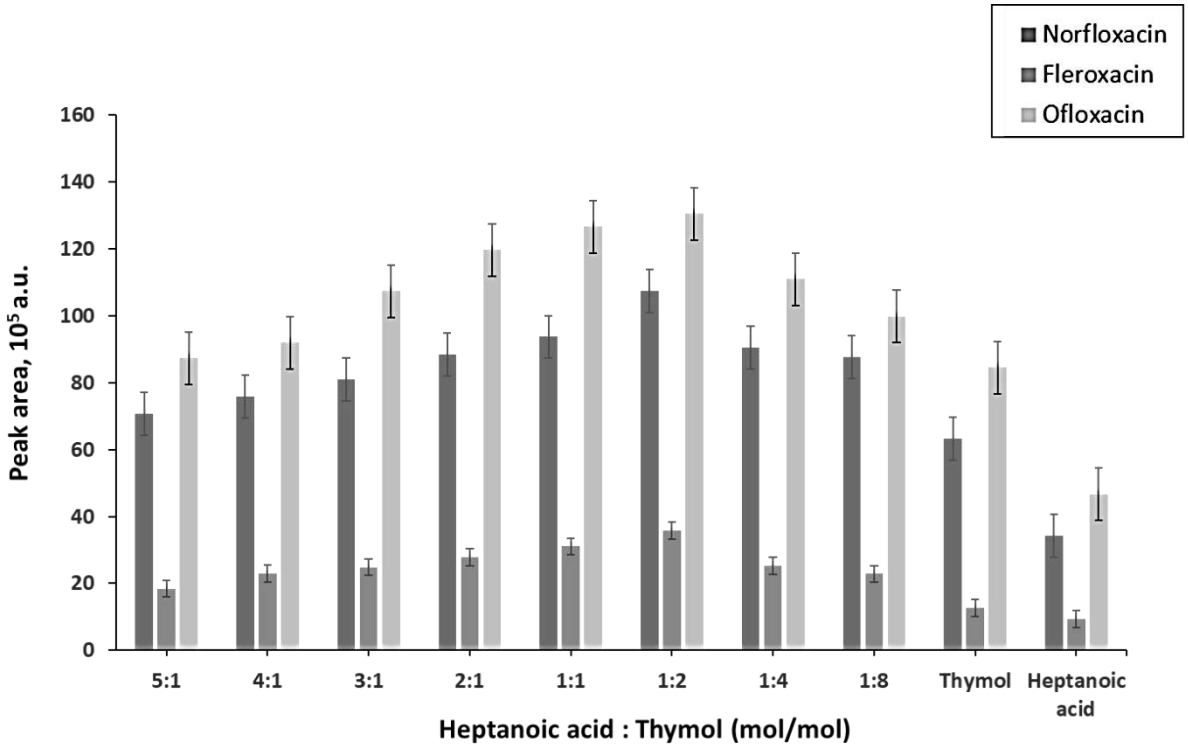

Figure 36. The influence of the ratio of DES precursors (heptanoic acid and thymol), as well as the individual precursors, on the areas of chromatographic peaks of fluoroquinolones ($C_{analyte}$ = 200 μg/L, volume of extractant 100 μL, n = 3).

Formic, acetic, oxalic, citric and malic acids were investigated as proton donor agents for generating CO2 bubbles during the EA-DLLME. It was established, that oxalic, citric and malic acids formed solid phases in the presence of thymol and heptanoic acid. Short-chain carboxylic acids (formic and acetic acids) formed homogeneous systems with thymol and heptanoic acid and provided fast reaction with the effervescent agent (carbonate ions) in aqueous phase. Thus, the extraction capabilities of DESs based on formic and acetic acids were studied. The highest analytical signals were observed in case of formic acid since formic acid (pKa 3.75) is stronger than acetic acid (pKa 4.76) and provides more effective effervescence and dispersion of the extractant.

Sodium carbonate was used as sources of CO_2 bubbles in this research. The amount of sodium carbonate has a significant impact on the extraction process. The concentration of the effervescent agent was varied within the range from 0.25 to 1.25 mol/L. Ir was established, that the optimal concentration of Na_2CO_3 for the proposed technique is 0.75 mol/L, while higher concentrations provide lower analytical signals. Generally, the addition of higher amount of Na_2CO_3 allows to provide more effective formation of CO_2 bubbles assisting the dispersion of DES and promoting the extraction of analytes. However, the higher amount of effervescent agent was added, the faster phase separation was observed and, as a consequence, lower extraction efficiency was achieved. Thus, 5 mL of sample solution with the concentration of 0.75 mol/L sodium carbonate and 390 μL the three-component DES consisting of 100 μL of heptanoic acid and thymol (1:2, mol/mol) and 290 μL of formic acid were chosen for the DLLME.

To establish the composition of the separated organic phase, it was withdrawn and analyzed by GC-FID (determination conditions and chromatogram are presented in section 2.5). It was established that the extractant consisted of heptanoic acid and thymol (1:2, mol/mol). Thus, the hydrophobic DES based on terpenoid and medium-chain carboxylic acid was formed during the DLLME.

The FT-IR spectra of the initial components and the extract phase were received (Figure 37). According to literary data [141], the formation of new intermolecular hydrogen bonds between donors and acceptors is confirmed by the appearance or change of absorption bands in the region of 3230–3550 cm^{-1}. Stretching vibration of –OH on pure

thymol was observed at 3321 cm^{-1} and in the phase of medium-chain carboxylic acid at 3036 cm^{-1}. In the DES phase this peak was shifted to 3420 cm^{-1}. At the same time, the stretching vibrations of –COOH in the medium-chain carboxylic acid phase were shifted from 1705 cm^{-1} to 1708 cm^{-1}. The spectral shifts confirmed the formation of hydrogen bonding between –COOH and –OH groups.

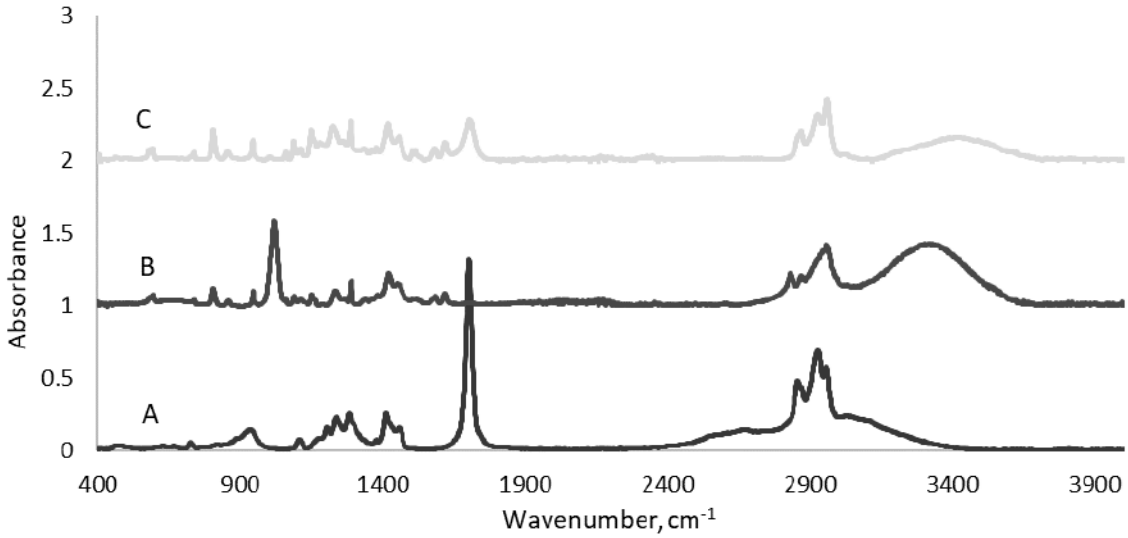

Figure 37. FT-IR spectra of heptanoic acid (A), thymol (B) (dissolved in methanol 1:1) and the obtained DES phase (C) after the DLLME.

The optimal sample volume was set at 5 mL and the extraction time was set at 5 min.

Under the optimal conditions, series of experiments were performed to estimate the analytical characteristics of the proposed procedure. Linear calibration ranges of 2.0 - 500.0 μg/L for norfloxacin and 1.0 - 500.0 μg/L for ofloxacin and fleroxacin were obtained with coefficients of determination in the range from 0.993 to 0.996. The LODs, calculated from the blank tests based on 3σ, were 0.06 μg/L for norfloxacin, 0.03 μg/L for ofloxacin and 0.03 μg/L for fleroxacin for milk sample and 0.6 μg/kg for norfloxacin, 0.3 μg/kg for ofloxacin and 0.3 μg/kg for fleroxacin for shrimp sample.

The enrichment factor values for norfloxacin, ofloxacin and fleroxacin were 46.5, 49.5 and 43.0, respectively. The repeatability, characterized by the value of the RSD value, did not exceed 10 %. The specificity of the determination of norfloxacin, ofloxacin and fleroxacin in the analysis of real samples was estimated by comparing the retention time of each analyte in the analysis of the real sample and the standard solution; the discrepancy in retention times did not exceed 2 %.

Using the developed method, norfloxacin, ofloxacin and fleroxacin were determined in a milk and shrimp samples; preliminary sample treatment was carried out in accordance with section 2.3. No traces of fluoroquinolone antibiotics were found in the original shrimp and milk samples (Table 11). The correctness of the results obtained was confirmed by the “add-found” method.

Table 11. Results of determination of norfloxacin, fleroxacin and ofloxacin in food products (n = 3, P = 0.95).

Sample	**Analyte**	**Concentration of analyte, µg/L**		**R, %**
		Added, µg/L	**Found, µg/L**	
Milk	norfloxacin	0	< LOD	-
		50	48.2 ± 2.7	4
		100	101.8 ± 2.1	2
	fleroxacin	0	< LOD	-
		50	47 ± 3	6
		100	102.1 ± 1.7	2
	ofloxacin	0	< LOD	-
		50	53 ± 3	6
		100	95 ± 3	5
		Concentration of analyte. µg/kg		
Shrimp	norfloxacin	0	< LOD	-
		18.0	16.6 ± 0.8	4
		25.2	22.5 ± 1.9	11
	fleroxacin	0	< LOD	-
		10.8	9.4 ± 1.3	13
		17.3	16.1 ± 1.4	7
	ofloxacin	0	< LOD	-
		15.2	13.7 ± 1.1	11
		21.7	19.8 ± 2.5	6

Compared with existing analogues, the developed microextraction method has a number of *advantages*: expressiveness (5 min), environmental friendliness (does not require the use of toxic organic solvents) and energy efficiency (does not require energy to intensify the process of mass transfer and phase separation). In addition, due to the absence of the centrifugation stage, its full automation becomes possible, which will be discussed in section 6.1. The *disadvantage* of the developed approach is the need to eliminate fats and proteins from the sample matrices during preliminary sample pretreatment, which makes the analysis relatively labor-intensive.

The results of the study were published in the journal Talanta [21].

4.4. Microextraction into the micellar phase

A promising sample preparation method for the analysis of suspended samples is micellar microextraction, which is characterized by environmental safety compared to sample preparation procedures based on conventional extraction technique, simplicity and availability, as well as the ability to extract dissolved substances with a wide range of polarity. However, due to the high viscosity of the micellar phase, the technique is more difficult to combine with detection methods. Most often, dilution of extracts is required, which leads to an increase in LOD.

To eliminate the above-mentioned shortcomings, a new method of micellar microextraction was developed, based on the extraction and concentration of polar analytes in the phase formed by a primary amine and a medium-chain carboxylic acid. Phase separation was ensured by a salting-out agent. The phase obtained during microextraction had low viscosity and did not require additional manipulations before analysis.

The proposed method was used to determine four polar pesticides that differ in chemical structure (Figure 38) and are widely used in agriculture in the Russian Federation: bifenthrin (water solubility (S) <1 mg/L), diazinon (S = 60 mg/L), triadimenol (S = 72 mg/L) and triadimefon (S = 70 mg/L) [150].

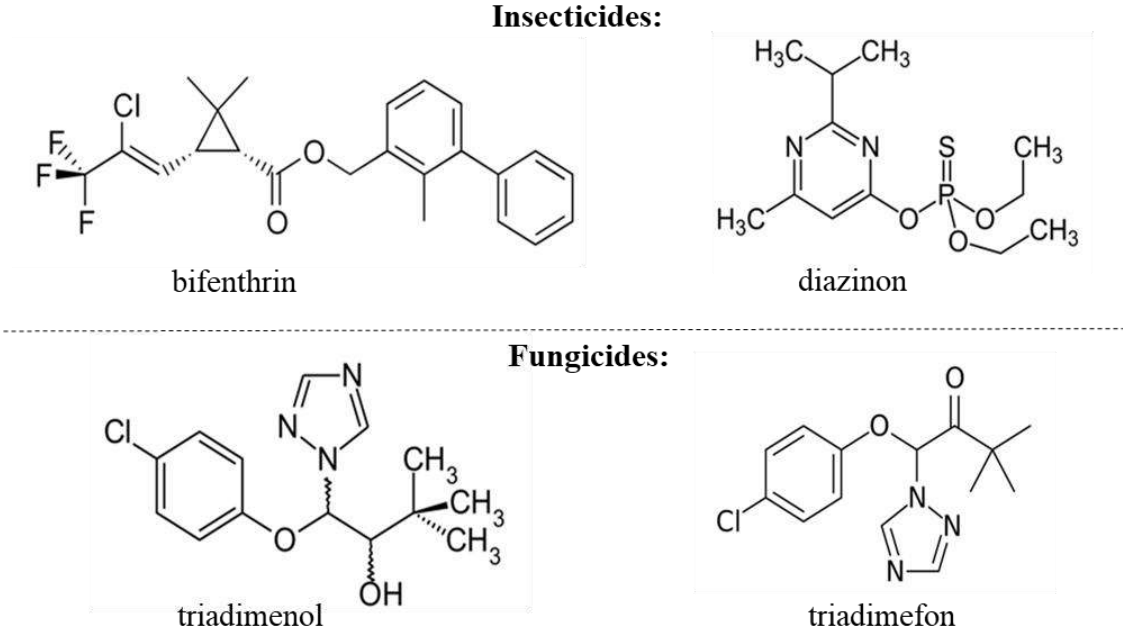

Figure 38. Structural formulas of bifenthrin, diazinon, triadimenol and triadimefon.

At the first stage, the composition of the extraction mixture was studied. It is known that when mixed with water, primary amines are capable of forming isotropic solutions [151]. The process occurs due to the formation of amine hydrates and their dissociation, resulting in the formation of positively charged amphiphiles, which, upon reaching the critical micelle concentration, are capable of spontaneous self-association with the formation of micelles. Similar to surfactant solutions, amine micelles form their own microphase in an aqueous medium and are homogeneous and single-phase on a macroscale, but microheterogeneous and two-phase on a nanoscale [152]. Short-chain fatty acids containing a hydrophilic carboxyl group (-COOH) can also act as amphiphiles in an aqueous medium, while the hydrocarbon chain is hydrophobic. Coacervating agents of such systems are quaternary ammonium salts, an aprotic solvent (tetrahydrofuran), and magnetic nanoparticles [153,154].

Considering the different polarity of the pesticides under the study (for example, the partition coefficient $\log P_{o/w}$ for diazinon is 3.8, while for bifenthrin it is 6.0 [155]), it was proposed to study primary amines in the range from butylamine to decylamine and such carboxylic acids as 2,2-dimethylpropanoic, hexanoic, octanoic and nonanoic.

The experimental investigations were provided as follows: 1 mL of pesticides solution with a concentration of 100 μg/L was mixed with 50 μL of primary amine, fatty acid or extraction mixture containing primary amine and fatty acid in different ratios from 1:4 to 4:1 (vol./vol.). Then, 200 μL of saturated aqueous NaCl solution was added and shaken for 3 min, and centrifuged at 5000 × g for 5 min. The obtained phase was analyzed by GC-MS.

It was found that homogeneous solutions were formed for all amines and systems based on primary amines and carboxylic acids in almost all ratios. Hexanoic, octanoic, nonanoic and 2,2-dimethylpropanoic acids formed hydrophilic emulsions, which can be explained by the low solubility of acids in the aqueous phase and their high concentration in the system. However, after the addition of a salting-out agent, the separation occurred only for a few systems: 1-heptylamine and 2,2-dimethylpropanoic acid (4:1, vol./vol.), 1-nonylamine and 2,2-dimethylpropanoic acid (3:2, vol./vol.), 1-heptylamine and nonanoic acid (4:1, vol./vol.). It was found that the introduction of 2,2-dimethylpropanoic acid into the system significantly improved the extraction efficiency for more water-soluble analytes (triadimefon and triadimenol) due to the lower hydrophobicity of 2,2-dimethylpropanoic acid (Figure 39).

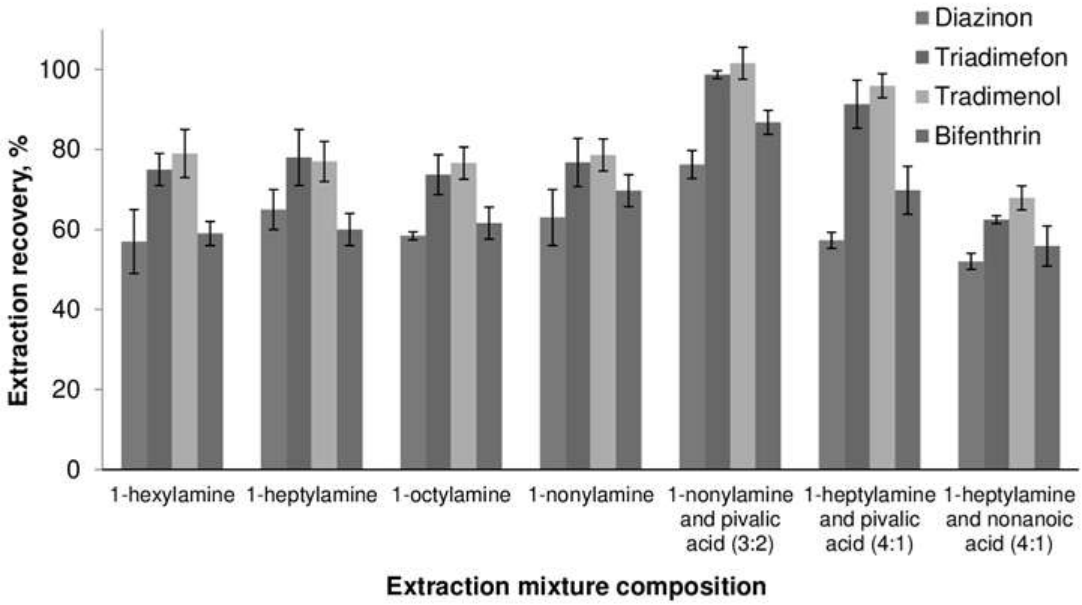

Figure 39. Effect of extraction mixture composition on the degree of pesticide extraction, *pivalic acid = 2,2-dimethylpropanoic acid* ($C_{analyte}$ = 100 μg/L, n = 3).

Based on the data obtained, a supramolecular solvent based on 1-nonylamine and 2,2-dimethylpropanoic acid (3:2, v/v) was selected, providing a high degree of extraction of target analytes (76 – 100 %).

To reduce the viscosity of the selected extraction mixture, it was proposed to add deionized water to the system. It was found that the ratio of 1-nonylamine, 2,2-dimethylpropanoic acid and deionized water equal to 3:2:5 (vol./vol./vol.) provided the maximum extraction recovery values. Thus, the introduction of deionized water into the extraction mixture allowed to significantly reduce its viscosity, which had a positive effect on the reproducibility of the results, and, in addition, simplified the sample preparation process. The optimal volume of the selected extraction mixture was also established, it was equal to 225 μL.

The most common salts (ammonium sulfate, ammonium acetate, sodium hydrogen phosphate, sodium chloride, potassium carbonate) were studied as salting-out agents. It was observed, that coacervate was formed only with the use of sodium chloride, while ammonium sulfate, sodium hydrogen phosphate and potassium carbonate were providing the formation of precipitates – coagulates, characteristic of colloidally unstable systems. Thus, sodium chloride as the salting-out agent was chosen for further experiments.

In order to avoid dilution of the sample solution, it was decided to use solid-phase sodium chloride. The salt mass was studied in the range from 80 to 250 mg, and 80 mg was established to be minimal for phase separation. It was shown, that the analytical signals were raised with increasing of salting-out agent mass till 200 mg of NaCl. Thus, this mass was chosen for further experiments due to the lower standard deviations and higher analytical signals. The sample weight was also determined experimentally to be 0.8 g.

For the analysis of plant-based foods, the scheme included several stages was proposed: 1) injection of the extraction mixture based on primary amine and carboxylic acid into the homogenized sample solution (suspension) and mixing of the system; 2) removal of solid sample particles using a filter paper (pore size, 4 μm); 3) addition of coacervating agent to the system; 4) centrifugation for complete phase separation; 5) analysis of the separated phase using GC-MS (Figure 40).

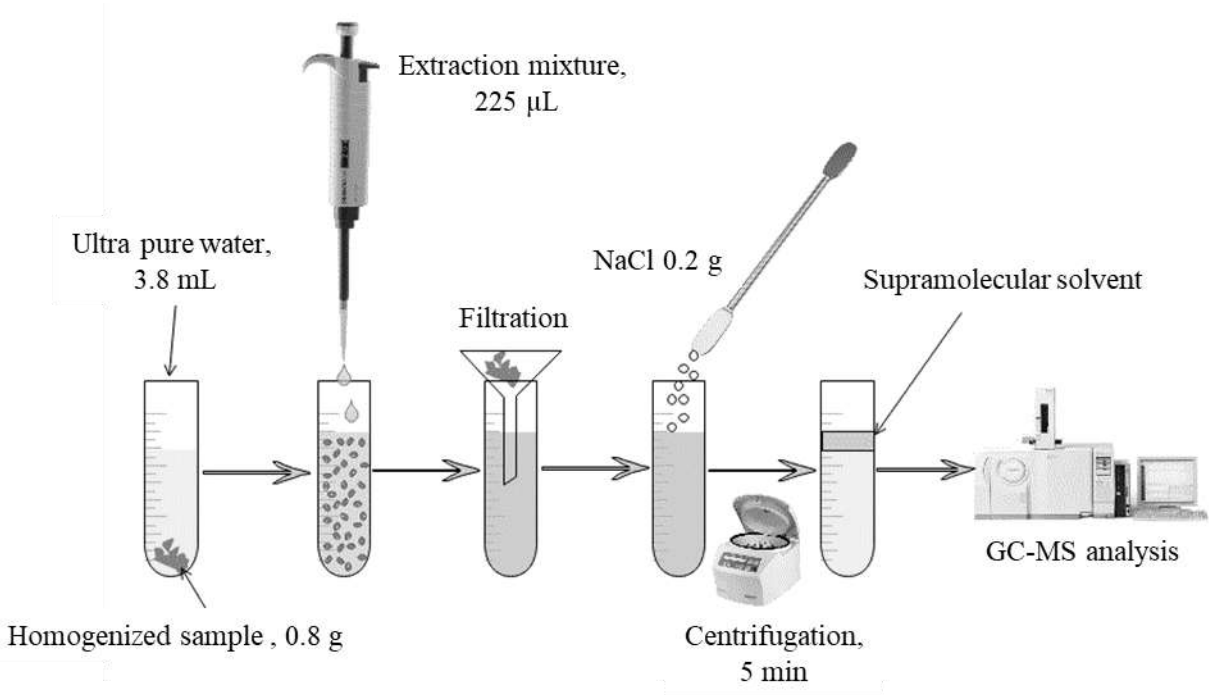

Figure 40. A combined scheme for the analysis of solid-phase food samples, including micellar microextraction and subsequent GC-MS analysis.

The next stage was devoted to the study of the separated phase. The content of water in the supramolecular phase was estimated by the Karl-Fischer method using an 831 KF Coulometer (Metrohm, Switzerland). It was found that the separated phase contained (75.0±1.0) % (v/v) of water. We assumed that a high water content provided low viscosity of the separated phase. The measured kinematic viscosity value with the use of an Ubbelohde viscometer was (15.3 ± 0.4) mm^2/s at 25 °C. Analysis of separated phase by GC-FID (section 2.5) confirmed, that the phase contained (25.0 ± 1.0) % (vol./vol.) of 1-nonylamine and 2,2-dimethylpropanoic acid in ratio of 3: 2 (vol./vol.) (which corresponded to the ratio of 1:1 (mol/mol)). Thus, it can be assumed that when a negatively charged ion (carboxylic acid) is added to a positively charged amine in the presence of an electrolyte, a coacervate is formed.

To confirm the formation of self-assembled systems in the extraction mixture and separated phase, the measurements by DLS method were performed. The method is based on phenomenon of light scattering by molecules aggregates in solution [156]. The scattering angle (θ) range of 40 - 100° was chosen as the optimal range for investigation of small supramolecular structures, which scatter equally at all angles.

The results showed that there are no stable mode in the case of pure water, 1-nonylamine and 2,2-dimethylpropanoic acid, where any specific diffusion coefficient can be associated with, so, supramolecular structures were not established for these liquids. The presence of a stable mode for the extraction mixture (water, 1-nonylamine and 2,2-dimethylpropanoic acid (5: 3: 2, vol./vol./vol.)) and phase separated was characterized. Distributions of the diffusion coefficient calculated from the data obtained at a scattering angle of 90° are shown in Figure 41. Each distribution with a single peak show that one type of supramolecular structure having similar diffusion behavior was formed. The diffusion coefficients of these aggregates in the extraction mixture $(31.9 \pm 0.7)\cdot10^{-8}$ cm^2 s^{-1} and in the phase separated $(22.2 \pm 0.2)\cdot10^{-8}$ cm^2 s^{-1} were calculated as the mean values (n = 7) from measurements acquired at scattering angles from 40 to 100° with the step of 10°. The diffusion coefficients of water and precursors of mixed system are substantially higher: $2.3\cdot10^{-5}$ cm^2s^{-1} for water [157], $0.59\cdot10^{-5}$ cm^2 s^{-1} for 1-nonylamine [158], $0.89\cdot10^{-5}$ cm^2 s^{-1} for 2,2-dimethylpropanoic acid [159]. It indicates that aggregation of the molecules took place and supramolecular structures were formed.

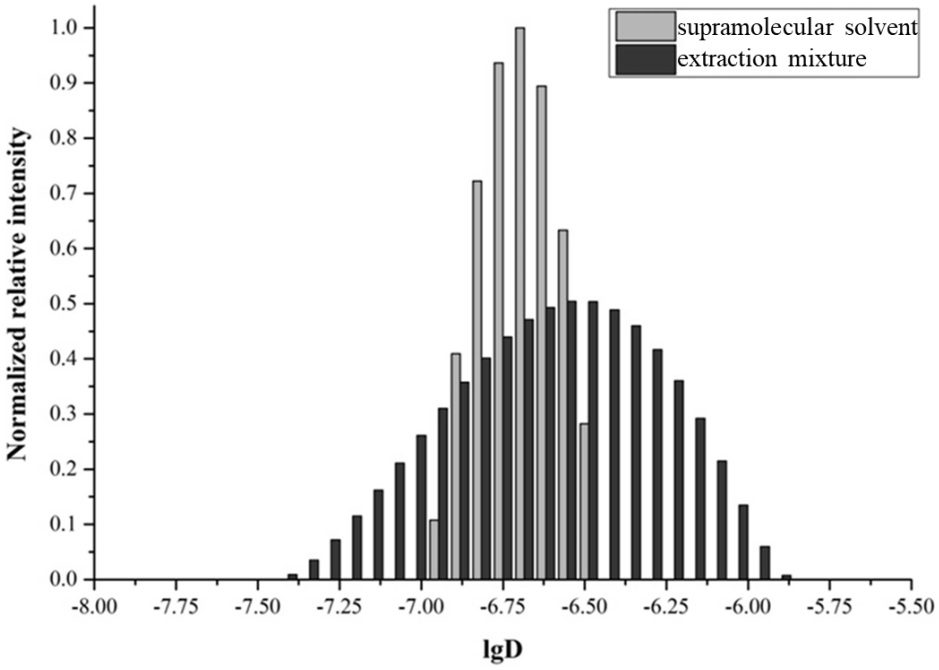

Figure 41. Distribution of the diffusion coefficients for supramolecular structures presented in the extraction mixture and the organic phase (T = 293 K, a scattering angle of 90°).

Thus, based on the obtained data the extraction mechanism was as follows (Figure 42): when the extraction mixture was introduced into an aqueous phase, the self-assembled system formed; extraction of analytes from solid-phase food sample into the self-assembled system took place; the change in the ionic strength promoted coacervation and final separation of supramolecular phase (1-nonylamine and 2,2-dimethylpropanoic acid in ratio of 3: 2 (vol./vol.), which corresponds to the ratio of 1:1 (mol/mol)) containing target analytes. The main mechanism of interaction between 1-nonylamine and 2,2-dimethylpropanoic acid is electrostatic attraction (via positive charge of 1-nonylamine and negative charge of 2,2-dimethylpropanoic acid). And the extraction of analytes into the supramolecular solvent is ensured by intermolecular interactions between the hydrophobic tails of 1-nonylamine/2,2-dimethylpropanoic acid molecules and the alkyl groups of pesticide molecules.

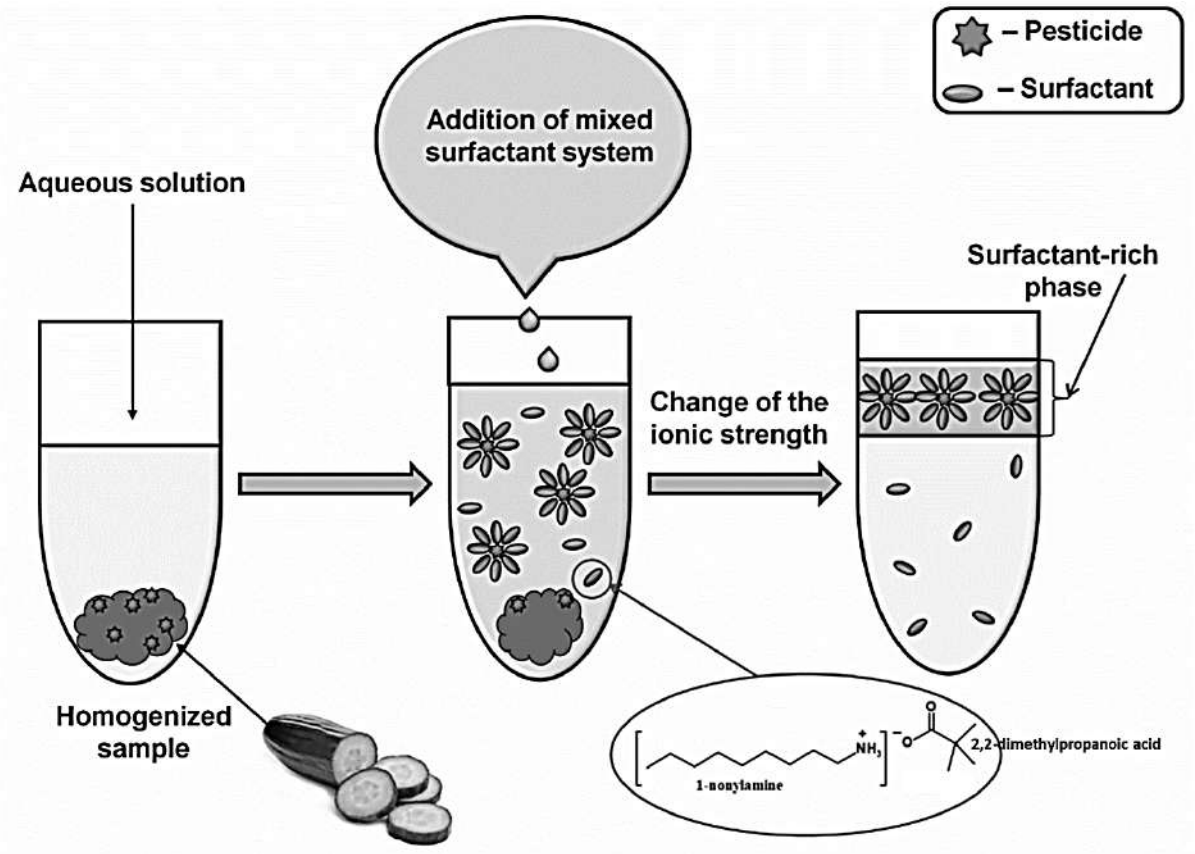

Figure 42. Micellar microextraction of pesticides from suspensions into a supramolecular solvent based on a primary amine and carboxylic acid.

An important feature of the obtained phase is its low viscosity, which allowed its direct introduction into the gas chromatograph without dilution, and therefore,

maintaining the sensitivity of the method as a whole. For comparison, the kinematic viscosity values at 25 °C were measured for obtained phases with Triton X-100 (30 %, w/vol.) from [93] and the extraction mixture used in this study. The values were (1070 ± 33) mm^2/s and (15.3 ± 0.4) mm^2/s, respectively.

Under the optimal conditions, the analytical performance of the developed procedure was evaluated. The calibration curves constructed by plotting the peak areas against six different concentrations of pesticides were obtained in the ranges of 25 - 800 μg/kg for diazinon, 30 - 1000 μg/kg for triadimefon, 15 - 500 μg/kg for triadimenol and 3 - 1200 μg/kg for bifenthrin with the correlation coefficient more than 0.991. The LODs, calculated from blank test based on 3σ, were 8 μg/kg for diazinon, 10 μg/kg for triadimefon, 5 μg/kg for triadimenol, and 1 μg/kg for bifenthrin. The repeatability, characterized by the value of the RSD, did not exceed 9 %. Time of extraction procedure is less than 7 min and GC-MS analysis – 12 min. The specificity of analyte determination in the analysis of real samples was assessed by comparing the retention time of each analyte in the analysis of a real sample and a standard solution; the discrepancy in retention times did not exceed 2 %.

The capabilities of the developed method were demonstrated using the example of determining diazinon, triadimefon, triadimenol and bifenthrin in food products of plant origin (cucumbers and baby vegetable puree). The accuracy of the results obtained was confirmed by the “add-found” method and reference method (Table 12). A combined method based on the classical procedure of liquid-liquid extraction with salting out and subsequent GC-FID analysis was chosen as the reference method [160].

Table 12. The results of the determination of diazinon, triadimefon, triadimenol and bifenthrin in vegetable baby food and cucumbers (n = 3, P = 0.95; F_{cr} = 19.00; t_{cr} = 2.78).

Sample	**Analyte**	**Added, μg/kg**	**Found, μg/kg**		**t-test**	**F-test**	**R, %**
			This method	**Reference method**			
	diazinon	0	<LOD	<LOD			-

Baby vegetable puree		250	243 ± 10	240 ± 11	0,67	1,10	3
		500	497 ± 13	475 ± 18	2,02	2,19	1
	triadimefon	0	<LOD	<LOD			-
		250	246 ± 12	252 ± 10	0,83	1,98	2
		500	501 ± 20	512 ± 19	0,80	1,16	1
	triadimenol	0	<LOD	<LOD			-
		50	52 ± 3	51 ± 4	0,32	2,22	1
		100	103 ± 10	108 ± 9	1,58	2,40	3
	bifenthrin	0	<LOD	<LOD			-
		500	496 ± 17	501 ± 11	0,44	2,44	1
		1000	968 ± 30	1011 ± 10	2,74	13,07	3
Cucumbers smooth long-fruited	diazinon	0	<LOD	<LOD			-
		250	257 ± 16	245 ± 9	0,73	3,26	3
		500	510 ± 10	496 ± 27	1,06	16,41	2
	triadimefon	0	<LOD	<LOD			-
		250	250 ± 10	251 ± 18	0.03	10.15	0
		500	513 ± 25	503 ± 8	0.75	9.28	3
	triadimenol	0	23 ± 2	23 ± 1	0.51	3.99	-
		50	76 ± 7	75 ± 2	0.35	17.26	4
		100	122 ± 2	123 ± 2	1.38	2.25	2
	bifenthrin	0	<LOD	<LOD			-
		200	209 ±19	196 ± 8	1.24	6.21	5
		400	403 ± 23	397 ± 5	0.50	18.94	1
Cucumbers prickly short-fruited	diazinon	0	<LOD	<LOD			-
		250	248 ± 30	250 ± 12	0.39	10.35	1
		500	511 ± 25	515 ± 11	0.30	6.02	2
	triadimefon	0	<LOD	<LOD			-
		250	256 ± 11	252 ± 10	0.54	5.38	2
		500	504 ± 18	494 ± 11	0.85	4.87	1
	triadimenol	0	<LOD	<LOD			-
		50	50 ± 2	50 ± 1	0.39	3.53	0
		100	103 ± 9	99 ± 3	0.79	13.32	3
	bifenthrin	0	<LOD	<LOD			-

		200	216 ± 17	202 ± 12	1.41	3.04	8
		400	420 ± 45	395 ± 19	1.13	5.07	5

Micellar extraction has been successfully implemented for the determination of pesticides in various food products. However, as already noted, self-assembled phases have one serious limitation – high viscosity. This property prevents the direct injection of the phase into the detection system and requires the inclusion of an additional procedure: dilution, precipitation or re-extraction, which significantly increases the analysis time. Unlike the methods presented in the literature (Table 13), the developed method does not require dilution and back extraction due to the low viscosity of the supramolecular solvent obtained. The extraction procedure is simple, easy, fast and environmentally friendly.

The results of the study were published in the Journal of Molecular Liquids [16].

Table 13. Comparison of the technique developed for the determination of pesticides in food samples based on micellar extraction.

Extraction solvent (volume/mass)	**Sample (mass)**	**Analyte**	**Detection method**	**LOD**	**Sample preparation time, min**	**Reference**
Triton X-100 (1.5 mL)	Vegetables (2 g)	Trichlorfon	HPLC-UV	2.0 μg/L	60	[93]
Sodium dodecyl sulfate/tetrabutylammonium bromide (0.233 g)	Fruit juices, water (5 mL)	Organophosphorus pesticides	HPLC-UV	1 - 30 μg/L	15	[161]
Sodium dodecyl sulfate (0.3 g)	Vegetables (10 g)	Carbaryl	SP-UV	50 μg/L	7	[162]
Polyethylene glycol 6000 (3 mL)	Fruit juices (2 g)	Organophosphorus pesticides	GC-FPD	0.5 - 3 μg/kg	27	[163]
Decanoic acid / magnetic NPs	Water (30 mL)	Triazine herbicides	HPLC-UV	0.3 - 0.5 μg/L		[154]

(0.2 g)					17	
Nonylamine/2,2-dimethylpropanoic acid/water (0.225 mL)	Vegetables, vegetable puree (0.8 g)	Diazinon, bifenthrin, triadimefon, triadimenol	GC-MS	1 - 10 µg/kg	7	This work

CHAPTER 5. DISPERSIVE LIQUID-LIQUID MICROEXTRACTION OF ANALYTES FROM LIQUID FOOD SAMPLES

One of the most popular and widely used microextraction methods in the analysis of water samples is DLLME. In the context of DLLME development, special attention is paid to the search for new extraction systems based on the principles of green analytical chemistry. Thus, natural terpenoids have been proposed as safe extractants for the implementation of DLLME [164]. Such terpenoids as menthol and thymol are in a solid-phase state at room temperature. With slight heating of terpenoids (the melting points of menthol and thymol are 43.0 °C [165] and 50.5 °C [166], respectively), viscous liquids are formed, for their dispersion in the aqueous phase the use of polar organic dispersive solvent is also required [167]. As have been noted in the literature review, the presence of a dispersive solvent in the system lead to a negative effect on the extraction efficiency of target analytes, promoting their better dissolution in the aqueous phase. Thus, the search for new approaches to the implementation of DLLME using green extraction solvents remains a relevant analytical task.

5.1. Microextraction based on phase transitions of the extraction solvents

For the analysis of liquid food samples, an express DLLME was developed. It involves injecting a liquid sample into a previously prepared extraction vial with a layer of solidified menthol on the bottom and keeping the system at a temperature above the melting point of the extraction solvent, that leads to melting organic phase and rising from the bottom of the vial to the surface of the aqueous phase (Figure 43). This simple to implement procedure ensured uniform distribution of menthol throughout the entire sample volume during its slow phase transition, and as a consequence, effective extraction of the target analyte. When diluting the phase separated with an organic solvent (methanol, acetonitrile), it is possible to combine the proposed approach with an HPLC system. The capabilities of the developed DLLME with phase transition of extraction solvent were demonstrated using the example of HPLC-UV determination of the preservative benzoic acid in soft drinks.

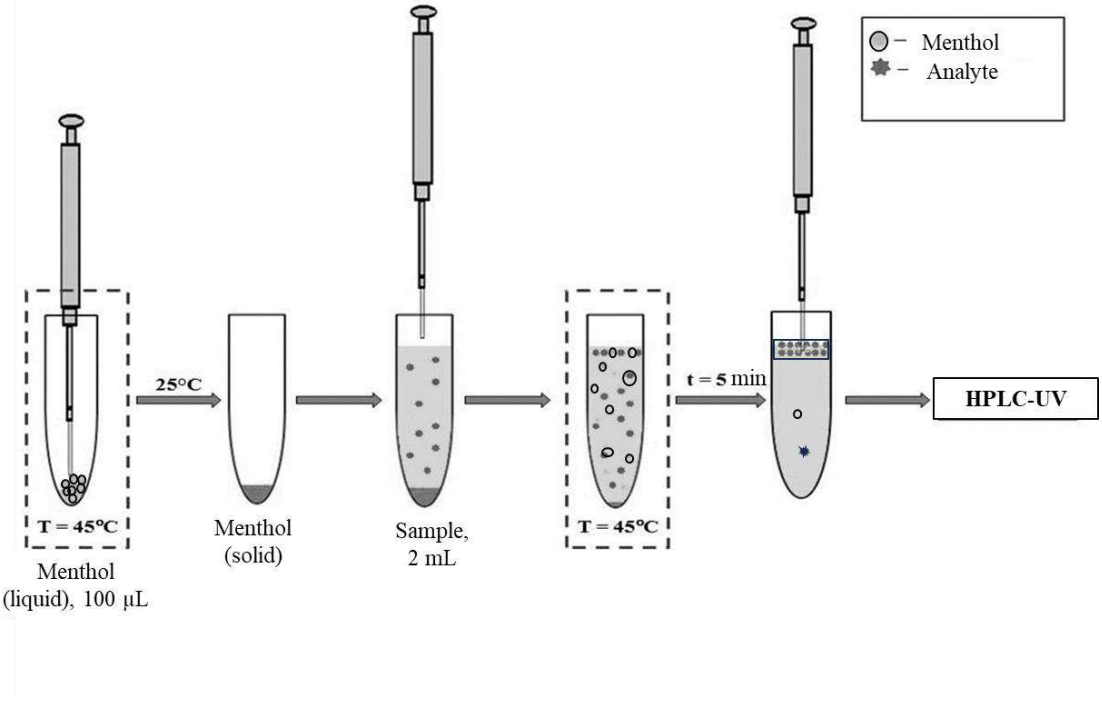

Figure 43. DLLME based on the phase transition of the extraction solvent (solid – liquid).

The main regularities of the microextraction process were revealed. For this purpose, various parameters of the system were studied: phase ratio, temperature regime, acidity of the medium and ionic strength of the solution. The use of chemometric algorithms (multifactorial design) allowed to reduce the number of experiments for choosing optimal conditions.

Each of the parameters was considered at two boundary levels; the coded values are given in Table 14: "+1" corresponded to the maximum value from the range under consideration, and "–1" to the minimum.

Table 14. Coded values of system parameters.

$V_{solvent}$, µL		V_{sample}, mL		*pH*		T, °C		Na_2SO_4	
100	300	1	10	1	7	45	70	with	without
–1	+1	–1	+1	–1	+1	–1	+1	–1	+1

The key parameter of the system with phase transition of the extractant is the temperature, therefore the minimum temperature was determined by the melting temperature of menthol (43.0 °C [165]), taken into account the error of heating the device.

The range of pH values was chosen in the acidic region to exclude the ionization of benzoic acid (pKa = 4.21).

The salting-out effect was studied at two categorical levels (with and without the salting-out agent Na_2SO_4). The minimum volume of the extractant was determined by the convenience of its selection for further HPLC-UV analysis. The sample volume was selected taking into account the concentration factors acceptable for effective extraction. Using fractional factor analysis, the number of experiments was set at 16. After each experiment (n = 3), responses (chromatographic peak area values) were obtained. The regression coefficients in the equation relating the experimental parameters to the analytical signal were calculated using the normal equation [168]. The obtained values for each of the coded factors are given in the equation:

$$\mathbf{AUP} = 3560.56 - 1116.06\times\mathbf{t} - 703.94\times\mathbf{pH} + 21.06\times\mathbf{salt} - 1020.56\times\mathbf{V_{ex}} - 248.94\times\mathbf{V_s} + +244.94\times\mathbf{t}\times\mathbf{pH} - 238.06\times\mathbf{t}\times\mathbf{salt} + 254.31\times\mathbf{t}\times\mathbf{V_{ex}} + 71.19\times \mathbf{t}\times\mathbf{V_s} - 396.44\times\mathbf{pH}\times\mathbf{salt} + 122.19\times\mathbf{pH}\times\mathbf{V_{ex}} + + 408.31\times\mathbf{pH}\times\mathbf{V_s} - 91.06\times\mathbf{salt}\times\mathbf{V_{ex}} - 2.44\times\mathbf{salt}\times\mathbf{V_s} - 449.94\times\mathbf{V_{ex}}\times\mathbf{V_s},$$

where **AUP** is the area under the peak of benzoic acid, $\mathbf{V_{ex}}$ – extractant volume, $\mathbf{V_s}$ – sample volume, **t** – temperature, **salt** – presence/absence of Na_2SO_4. The absolute values of the coefficients indicate the importance of corresponding factors and their interactions for the experiment outcome.

Examination of the values of resulted coefficients reveals the following:

- the most important parameter is the temperature and since the corresponding coefficient has negative sign – the lower the temperature, the higher is the extraction efficiency. No further lowering of temperature is possible due to the menthol melting point value. Thus, a temperature of 45 °C was chosen;

- the lower volume of the extraction solvent also provides for better efficiency from the point of view of enrichment factor obtained. No further reducing of extraction solvent volume is possible due to the handling of solidified extract;

- the same holds for pH – the lower it is, the better is the extraction due to the suppression of benzoic acid ionization;

- the next most important coefficient after pH is the one for the interaction term between sample and solvent volumes;

- cooperative effects of pH and solvent volume, and of pH and salt are also important;

- the rest of the coefficients are at least two times smaller and, thus, these parameters and interactions have very minor influence on the experiment outcome;

- no effect of Na_2SO_4 addition (salting-out effect) was observed, thus it was not used in further experiments.

Taking into account the values of interaction terms between pH, V_{ex} and V_s another experimental design including these parameters was made. This time it was full factorial design 2^3 for three parameters at two levels. pH values were 1 and 2 in order to explore the effect of pH at lower region, solvent volume was 100 and 200 μL, sample volume was 1 and 2 mL. Thus, another nine experiments were run at the fixed temperature of 45 0C and without salting-out agent (taking into account the results of the previous run). Based on the registered outputs of these experiments (benzoic acid peak area) corresponding regression coefficients were calculated. They are presented in the equation:

$$\mathbf{AUP} = 6248.25 + 117.75\times\mathbf{pH} - 867.25\times\mathbf{V_{ex}} + 1662.5\times\mathbf{V_s} + 7.75\times\mathbf{pH}\times\mathbf{V_{ex}} - 253\times\mathbf{pH}\times\mathbf{V_s} - 160\times\mathbf{V_{ex}}\times\mathbf{V_s} - 46\times\mathbf{pH}\times\mathbf{V_{ex}}\times\mathbf{V_s},$$

The following conclusions can be made from these results:

- sample volume of 2 mL provides better conditions for the DLLME;

- menthol volume should be kept minimal;

- in this experimental layout pH changes did not have significant influence on the extraction efficiency;

- interaction terms were also not very significant.

Based on the results of these two studies the following conditions were chosen for all further HA-LLME experiments: T= 45 ^{0}C, pH = 2, V_{ex} = 100 μL, V_s = 2 mL.

The effects of such compounds as ascorbic, lemon, oxalic, malic, succinic, and tartaric acids, rutine, inorganic salts (aluminum chloride, sodium chloride, sodium sulfate), glucose, fructose and sucrose; rutin, as well as various dyes (indigo carmine

(E132), brilliant blue (E133), azorubin (E122), sunset yellow (E110), caramel color (E150a), and tartrazine (E102), that can be found in juices, on the determination of benzoic acid by the developed method were investigated. The tolerable concentration of each interfering compound is considered to be less than 5 % of relative error in the signal. All these substances were found to be not interfering even at their 1000-fold excess.

In addition, it is clearly visible (Figure 43) that the color pigments did not interfere with the determination of benzoic acid; a single peak of benzoic acid was recorded on the chromatogram of real spiked sample (Figure 44).

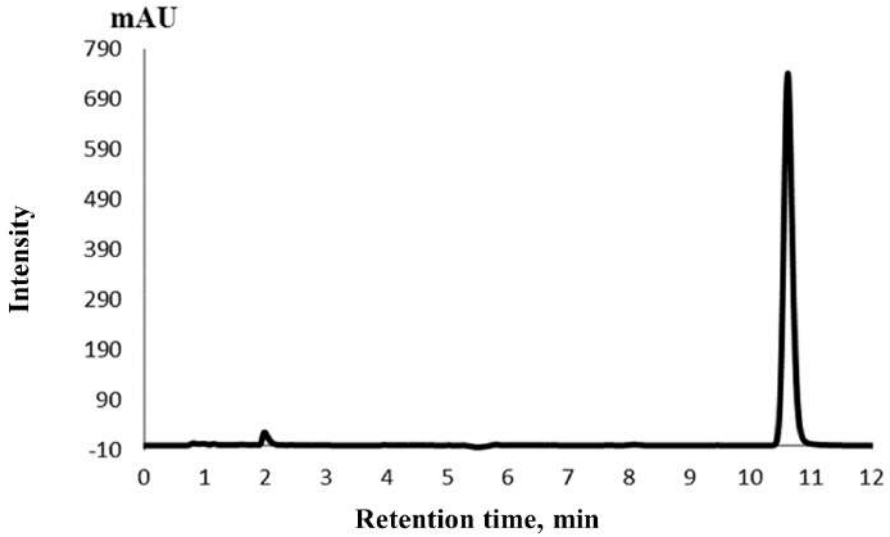

Figure 44. Chromatogram obtained during the determination of benzoic acid ($C_{analyte}$ = 25 mg/L) in spiked juice sample using the developed method.

The developed approach of the DLLME ensured high selectivity, simplicity, rapidity (3 min) and reliability of sample preparation. The linear calibration range of 0.5 – 50 mg/L for benzoic acid was obtained with a correlation coefficient of 0.996. The LOD, calculated from a blank test, based on 3σ, was found to be 0.15 mg/L. Repeatability, characterized by the RSD values, did not exceed 12 %.

The method was used to determine the concentration of benzoic acid in fruit and berry juices (Table 15). The accuracy of the results obtained was assessed using the "add-found" method and the reference method [169]. There was only one sample (berry juice) with benzoic acid detected (13.5 mg/L). It can be explained by the fact that some berries naturally contain benzoic acid, for example, cloudberries.

Table 15. The results of determination of benzoic acid in juices (n= 3, P = 0.95; F_{cr} = 19.00; t_{cr} = 2.78).

Sample	Added, mg/L	Found, mg/L		t-test	F-test	R, %
		This method	Reference method [169]			
Apple juice	0	< LOD	< LOD			-
	1	1.17 ± 0.26	1.3 ± 0.3	0.44	1.47	17
	10	10.4 ± 0.6	9.67 ± 0.22	2.77	6.86	4
	25	24.7 ± 0.5	24.9 ± 0.7	0.42	1.97	1
Multifruit juice	0	< LOD	< LOD			-
	1	1.07 ± 0.22	1.0 ± 0.4	0.20	2.71	7
	10	10.0 ± 0.7	10.8 ± 0.7	2.07	1.03	0
	25	24.7 ± 0.3	24.67 ± 0.22	0.45	1.86	1
Orange juice	0	< LOD	< LOD			-
	1	1.1 ± 0.3	1.1 ± 0.3	0.67	1.95	10
	10	10.6 ± 0.6	10.9 ± 0.8	0.66	2.02	6
	25	24.9 ± 0.8	25.1 ± 0.3	0.59	7.00	1
Cherry juice	0	< LOD	< LOD			-
	1	0.93 ± 0.3	0.87 ± 0.08	0.53	13.00	7
	10	10.0 ± 0.6	10.1 ± 0.5	0.40	1.70	0
	25	25.2 ± 0.6	24.67 ± 0.22	1.35	6.86	2
Berry juiceh	0	13.5 ± 0.4	13.3 ± 0.6	0.84	2.33	-
	1	14.6 ± 0.4	14.6 ± 0.5	0.73	2.58	10
	10	23.8 ± 07	23.9 ± 0.6	0.19	1.21	3
	25	38.8 ± 0.6	38.0 ± 0.5	1.99	1.33	1

There are several *advantages* of the developed DLLME based on the use of a low-melting natural terpenoid compared to the conventional version of DLLME: the absence of a dispersive solvent; no need for dilution or filtration of samples, as well as the centrifugation, which significantly affects the time of the entire analysis. Thus, the

developed sample preparation method can be considered as a tool for the extraction of analytes with acidic properties (e.g., monobasic carboxylic acids) into the organic phase in order to eliminate the interfering effect of a complex matrices of liquid samples for subsequent analysis by liquid chromatography methods with various detection options. The use of disposable extraction vials with a long shelf life eliminates cross-contamination of samples and ensures reproducibility of results. The rapid and simple sample preparation procedure may be of interest for mass sample analysis. However, this method has a *limitation* such as clogging of the HPLC-UV system with the menthol phase, so it must be pre-dissolved in an organic solvent or in the mobile phase before analysis. Menthol, like many other terpene alcohols, slightly absorbs electromagnetic radiation in the UV region [170], so it does not interfere with UV detection of analytes.

The results of the study were published in the Journal of Molecular Liquids [12].

During the research process it was found that dispersion of the system by the gas phase with subsequent solidification of organic phase leads to an increase in the extraction efficiency of benzoic acid by almost 2 times compared to the approach proposed above. This can be explained by the partial dissolution of menthol in the aqueous phase of the sample at high temperatures (435.5 mg/L at 25 °C [170]), leading to less efficient extraction of the analyte into the terpenoid phase, which is eliminated in the approach with solidification of the organic phase. As a result, all menthol in the system is collected on the surface of the sample (the density of menthol is 0.890 g/cm^3). Moreover, the solubility of analytes in the aqueous phase decreases with lowering the temperature.

Thus, a simple and highly-available air-assisted dispersive liquid-liquid microextraction procedure with organic phase solidification was developed as new approach for pretreatment of complex sample matrix (Figure 45).

At the first stage, the scheme involved introducing molten menthol into a liquid-phase sample, thermostated at the temperature higher the melting point of the extractant (menthol), and subsequent aspiration of the system by taking the mixture from the extraction container using a dispenser and pushing out into the vial (repeated three times).

This dispersion approach is well known and was discussed in the literature review. In the second stage, the suspension was cooled, resulting in the formation of a thin film

of the terpenoid phase with the analytes released into it on the surface of the system. To analyze the resulting extract, the menthol film was pierced with a needle and the aqueous phase was removed with a syringe. After that, methanol (1:1) was added to the extraction solvent and thermostated for 30 s for its complete dissolution and subsequent introduction into the HPLC-UV system.

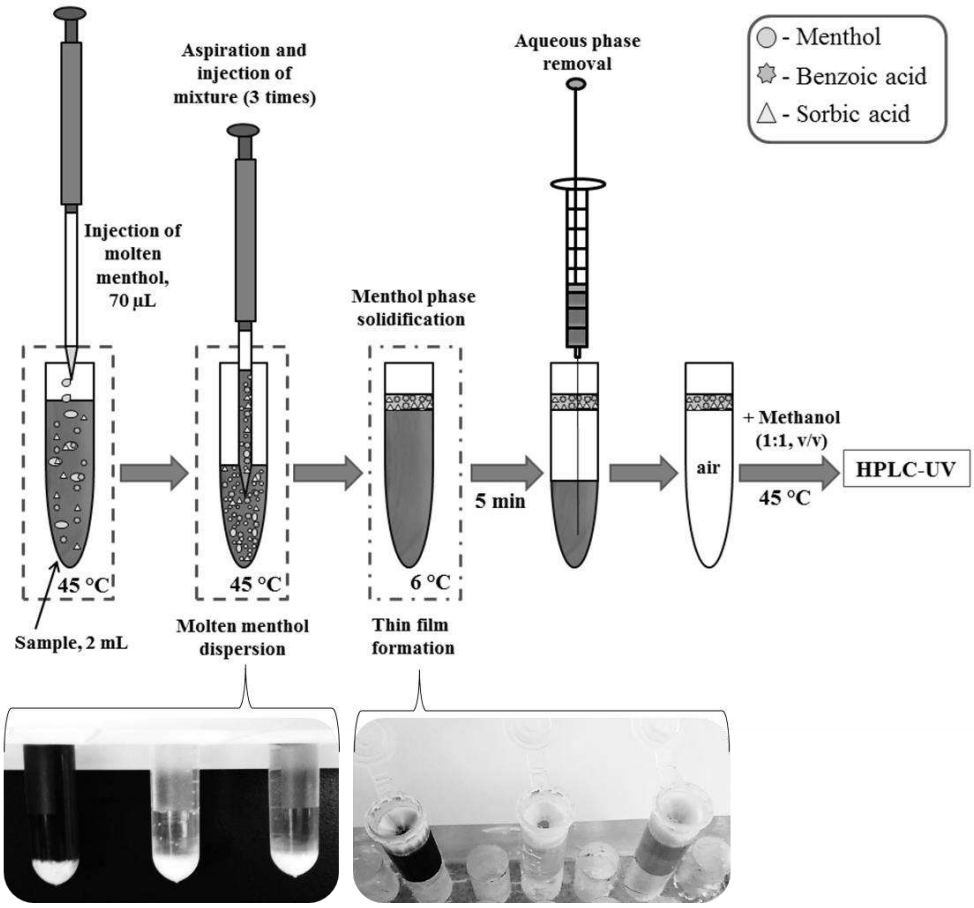

Figure 45. DLLME based on the phase transition of the extraction solvent (liquid – solid).

It was experimentally established that the optimal sample volume for implementing the proposed procedure is 2 mL, and the volume of the extraction solvent is 70 µL. Using a menthol volume of less than 70 µL resulted in discontinuity of menthol films and high RSD values . Three aspiration cycles were sufficient to disperse the system with the gas phase, which was confirmed by a stable analytical signal and reproducibility of the results. To accelerate the process of solidification of the menthol phase, the extraction container was placed in a refrigerating chamber (6 °C) for 5 min. A lower temperature of the freezer (-15 °C) did not contribute to accelerating the process.

The linear calibration ranges of 0.1 - 150 mg/L for benzoic and 0.05 - 100 mg/L for sorbic acids were obtained with correlation coefficients of 0.998 for both analytes. The LODs, obtained from a peak 3 times the signal-to-noise ratio, was found to be 0.03 mg/L and 0.02 mg/L, respectively.

The developed procedure was applied for the determination of benzoic and sorbic acids in two juice samples (fruit and berry), three soft drinks and soy sauce. Samples were used without any pre-treatment except of cases with high content of analytes, where dilution was required. According to juice and soy sauce producers, all samples were manufactured without preservatives, as it was indicated on the packages. As for the soft drinks, E210 (benzoic acid) and E211 (benzoate) were added in these samples. The concentrations of the target analytes in real samples obtained by using calibration curve are shown in Table 16. The concentrations of sorbic acid in all samples were less than LOD. All concentrations of benzoic acid found in soft drinks were within the limitation (150 mg/L) determined by European Union Directive 95/2/CE. For sorbic acid this limitation is 2 times higher – 300 mg/L. Accuracy and reliability of the resulting information were further studied by analyzing the mentioned samples using referent extraction procedure described in [169].

Table 16. The results of determination of benzoic and sorbic acids in beverages and soy sauce (n=3, P=0.95; F_k = 19.00; t_k = 2.78).

Sample	**Analyte**	**Added, mg/L**	**Found, mg/L**		**t-test**	**F-test**	**R, %**
			This method	**Reference method [169]**			
Apple and grape juice	benzoic acid	0	< LOD	< LOD			-
		50	46.5 ± 1.8	49.8 ± 2.2	2.19	1.53	7
		100	99 ± 3	97.9 ± 2.7	0.67	1.24	1
	sorbic acid	0	< LOD	< LOD			-
		50	49.9 ± 1.4	52.1 ± 1.2	2.17	1.59	0
		100	99.9 ± 2.6	100.6 ± 0.9	0.44	7.64	0
Fruit and berry juice	benzoic acid	0	< LOD	< LOD			-
		50	49.4 ± 1.4	47.7 ± 1.6	1.55	1.38	1

		100	100.7 ± 2.3	101 ± 4	0.06	3.76	1
	sorbic acid	0	< LOD	< LOD			-
		50	48.8 ± 1.9	51.6 ± 0.7	1.75	8.82	2
		100	99.2 ± 0.9	99.8 ± 1.5	0.71	2.74	1
Soft drink №1	benzoic acid	0	135.4 ± 1.1	141 ± 4	2.71	13.45	-
		50	186.4 ± 2.9	190.0 ± 2.4	1.72	1.91	2
		100	238 ± 3	240.8 ± 2.8	2.07	1.26	3
	sorbic acid	0	< LOD	< LOD			-
		50	49 ± 4	49.9 ± 1.2	0.18	12.63	2
		100	100 ± 3	100.6 ± 1.8	0.24	3.62	0
Soft drink №2	benzoic acid	0	117 ± 7	116 ± 7	0.14	1.18	-
		50	169 ± 4	167 ± 2.5	0.85	2.54	4
		100	218 ± 5	216.12±2.66	0.50	3.72	1
	sorbic acid	0	< LOD	< LOD			-
		50	49 ± 3	49.3 ± 1.9	0.13	3.00	1
		100	105 ± 3	98.4 ± 1.3	1.14	5.85	5
Soft drink №3	benzoic acid	0	122 ± 7	127.9 ± 9.2	1.05	1.52	-
		50	176 ± 3	178.5 ± 3.8	2.49	1.59	8
		100	222.5 ± 1.8	225.9 ± 1.9	2.58	1.08	1
	sorbic acid	0	< LOD	< LOD			-
		50	49.6 ± 2.1	49.7 ± 1.9	0.07	1.07	1
		100	100.2 ± 0.4	98.8 ± 1.4	1.89	13.52	0
Soy sauce	benzoic acid	0	< LOD	< LOD			-
		50	49.0 ± 2.1	49.0 ± 0.6	0.55	11.63	2
		100	99.2 ± 1.2	50.4 ± 0.8	0.30	3.22	1
	sorbic acid	0	< LOD	< LOD			-
		50	49.1 ± 1.2	50.4 ± 0.8	1.77	2.11	2
		100	103 ± 3	100.4 ± 1.1	0.40	8.51	3

Advantages. Compared with the known methods in the literature for determining benzoic and sorbic acids in liquid samples of food products based on LLE and LLME (Table 17), the developed approach of DLLME provides relatively low LOD, rapidity, ease of implementation (does not require solvent replacement or centrifugation).

Table 17. Comparison of methods for the determination of benzoic (E 210) and sorbic acids (E 200) in liquid food products based on liquid-liquid extraction and microextraction.

Sample (mass/volume)	**Sample preparation (time)**	**Extraction solvent (volume, µL)**	**Detection method**	**LOD, mg/L**	**Reference**
Soy sauce (1 g)	LLE, solvent exchange (-)	Diethyl ether (5000)	HPLC-UV	0.2 (E 210), 0.1 (E 200)	[171]
Soy sauce (1 mL)	LLE, solvent exchange (> 22 min)	Diethyl ether (5000)	CE-CD	9.8×10^{-3} (E210), 5.6×10^{-3} (E 200)	[172]
Soft drinks, milk, tomato paste (5 mL)	DLLME, centrifugation (12 min)	Chloroform / acetone (250 / 1200)	HPLC-UV	0.1 (E210), 0.08 (E 200)	[173]
Soft drinks, juices (6 mL)	DLLME, centrifugation (> 10 min)	Octanol (200)	SP	0.03 (E 210), 0.04 (E 200)	[174]
Drinking yoghurts (10 mL)	DLLME, centrifugation (12 min)	Octanol/ ethanol (60 / 450)	HPLC-UV	0.06×10^{-3} (E 210), 0.15×10^{-3} (E 200)	[169]
Juices (2 mL)	DLLME (3 min)	Menthol (100)	HPLC-UV	0.15 (E210)	This work 1
Soft drinks, juices, soy sauce (2 mL)	DLLME (7 min)	Menthol (70)	HPLC-UV	0.03 (E210), 0.02 (E200)	This work 2

In addition, the use of microvolumes of an environmentally friendly extraction solvent (menthol) and the absence of a dispersive solvent make it attractive for green analytical chemistry. A *limitation* of the method, as noted earlier, is the impossibility of direct injection of the obtained extract into the chromatograph system, which is easily eliminated by preliminary dissolution of the phase in methanol (1:1, vol./vol.).

The data obtained during the study were published in the Journal of Chromatography A [13].

5.2. Microextraction based on phase transition of dispersive solvent

To extract and concentrate organic analytes from liquid food samples, a DLLME technique was developed that provides the possibility of dispersing the extraction solvent in a wide acidity range. The procedure assumes the dispersion of the extractant with a gas phase that is formed as a result of a phase transition of dispersive solvent when heating the extraction system. In this case, the selectivity of mass transfer can be varied by changing the forms of the substances being separated when regulating the acidity of the aqueous phase, as well as by choosing the extractant. The analytical capabilities of the proposed approach were demonstrated using the following examples: 1) HPLC-UV determination of preservatives (sorbic (additive E200) and benzoic (additive E210) acids) in soft drinks; 2) HPLC-MS/MS determination of insecticides (malathion, diazinon and phosalone) in wine.

To implement the proposed approach, it was decided to use a natural terpenoid – menthol as an extraction solvent, which has proven itself as extraction solvent that ensures efficient and selective extraction of benzoic and sorbic acids from liquid samples (section 5.1). Dichloromethane was studied as a dispersive agent. Dichloromethane belongs to hazard class 4 (low-hazard substances), but nevertheless requires careful handling, so it's using had to be minimized. The effect of dichloromethane volume on the efficiency of microextraction was studied in the range from 35 to 145 μL, while the volumes of the aqueous phase (2.0 mL) and extraction solvent (70 μL) were constant. It was found that 100 μL of dichloromethane ensures efficient mass transfer of analytes to the organic phase.

An inorganic salt (sodium chloride) and a monosaccharide (glucose) were studied as initiators of evaporation of the dispersive solvent. It is worth noting, they can promote salting-out and sugaring-out effects. Addition of each of the substances in dry form to the extraction system led to intensive evaporation of dichloromethane, but higher analytical signals (chromatographic peak areas) were observed in the case of sodium chloride. It was found that when adding less than 10 mg of salt, the initiating effect was not observed, since the salt immediately dissolved in the aqueous phase. Salt mass in the range from 10 to 50 mg provided comparable analytical signals, therefore, the minimum initiator mass of 10 mg was chosen for further studies.

The boiling point of dichloromethane (40.0 °C [175]) is lower than the melting point of menthol (43.0 °C [165]), therefore, the evaporation of the dispersant occurs under conditions when menthol is in a liquid state. Considering that the temperature of the extraction system affects the intensity and rate of dichloromethane evaporation and, as a consequence, the efficiency of dispersion of organic phase in the aqueous sample, it was studied in the range from 45 °C to 80 °C. It was noted that intensive evaporation of dichloromethane and effective dispersion of the menthol were possible only with heating more than 70 °C, therefore, a temperature of 70 °C was chosen.

It should be mentioned that the choice of dispersive solvent is limited not only by its low boiling point, but also by its high density. To implement the proposed approach, the solvent should be located at the bottom of the extraction vial to ensure effective mixing of the entire sample volume during evaporation. Thus, the density of pure dichloromethane is 1.33 g/mL. In the proposed procedure of DLLME, the density of the mixture of menthol and dichloromethane (7:10, vol./vol.) was 1.197 g/mL, which is greater than that of water, so the organic phase did not rise to the surface when the sample was added. During the evaporation of dichloromethane, the density of the organic phase decreased to 0.940 g/mL, thus, the extraction solvent was released as a separate phase from the top. At the same time, the density of menthol is 0.890 g/mL, which indicates the formation of a eutectic mixture, which terpenoids are capable to form in the presence of water [176]. Before introducing the isolated phase into the chromatographic system, it

was diluted with methanol in a volume ratio of 1:1 (vol./vol.). The implementation of the proposed DLLME is shown in Figure 46.

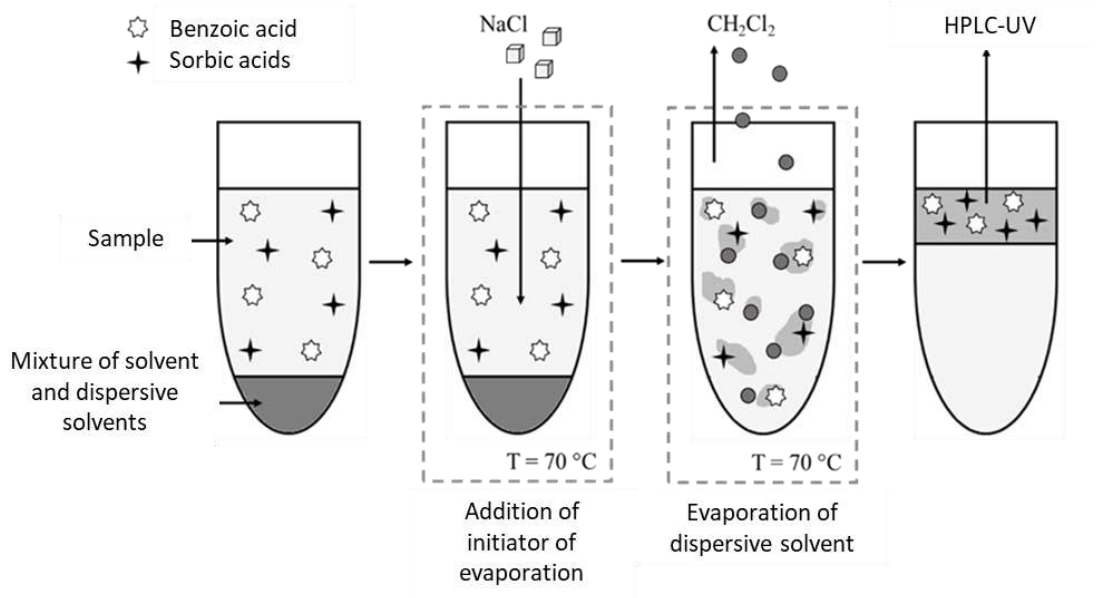

Figure 46. Scheme of DLLME with phase transition of dispersive solvent.

The developed method, including DLLME and subsequent HPLC-UV analysis, provided the linear calibration ranges of 1 - 300 mg/L for benzoic acid and 1 - 120 mg/L for sorbic acid. The detection limits (3σ) for two analytes were 0.3 mg/L, respectively. Repeatability and reproducibility were characterized using the relative standard deviation (RSD) and did not exceed 10 %. Sample preparation time was 5 min. Chromatographic analysis time was 13 min.

The proposed method was used to determine preservatives in canned soft drinks (juices and carbonated drinks) recommended for baby nutrition. The results of the determination are presented in Table 18. The accuracy of the results obtained was confirmed by “add-found” method. According to the data obtained, benzoic and sorbic acids were present in the samples in insignificant concentrations, which is explained by their natural presence in the original raw materials. The results of the studies were published in the Journal of Analytical Chemistry [23].

Table 18. Results of determination of preservatives in soft drinks (n = 3, P = 0.95).

Sample	Analyte	Added, mg/L	Found, mg/L	R, %
Fruit juice	benzoic acid	0	2.5 ± 0.5	-
		5	7.2 ± 0.9	6
		50	55 ± 7	4
		100	109 ± 24	6
		200	207 ± 17	2
	sorbic acid	0	1.90 ± 0.15	-
		5	6.6 ± 0.5	6
		50	50 ± 6	3
		100	105 ± 11	3
Berry juice	benzoic acid	0	1.90 ± 0.15	-
		5	6.7 ± 1.2	4
		50	54 ± 9	5
		100	99 ± 11	3
		200	213 ± 7	6
	sorbic acid	0	1.223 ± 0.017	-
		5	6.3 ± 1.0	1
		50	51 ± 6	0
		100	99 ± 19	2
Sparkling fruit soft drink	benzoic acid	0	1.45 ± 0.16	-
		5	6.3 ± 0.4	2
		50	49 ± 5	5
		100	98 ± 17	3
		200	209 ± 18	4
	sorbic acid	0	< LOD	-
		5	4.8 ± 0.5	3
		50	48.0 ± 2.0	4
		100	94 ± 19	6

The possibility of combining the DLLME based on phase transition of volatile dispersive solvent (dichloromethane) with the HPLC-MS/MS system was also demonstrated for the determination of three organophosphorus insecticides (malathion, diazinon and phosalone) in wine. These pesticides are widely used in agriculture.

The main requirements for the extraction solvents in the developed approach are: low solubility in the aqueous phase, low density (less than the density of water), and high extraction efficiency with respect to target analytes. In addition, an important criterion for selection of solvent is its compatibility with the final method of determination. It is known that for the extraction of pesticides, regardless of the nature of analytes (if they do not have strong acidic or basic properties) solvents are arranged in the following order in ascending order of extraction capacity: limited hydrocarbons < unsaturated hydrocarbons < chlorinated hydrocarbons < aromatic hydrocarbons < simple esters < alcohols < esters < vegetable oils.

Chlorine-containing hydrocarbons, which form hydrogen bonds with pesticides, have a high extraction capacity. For the same reason, alcohols and ethers extract pesticides well; their different nature allows the action of extraction solvents to be differentiated [177]. Taking into account the above requirements, a homologous series of aliphatic alcohols with carbon atoms from 4 to 7 was considered in order to select an extraction solvent for target analytes.

Initially, the distribution coefficients (K) were estimated for alcohol-aqueous phase systems (1:1, vol./vol.)). The estimated K (at 25 °C) for malathion, diazinon, and phosalone are presented in Table 19. As it can be seen from the table, alcohols investigated provided similar extraction efficiency. However, hexanol and heptanol have lower solubility in water compared to the other two alcohols, therefore they provided higher enrichment factors of the analytes. In addition, the results obtained using butanol and pentanol at higher at a higher ratio of organic solvent/aqueous phase were characterized by high RSD values (15 - 20 %) due to the volumes of the extracts depend on temperature fluctuations. The determining factor in the choice between hexanol and heptanol was the lower viscosity of the former, which is preferable for further combination with the HPLC-MS/MS system.

Table 19. The distribution coefficients of insecticides ($C_{analyte}$ = 10 μg/L, 25 °C, aqueous phase/organic phase – 1:1 (vol./vol.), n = 3).

Alcohol	**K**		
	Malathion	Diazinon	Phosalone
Butanol	45 ± 3	45 ± 2	31 ± 3
Pentanol	47 ± 3	48 ± 3	24 ± 4
Hexanol	48 ± 2	50 ± 2	25 ± 2
Heptanol	49 ± 2	52 ± 3	23 ± 4

Dichloromethane volume influences on density of the extraction mixture, rate of its evaporation from the system, and as a result, time of analysis. To find the optimal ratio, dichloromethane was mixed with hexanol in different ratios, and it was found that dropping the mixture to the bottom of aqueous phase was observed from ratio 1:1 (v/v) and higher volume of dichloromethane in the mixture. It was found that all the necessary requirements are provided with a sample volume of 10 mL, an extraction mixture with a volume of 0.4 mL and the ratio of dispersive and extraction solvents equal to 1:1 (vol./vol.).

Another important parameter for the extraction process is glucose mass, which initiates evaporation of dichloromethane. The concentration of saccharides in the samples do not effect negatively on the extraction of insecticides by the method developed, furthermore, the positive impact of glucose as sugaring-out agent was observed. It also should be mentioned, that semi sweet wine samples contain 18 - 45 g/L of sugar. To find the optimal glucose mass, 10 mL of standard solution (1 μg/L of each insecticide) was mixed with 0.4 mL of extraction mixture (hexanol and dichloromethane (1:1, vol./vol.)) and glucose (0.01 - 0.3 g) followed by mixture thermostating at 70 ºC for 1 min. The sufficient extraction efficiency and low RSD were found at 0.15 g of glucose.

The effect of temperature on the evaporation process was studied in the range of 40 - 90 °C. It was found that a temperature of 70 °C ensures efficient extraction and minimum values of the standard deviation (Figure 47), while the time required for extraction is 1 min.

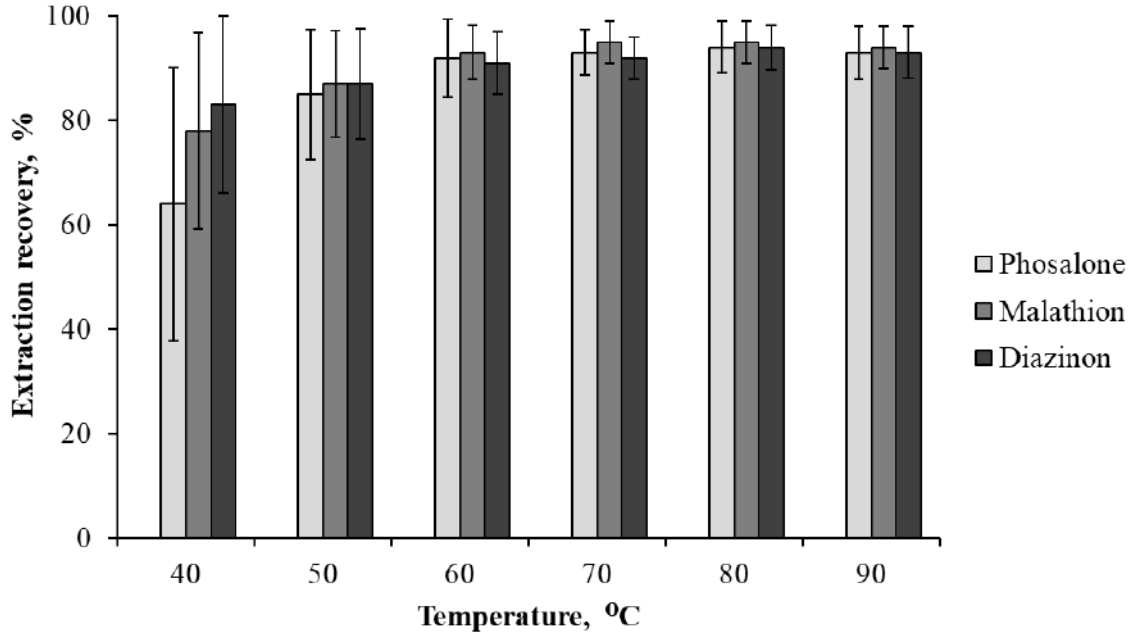

Figure 47. Effect of heating temperature on the extraction recovery of insecticides ($C_{analyte}$ = 1 μg/L, n = 3).

When selecting the pH value, the maximum values of the analytical signal were obtained in the range from 3 to 7, since strongly acidic and strongly alkaline media promote the hydrolysis of malathion and diazinon. It is worth noting that the pH of the wine samples was in the range of 3 - 5, so the samples were not subjected to additional sample pretreatment before extraction. It was also found that ethyl alcohol, which is part of the wines (9 – 16 %) at a content of 8 % and higher, has a negative effect on the extraction efficiency, increasing the solubility of analytes in the aqueous phase. Therefore, preliminary dilution of the wine samples was required to achieve effective extraction of analytes.

Taking into account the selected parameters, the scheme of the DLLME for the determination of insecticides in wine assumed the following stages (Figure 48). At the first stage, 10 mL of sample (diluted 3 times) was placed into a glass conical tube and 0.4 mL of extraction mixture (1:1) was added. Then 0.15 g of glucose as the initiator was added into the conical tube with sample mixture and the conical tube was placed into the incubator and thermostated at 70 ºC for 1 min. Dichloromethane was evaporated completely in 1 min and the top hexanol phase was immediately observed. After that 100

μL of the top alcohol phase was handled and transferred into an autosampler vial of the HPLC-MS/MS system.

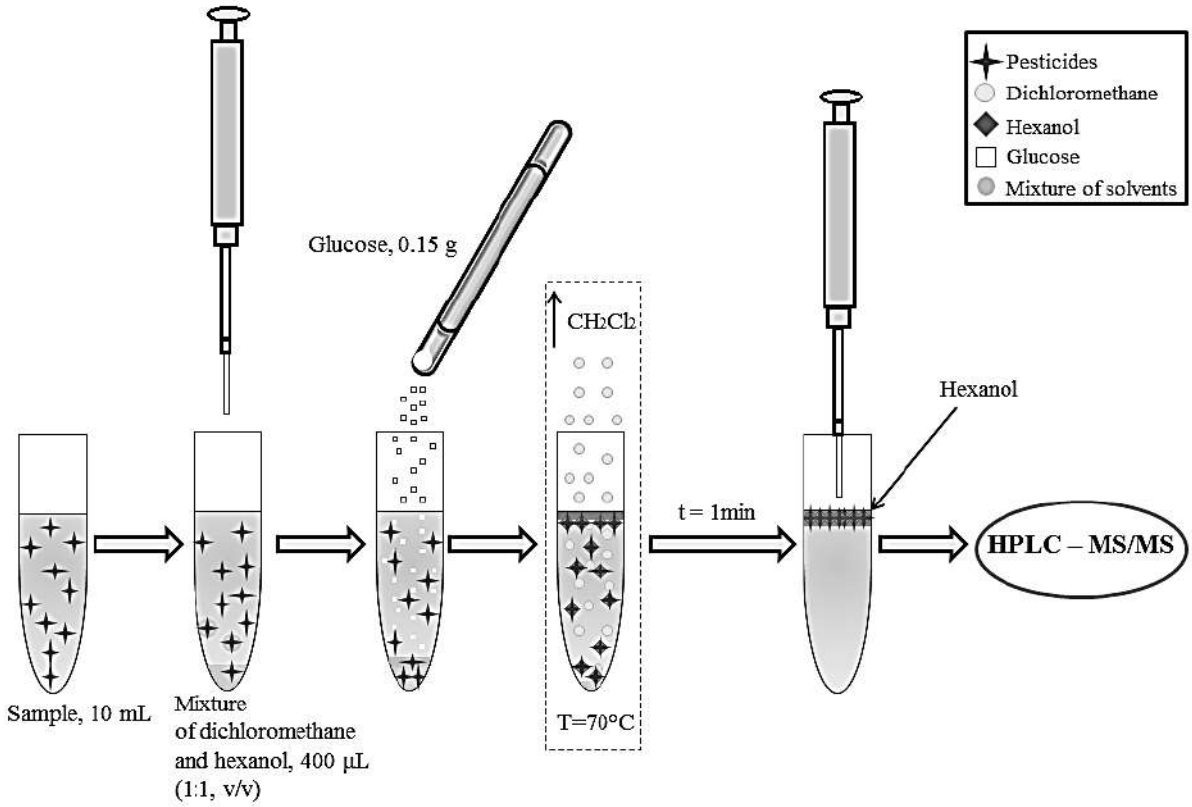

Figure 48. Scheme of the DLLME based on phase transition of volatile dispersive solvent for the determination of insecticides in wine.

The linear calibration ranges of 10^{-7} - 10^{-3} g/L for malathion, 10^{-9} - 10^{-4} g/L for diazinon, and 10^{-6} - 10^{-2} g/L for phosalone were obtained with the coefficient of determination more than 0.996. The LODs, calculated from a blank test, based on 3σ, found to be $3 \cdot 10^{-8}$ g/L for malathion, $3 \cdot 10^{-10}$ g/L for diazinon and $3 \cdot 10^{-7}$ g/L for phosalone. The method's repeatability expressed as RSD (n = 3) was from 4 to 7 %.

The maximum residue levels for most pesticides in various food products can range from 0.001 to 100 mg/kg, but, as a rule, for vegetables and fruits they are about 0.01 mg/kg [178]. The proposed method provides LOD below these values (in mg/kg terms).

The analytical possibilities of the method were successfully demonstrated using the example of determining malathion, diazinon, and phosalone in semi-sweet wines (Table

20). The accuracy of the results obtained was confirmed by the “add-found” method and the reference method [179].

Table 20. The results of determination of malathion, diazinon, and phosalone in wines (n=3, P=0.95; F_{cr} = 19.00; t_{cr} = 2.78).

Sample	Analyte	Added, µg/L	Found, µg/L		t-test	F-test	R, %
			This method	Reference method			
White wine 1	malathion	0	< LOD	< LOD			-
		1	1.06 ± 0.02	1.05 ± 0.03	0.24	14.9	6
	diazinon	0	0.044 ± 0.013	0.050 ± 0.012	2.75	1.2	-
		1	1.02 ± 0.07	1.08 ± 0.03	1.53	3.4	2
	phosalone	0	< LOD	< LOD			-
		1	0. 975 ± 0. 023	1.03 ± 0.07	1.69	10.3	2
White wine 2	malathion	0	< LOD	< LOD			-
		1	1.00 ± 0.03	1.02 ± 0.06	0.49	7.6	0
	diazinon	0	< LOD	< LOD			-
		1	1.02 ± 0.03	0.96 ± 0.05	2.33	3.5	2
	phosalone	0	< LOD	< LOD			-
		1	1.01 ± 0.04	0.962 ± 0.021	1.91	3.4	1
White wine 3	malathion	0	< LOD	< LOD			-
		1	0.961 ± 0.011	0.99 ± 0.05	1.52	17.9	4
	diazinon	0	< LOD	< LOD			-
		1	1.01 ± 0.11	1.04 ± 0.03	0.42	12.9	1
	phosalone	0	< LOD	< LOD			-
		1	1.01 ± 0.03	0.96 ± 0.03	2.33	1.4	1
Red wine	malathion	0	< LOD	< LOD			-

		1	0.96 ± 0.03	1.03 ± 0.03	2.75	1.4	4
	diazinon	0	< LOD	< LOD			-
		1	1.01 ± 0.05	0.96 ± 0.03	1.64	8.7	1
	phosalone	0	< LOD	< LOD			-
		1	1.06 ± 0.03	1.10 ± 0.04	1.47	18.1	6
Pear wine	malathion	0	< LOD	< LOD			-
		1	0.96 ± 0.04	0.99 ± 0.04	1.26	1.3	4
	diazinon	0	< LOD	< LOD			-
		1	1.03 ± 0.03	1.01 ± 0.04	1.11	12.3	3
	phosalone	0	< LOD	< LOD			-
		1	0.94 ± 0.05	0.96 ± 0.06	0.43	1.2	6

Advantages. The developed approach of DLLME is characterized by simplicity of implementation, rapidity (sample preparation time is only 2 min) and economy (inexpensive reagents, does not require complex equipment). Due to the use of an extraction solvent with a low viscosity, direct analysis of the extract is possible without preliminary dilution with an organic solvent or mobile phase. The combination with the highly sensitive HPLC-MS/MS method made it possible to obtain record low values of LOD (Table 21). No need for centrifugation opens up the opportunity for automation of the proposed approach based on flow methods (section 6.1). The *limitation* of the technique includes the toxicity of the dispersive solvent (dichloromethane), which introduces an additional condition for carrying out this approach – the use of an exhaust ventilation system.

The research data were published in the Journal of Chromatography A [10].

Table 21. Comparison of the techniques based on DLLME for the determination of pesticides in food samples.

Sample	Extraction solvent / dispersive solvent	Analyte	Detection method	LOD, µg/L	Reference
Hawthorn juice	Tetrachloroethane/acetonitrile	Organophosphorus pesticides	GC-FPD	0.05 - 0.1	[180]
Pear juice	Tetrachlorethylene/acetonitrile	Cypermethrin, permethrin	GC-FID	2.2 - 3.1 µg/kg	[181]
Wine	Tetrachloroethane/-(US- and vortex assisted)	Organophosphorus and triazine pesticides	GC- NPD	0.007 - 0.07	[182]
Honey and tea	IL (NPs TiO_2)/-(CO_2-mixing)	Acaricides	HPLC-UV	0.04 - 0.18	[183]
Grape juice, cucumbers, tomatoes	Toluene/-(air-assisted)	Triazole pesticides	GC-FID	0.53 - 1.13	[160]
Wine	Hexanol/ dichloromethane	Organophosphorus pesticides	HPLC-MS/MS	0.0003 – 0.3	This work

Thus, it can be concluded that the developed approach of the DLLME based on dispersion of the extraction solvent by the gas phase formed *in situ* as a result of the phase transition – evaporation of a volatile organic solvent, has a number of *advantages* over the conventional DLLME:

1) due to the removal of the dispersive organic solvent from the system, it is possible to exclude its negative impact on the extraction efficiency;

2) due to the simplicity of the proposed approach and the absence of a centrifugation stage, it is possible to carry it out in an automated mode.

In general, the developed technique can be combined with various detection methods, and also has potential for studying the use of new generation solvents (DES, IL, supramolecular liquids) as extraction solvents. The *limitation* of the method is associated with the requirements for the physical properties of the dispersive solvents, and significantly narrows the range of such substances.

CHAPTER 6. AUTOMATION OF MICROEXTRACTION BASED ON FLOW ANALYSIS

Automation of chemical analysis, as well as miniaturization, are the concepts of green analytical chemistry and a promising direction for its development [184]. To increase the productivity of analysis, flow methods are used, as discussed in the literature review (section 1.3). Considering that sample preparation is most often the limiting stage of analysis, its automation is energetically and economically justified. The extraction of analytes into the extraction solvent phase is carried out in special extraction chambers, and the sample, reagent solutions and extraction solvent are fed using peristaltic or syringe pumps, switching the flows through a solenoid valve. The addition of special mixing devices in flow scheme allows to achieve complete mass transfer and a high rate of equilibrium.

This chapter is dedicated to the automation of the DLLME, HLME and micellar microextraction based on principles of flow methods, intended for the analysis of liquid and solid-phase food samples. The possibility of combining automated approaches with highly sensitive detection methods will also be demonstrated. The variety and features of automated techniques for food analysis, including those described in this chapter, are presented in reviews [6, 11, 15, 20, 24].

6.1. Automated dispersive liquid-liquid microextraction

The DLLME based on phase transition of dispersive solvent (dichloromethane) presented in section 5.2 was automated based on the principles of stepwise injection analysis, including several stages (Figure 49). At the first stage, using a peristaltic pump and an 8-port valve, a portion of the sample (10 mL) was fed into an extraction chamber heated at 70 °C through the channel 1, then an extraction mixture (0.4 mL) consisting of hexanol and dichloromethane (1:1, vol./vol.) was injected there through the channel 2. At the second stage, when the system was warming up, 0.5 mL of 315 g/L glucose solution was fed into the container via the channel 3, followed by the supply of air flow (channel 4) for uniform distribution of the sugaring-out agent throughout the system. As a result,

intensive evaporation of dichloromethane was observed followed by moving extraction solvent microdroplets from down to the top of aqueous phase due to the decrease of the extraction mixture density. The extraction of analytes into hexanol phase was carried out. At the next stage, the lower (aqueous) phase was drained from the chamber using a peristaltic pump (channel 5), and the extract was pumped into a vial for subsequent HPLC-MS/MS analysis. At the final stage, all system communications were washed with a mixture of methanol and distilled water (1:1, vol./vol.) (channel 6). All operations were performed using software installed on a computer.

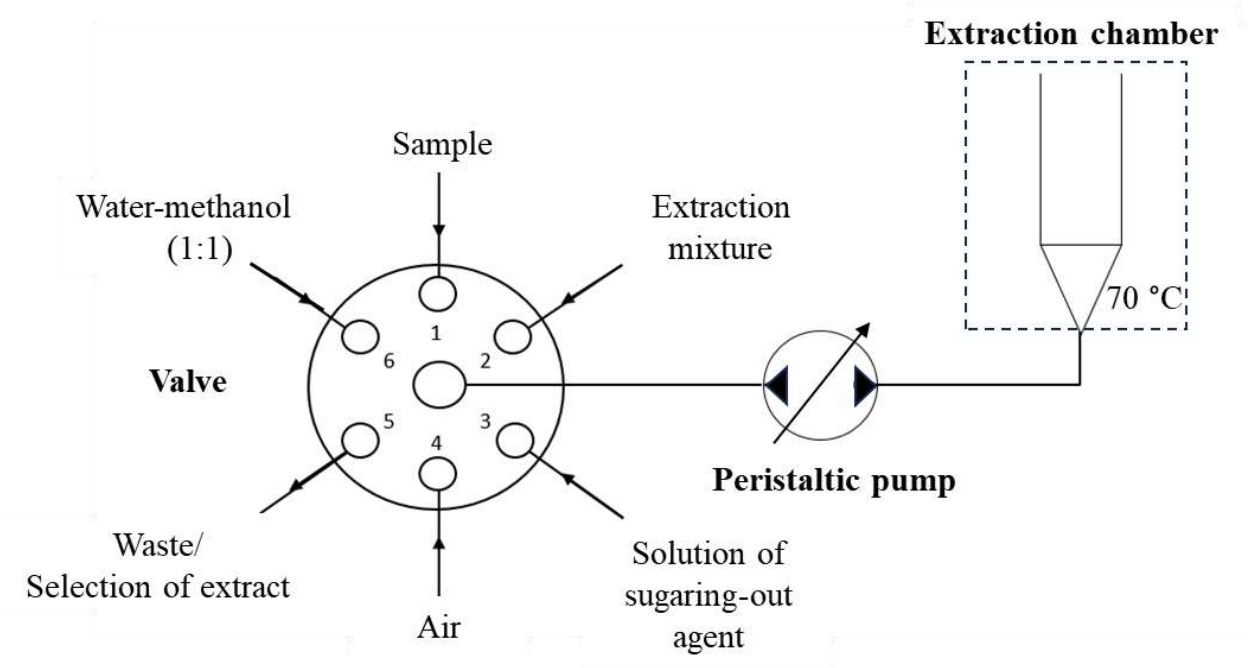

Figure 49. Automated DLLME based on phase transition of dispersive solvent for the analysis of water samples.

The developed analysis scheme was applied to determine malathion, diazinon and phosalone in semi-sweet wines, the results confirmed the previously obtained results using the manual procedure (Table 20). Automation of the sample preparation process allowed to reduce labor costs and make the analysis more environmentally friendly, reducing the analyst's contact with reagents.

Several tools for greenness assessment such as Analytical GREEnness metric (AGREE) [185], National Environmental Methods Index (NEMI) [186], Eco-Scale Assessment (ESA) [187], Green Analytical Procedure Index (GAPI) [188], etc. were currently proposed. For clarity, the greenness assessment of the approach proposed

(manual and automated procedure) were calculated using the AGREE system. The idea of the AGREE method is to calculate the degree of impact for each of the 12 principles of green analytical chemistry on a scale from 0 to 1, where 1 is the maximum score, indicating full compliance with the principle. The effect is transformed into simple scores with stepwise, linear or geometric functions. The resulting scores are summed up and averaged by the calculator app producing an easy-to-read pictogram divided into 12 segments and colored with an intuitive traffic light. Due to such systems of greenness assessment, it is possible to quickly identify weak points in analytical procedures, and also to compare developed methods with others.

Thus, the calculated indices of greenness assessment for the two methods of determination of insecticides with manual and automated approaches of DLLME based on phase transition of dispersive solvent presented in the work were 0.56 and 0.61, respectively (Figure 50).

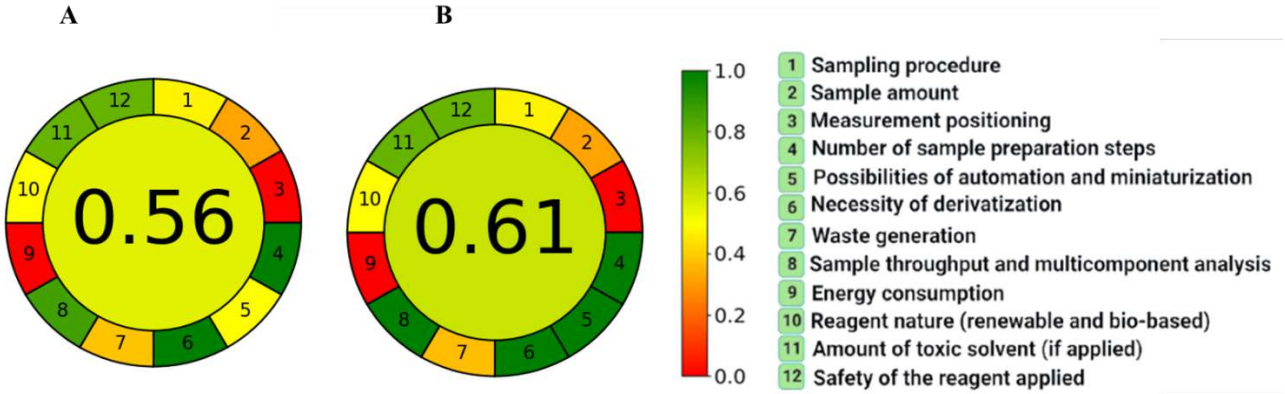

Figure 50. Indexes of greenness assessment for manual (A) and automated (B) approaches of DLLME based on phase transition of dispersive solvent (dichloromethane).

The proposed in section 4.3 DLLME of fluoroquinolones in DES with phase mixing by carbon dioxide formed *in situ* as a result of the chemical reaction of the sample's hydrocarbonate ions with one of the components of the extraction mixture was automated based on the principles of cyclic injection analysis. The scheme of the SWIA (Figure 51) included: a multi-way valve (Cole-Parmer, USA); a peristaltic reversible pump (MasterFlex L/S, Cole-Parmer, USA) with flow rate ranged from 0.5 to 6.0 mL/min; an extraction chamber – a cone-shaped polypropylene tube with a volume of 5 mL;

polytetrafluoroethylene commutating tubes (internal diameter 0.5 mm); a computer equipped with the LabVIEW 8.5 program.

The automated sample preparation scheme involved supplying portions (1.9 mL) of a 2 mol/L sodium carbonate solution (channel 1) and 3.1 mL of sample (channel 2) into the extraction chamber using the peristaltic pump and the valve, after that the mixture was mixed with bubbles of atmospheric air, which was fed through channel 3 for 20 s. Then, 390 μL of extraction mixture (heptanoic acid, menthol and formic acid, 1:2:48, mol./mol./mol.) was fed into the extraction chamber via channel 4, after which atmospheric air was again supplied for 5 min (the flow rate was 3.0 mL/min). In this case, an intensive release of carbon dioxide babbles was observed in the extraction chamber, played a role of dispersive solvent and facilitated further phase separation. After phase separation, the lower (water) phase was drained from the chamber using a peristaltic pump, and the extract phase was pumped into a vial for subsequent analysis. At the final stage, all system communications were washed with a mixture of methanol and distilled water (channel 6).

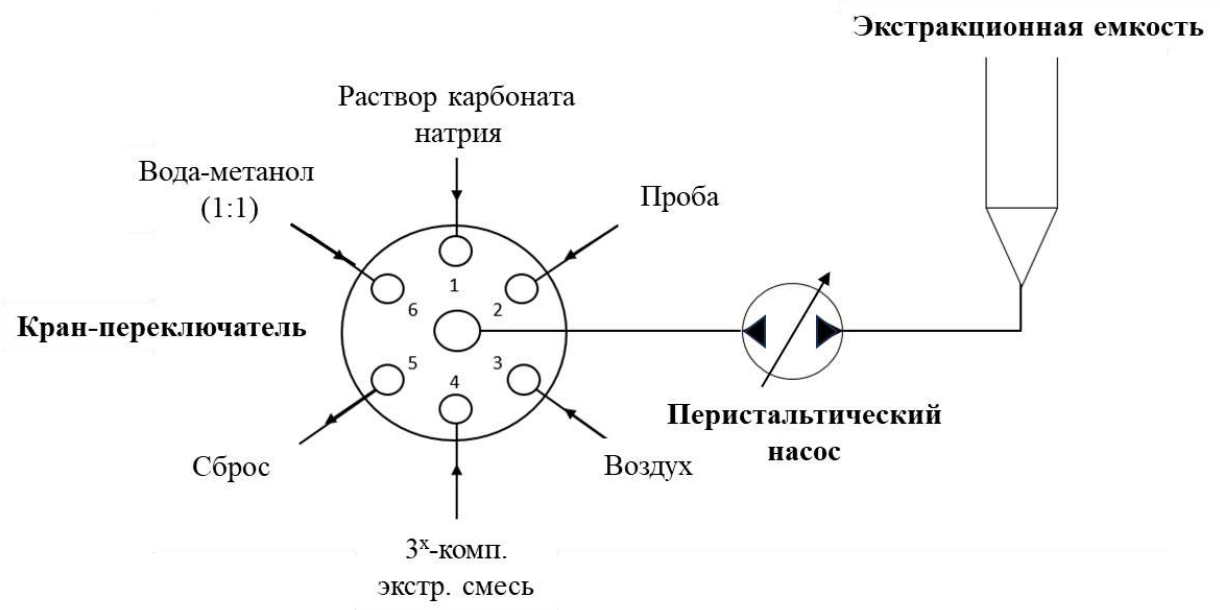

Figure 51. Scheme of automated DLLME in DES with phase mixing by carbon dioxide formed *in situ* for the analysis of water samples.

The technique provided the linear calibration ranges of 1 to 200 μg/L for ofloxacin, fleroxacin and norfloxacin at a sample volume of 5 mL. The LOD (3σ) for all analytes

were 0.3 μg/L. The RSD values did not exceed ± 20 % (P = 0.95). Sample preparation throughput was 10 samples per hour. Chromatographic analysis time – 20 min.

The capabilities of the automated microextraction technique were demonstrated in the HPLC-FLD determination of ofloxacin, fleroxacin and norfloxacin in water (Table 22). The accuracy of the results obtained was confirmed by the "add-found" method. The biases (%) did not exceed 20 %, which is acceptable for this concentration level according to [128].

Table 22. Results of determination of ofloxacin, fleroxacin and norfloxacin in water samples (n = 3, P = 0.95).

Water sample №	**Analyte**	**Added, μg/L**	**Found, μg/L**	**R, %**
1	Norfloxacin	0	<LOD	
		15	12.1 ± 1.2	19
		30	25.0 ± 1.5	17
		45	38.6 ± 1.9	16
	Fleroxacin	0	<LOD	
		15	13.5 ± 1.4	10
		30	24.4 ± 0.8	19
		45	38.7 ± 2.1	14
	Ofloxacin	0	<LOD	
		15	13.2 ± 0.9	12
		30	25.6 ± 1.2	15
		45	37.4 ± 0.9	17
2	Norfloxacin	0	<LOD	
		15	14.7 ± 0.8	2
		30	27.1 ± 1.0	10
		45	41.7 ± 1.5	7
	Fleroxacin	0	<LOD	
		15	13.7 ± 0.7	9
		30	28.6 ± 1.3	5
		45	40.5 ± 1.0	10

	Ofloxacin	0	<LOD	
		15	14.8 ± 0.9	1
		30	27.7 ± 0.6	8
		45	41.2 ± 1.4	8
3	Norfloxacin	0	<LOD	
		15	12.6 ± 0.7	16
		30	24.7 ± 1.2	18
		45	40.0 ± 1.4	11
	Fleroxacin	0	<LOD	
		15	13.4 ± 1.1	11
		30	26.0 ± 0.9	13
		45	39.7 ± 1.3	12
	Ofloxacin	0	<LOD	
		15	13.9 ± 0.6	7
		30	26.7 ± 1.0	11
		45	37.3 ± 0.6	17

The calculated indices of greenness assessment for the manual (section 4.3) and automated approaches of the DLLME were 0.50 and 0.63, respectively (Figure 52). It can be concluded that the automated method is more environmentally friendly, which is consistent with the principles of green analytical chemistry. The method made it possible to eliminate manual manipulations and reduce labor costs. The results of the work were published in the Journal of Analytical Chemistry [22].

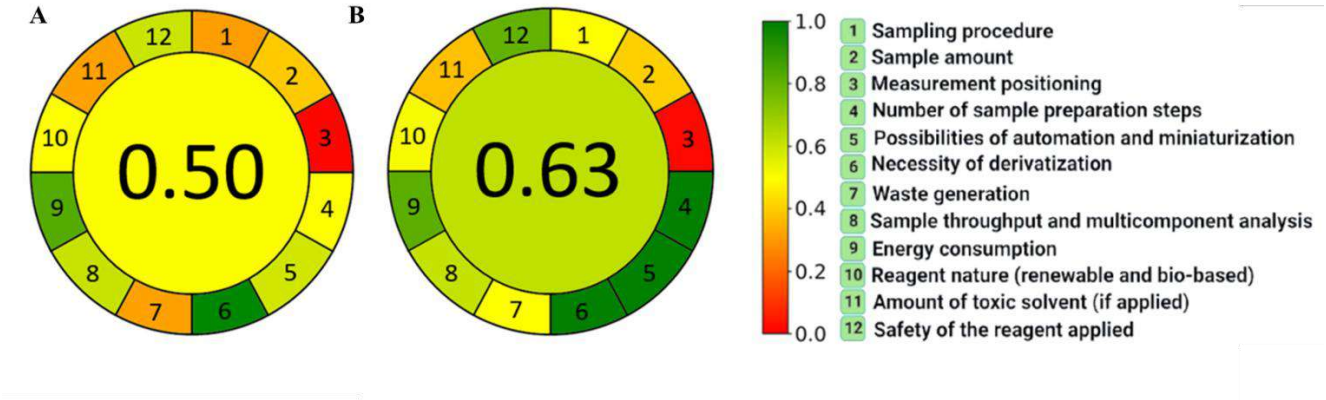

Figure 52. Indexes of greenness assessment for manual (A) and automated (B) approaches of DLLME with phase mixing by carbon dioxide formed *in situ*.

A fundamentally new approach in the analysis of solid-phase food products was implemented in an attempt to eliminate the shortcomings of conventional DLLME and to solve the issue of complete automation of the sample preparation procedure. As mentioned in the literature review, the analysis of solid-phase samples is a time-consuming process, often requiring prior sample treatment. In addition, the analysis of animal products is complicated by the presence of large amounts of proteins, fats and fibrous structure. Therefore, almost all techniques involve a centrifugation step, which makes difficulties for automation.

Taking into account all the above-mentioned features, a fully automated scheme for the analysis of solid-phase food samples was implemented, including extraction of polar analytes from the sample into the aqueous phase in the ultrasonic field followed by microextraction of analytes into the organic solvent phase. The possibility of combining the new method of sample preparation with the HPLC-FLD system was shown and illustrated on determination of ofloxacin in chicken meat.

The extraction of ofloxacin in different classes of organic solvents was preliminarily studied: alkanes (*n*-decane, *n*-dodecane, n-hexadecane), aryl halides (bromobenzene, chlorobenzene), esters (tributyl phosphate, dibutyl phthalate), aromatic hydrocarbons (o-xylene), fatty acids (oleic, nonanoic and hexanoic acids), alcohols (1-heptanol, 1-octanol) and organochlorine solvents (tetrachloroethylene, chloroform, dichloromethane). Based on the calculated distribution coefficients, dichloromethane was selected. In addition, the use of a volatile extractant allows to provide solvent replacement by evaporation of the organic solvent from the system, as well as increasing the concentration ratios by using a smaller volume of replacement solvent.

The possibility of using various carboxylic acids (capable of ionization when changing the acidity of the solution) as dispersive solvents was studied. It was shown that when introducing a mixture of various organic extraction solvents and carboxylic acids into an aqueous alkaline medium, neutralization of carboxylic acids and rapid and effective dispersion of the organic phase with subsequent separation of the phases without centrifugation occurs.

It was found that the use of medium-chain carboxylic acids for the implementation of the proposed DLLME approach is not possible, since no phase separation is observed (salts of medium-chain fatty acids exhibit the properties of anionic surfactants, in the presence of which stable emulsions are formed). Therefore, the possibility of using organic acids with a smaller length of carbon chain (acetic (pK_a = 4.76), methacrylic (pK_a = 4.66) and acrylic (pK_a = 4.23) acids) was studied. It was found that all the studied acids ensured dispersion of extraction solvents in an alkaline medium, however, the maximum values of analytical signals were obtained in the case of acrylic acid, which has a higher degree of dissociation. In addition, sodium acrylate formed during the extraction process additionally had a positive salting-out effect, which is absent in the case of using classical dispersive solvents (methanol and acetonitrile). The use of classical organic solvents led to a decrease in the analytical signal by an order of magnitude, which is explained by increase in the solubility of analytes in aqueous phase – the main disadvantage of conventional DLLME.

It is known that fluoroquinolones exist in different forms depending on pH. Thus, pH values less than 6.0 lead to a sharp decrease in extraction efficiency, since the analytes in this state exist in the protonated form, which preferentially remains in the aqueous phase [189]. Therefore, in this work, the pH values of aqueous phase after the addition of acrylic acid were studied and found to be in the range from 6.0 to 7.0.

Various ratios of the extraction and dispersive solvents were studied. It was found that the ratio of dichloromethane and acrylic acid equal to 1:2 (vol./vol.) ensures the highest extraction efficiency. The effect of the sample and extraction mixture ratio was also studied. Better extraction efficiency of the analyte was observed when using 600 μL of the extraction mixture (1:2, vol./vol.) and 1.4 mL of aqueous solution.

Among the wide variety of flow methods, stepwise injection analysis was chosen to implement the proposed approach, which provides the possibility to analyze solid-phase samples due to the inclusion of mixing chambers in the analysis scheme [6]. The presence of mixing chambers directly connected to the atmosphere opens up possibilities for the implementation of complex sample preparation, including analyte extraction from

a solid-phase sample, elimination of the interfering effect of proteins in sample matrices, extraction and pre-concentration of the analyte.

For the determination of ofloxacin in chicken meat samples, it was proposed to extract the analyte in special disposable cartridges (polypropylene syringe) equipped with PTFE filters to eliminate the interfering effect of proteins that coagulate in the presence of electrolytes in the aqueous phase. It was found that 1.5 mL of 2 mol/L NaOH solution containing 20 wt. % Na_2SO_4 ensures effective extraction of the target analyte from chicken meat samples without the formation of a colloidal solution.

It is known that extraction under ultrasonic field facilitates effective extraction of target analytes from solid-phase samples. The effect of US on the extraction process can be explained by the implosion of the bubbles generated by cavitation, which results in intense shock waves being produced in the immediate vicinity of the wave sources. In this case, intensive mixing of the sample contents occurs, which is the main advantage of ultrasonic exposure compared to other extraction methods. A significant benefit of the extraction in an ultrasonic field is the fast speed of the process, and therefore high productivity [136]. Thus, the source of ultrasonic waves (ultrasonic bath) was included in the automated scheme. The selected exposure time was 15 min. Additionally, the effect of temperature on the efficiency of US-assisted extraction of ofloxacin was studied. It was found that heating the system by 5 °C already leads to expansion of air in the channel, and as a consequence to a violation of the integrity of the filtration system. So, the process was carried out at room temperature.

To remove suspended particles from aqueous alkaline extract the special designed disposable cartridges (polytetrafluoroethylene (PTFE), 70 mm height and 15 mm i.d.) were used (Figure 51). The cartridge was filled with the PTFE filter (10 mm × 15 mm) and ash-free paper filters (pore size 2-3 μm). The PTFE fraction was prepared by sintering the powder (particle size 0.15 - 0.20 mm) at 385 °C for 5 hours. This filter system allowed to automate filtration and obtain a clear (transparent) filtrate.

Thus, the flow manifold consists of an eight-port selection valve, a syringe and peristaltic pump, the filter cartridge placed in an ultrasonic bath, a mixing coil (80 cm length), a mixing chamber-1 (MC-1) (glass tube of 13 mm i.d. and 50 mm length), and a

mixing chamber-2 (MC-2) (polypropylene vial of 9 mm i.d. and 40 mm length) placed in a thermostat and a computer equipped with LabVIEW 8.5 software. The scheme is presented in Figure 53.

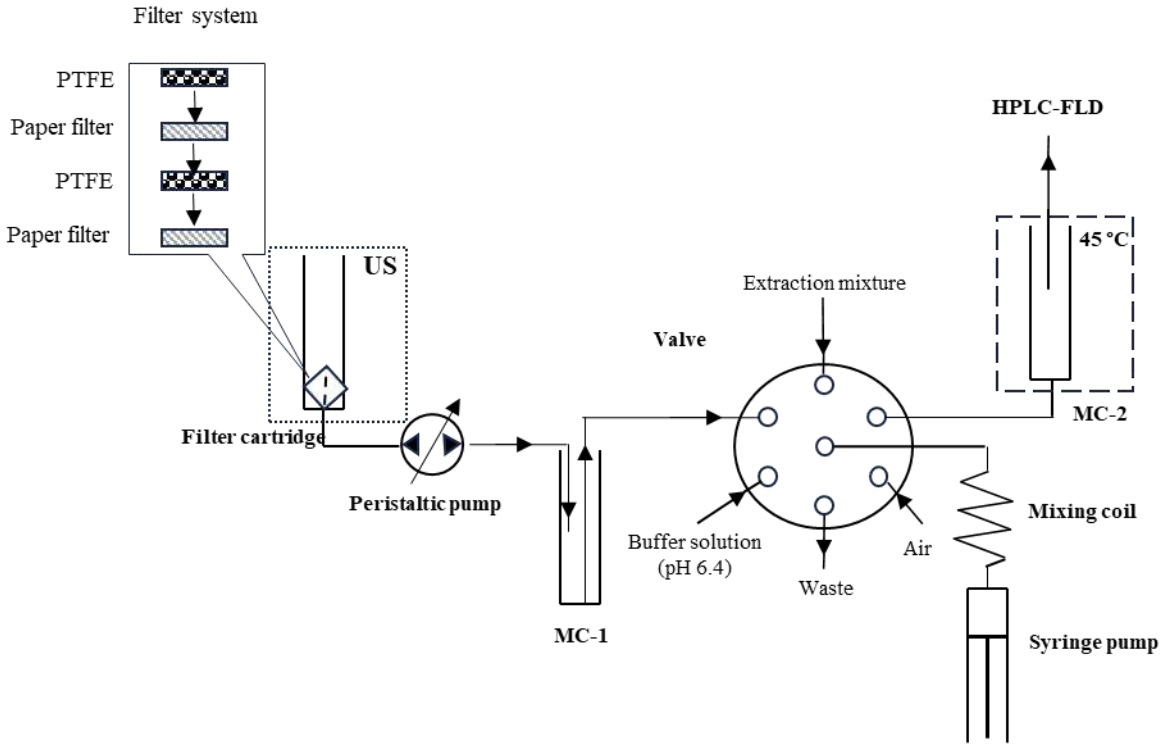

Figure 51. Automated sample preparation scheme including solid-liquid extraction of ofloxacin from a solid-phase sample, DLLME of the analyte from an aqueous solution, solvent exchange and subsequent HPLC-FLD analysis.

According to the developed scheme, at the first stage, 1 g of chicken meat sample and 1.5 mL of 20 % Na_2SO_4 (in 2.0 mol/L NaOH) were placed on the filter of the disposable cartridge. The ultrasound assisted solid-liquid extraction of ofloxacin was carried out during 15 min. Then the solution was passed through the filters and transferred into the MC-1 by the peristaltic pump. At the second stage, the syringe pump was set to the "Out" position and 600 μL of mixture of dichloromethane and acrylic acid (1:2, vol./vol.) was aspirated to the mixing coil by backward movement of the syringe pump plunger. The mixture was then transferred by forward movement of the syringe pump from the mixing coil into the MC-1 at the maximum speed value (7.5 mL/min). After that 1.5 mL of air was aspirated to the mixing coil by backward movement of the syringe pump plunger. Then air was transferred by forward movement of the syringe pump from

the mixing coil into the MC-1 for mixing. The formation of a cloudy solution was observed and sedimentation of organic phase was carried out during 1 min.

After that, 100 μL of 1 mol/L solution of phosphate buffer solution (pH = 6.4) was aspirated to the mixing coil by backward movement of the syringe pump and transferred in the thermostated MC-2 (45 °C) by forward movement of the syringe pump. Then the dropped organic phase (100 μL) was aspirated from the MC-1 to the mixing coil and then moved to the MC-2. After that 1.5 mL of air was aspirated into the MC-2 by the technique described above for mixing by bubbling. Solvent exchange was performed by the evaporation of dichloromethane (boiling point is 39.8 °C), at 45 °C. The aqueous solution obtained was analyzed by HPLC-FLD.

Taking into account the selected conditions, the linear calibration range of ofloxacin was 6×10^{-9} - 5×10^{-7} mol/L with the LOD equal to 2×10^{-9} mol/L. The full sample preparation cycle took about 30 min.

The capabilities of the automated technique are demonstrated by the determination of ofloxacin in chicken meat samples (Table 23). The accuracy of the results obtained is confirmed by the “add-found” method and the reference method [190].

Table 23. Results of determination of ofloxacin in chicken meat samples (n = 3, P = 0.95; F_{cr} = 19.00; t_{cr} = 2.78).

Sample №	Added, μg/kg	Found, μg/kg		t-test	F-test	R, %
		This method	Reference method [190]			
1	0	< LOD	< LOD	-	-	-
	5	4.5 ± 0.5	4.7 ± 0.5	1.46	2.01	10
	50	51.0 ± 1.0	49.5 ± 0.9	3.18	1.96	2
2	0	< LOD	< LOD	-	-	-
	5	4.6 ± 0.5	4.5 ± 0.4	2.33	0.98	8
	50	49.4 ± 1.1	50.9 ± 1.0	2.47	2.71	1

Advantages. The developed method is the first automated procedure for the determination of ofloxacin in solid-phase samples, eliminating the centrifugation stage by including the filtration in the flow mode. The use of disposable cartridges for sample filtration, as well as washing the flow system after each experiment, made it possible to eliminate cross-contamination of samples and ensured high reproducibility. The proposed DLLME approach allowed to eliminate the use of polar organic dispersive solvent, which increases the solubility of analytes in aqueous phase. However, the method has *limitations*: the proposed approach can be used to extract analytes only in alkaline and neutral media.

The results of the work were published in the journal Analytica Chimica Acta [7].

6.2. Automated homogeneous liquid microextraction

Sugaring-out homogeneous liquid microextraction was automated for the first time based on the principles of flow analysis, and the possibility of its direct combination with the HPLC-MS/MS system was demonstrated and substantiated in this work.

Sample preparation was carried out directly in the syringe pump. It involved several stages (Figure 54). At the first stage, using the valve, portions of sample (channel 1) and polar extraction solvent – acetonitrile (channel 2) were alternately withdrawn into the syringe pump, while observing the formation of a homogeneous solution. At the second stage, the same syringe pump was used to inject glucose solution (channel 3), and the system mixing was carried out due to the magnetic stirrer placed inside the syringe, thus, emulsion formation was observed. The extraction of analytes into the acetonitrile phase was occurred. After stopping the magnetic stirrer mechanism, spontaneous phase separation was observed for a few seconds and then, at the final stage, the extracted upper phase was injected into the HPLC-MS/MS system.

The capabilities of the developed automated technique were demonstrated on the HPLC-MS/MS determination of four pesticides frequently used in agriculture (malathion, diazinon, imidacloprid and bayleton) in juices.

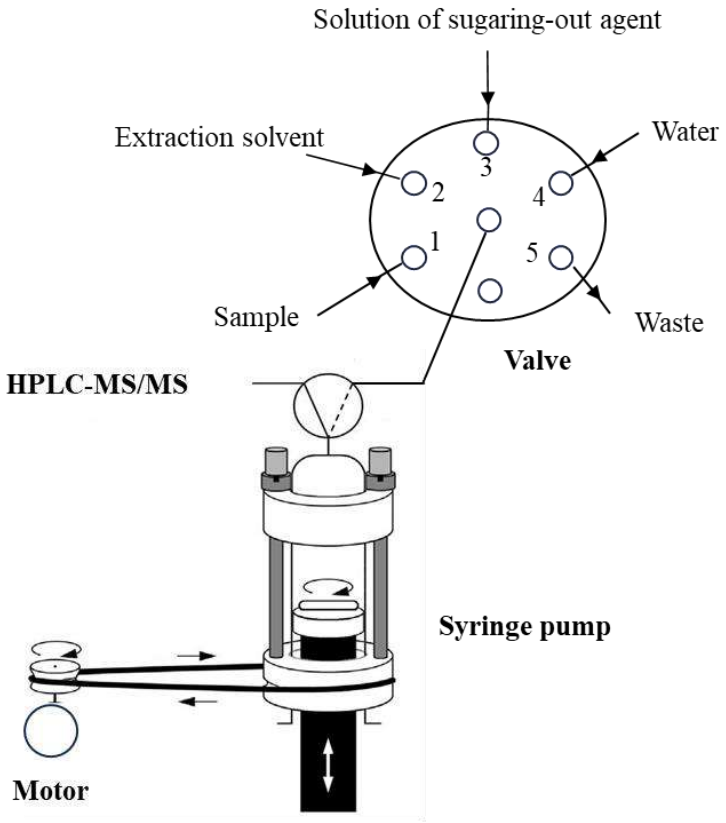

Figure 54. Scheme of in-syringe sugaring-out homogeneous liquid microextraction coupled with HPLC-MS/MS for the determination of pesticides.

Considering the features of the extraction process (low enrichment factors), an important criterion for the choice of extractant was its compatibility with the final determination method. From this point of view, acetonitrile used in the sugaring-out HLME is of interest for reversed-phase HPLC, which often uses this solvent as a component of the mobile phase.

During the studies, the distribution coefficients (at 25 °C) of the selected analytes in the aqueous phase-acetonitrile system were estimated to be 11.8 ± 0.9 for malathion; 68.8 ± 1.9 for diazinon; 7.2 ± 0.6 for imidacloprid and 8.9 ± 0.7 for triadimefon. Diazinon (O,O-diethyl-O-(2-isopropyl-6-methyl-pyrimidin-4-yl)thiophosphate) and malathion (O,O-Dimethyl-S-(1,2-dicarbethoxyethyl)dithiophosphate) are organophosphorus insecticides, imidacloprid (4,5-dihydro-N-nitro-1-[(6-chloro-3-pyridyl)-methyl]-imidazolidin-2-ylene-amine) is a neonicotinoid insecticide, triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-one) is a systemic triazole fungicide containing a 1,2,4-triazole fragment. Since the analytes differ greatly

in their polarity, as can be seen from the distribution coefficients, their general determination by HPLC-MS/MS in a reasonable time in the isocratic elution mode is difficult. Therefore, the gradient elution mode was chosen (section 2.5). It was found that the use of deionized water (mobile phase component A) and methanol (component B) containing 0.1 % (vol.) formic acid increases the intensity of pesticide ionization and, as a consequence, the sensitivity of the method compared to the use of acetonitrile (component B).

It was also found that the use of mono- (fructose, glucose) and disaccharides (sucrose) for phase separation is much more effective than the use of a salting-out agent (sodium sulfate), which led to a decrease in the analytical signal in the case of the selected analytes. Therefore, glucose was chosen as a sugaring-out agent. It should be noted that the salt content in the original fruit and berry juices is insignificant (the labeled salt content in the studied samples was less than 0.2 (w/vol.)), therefore, the effect of sample salts on the efficiency of pesticide extraction was not detected.

Complete phase separation of acetonitrile and the studied juices was observed at a phase ratio of 1:1 (vol./vol.) and a glucose concentration in the aqueous solution of more than 200 g/L. For a fully automated procedure, 0.6 mL of acetonitrile, 0.3 mL of the sample and 0.3 mL of 400 g/L glucose solution were selected. It was found that 1 min of phase mixing was sufficient to ensure effective extraction (extraction recovery > 90 %), while spontaneous phase separation was observed within 20 s.

The effect of temperature on the extraction efficiency of target analytes was studied in the range of 20 - 50 °C. Heating above 25 °C resulted in the formation of a stable emulsion requiring an additional centrifugation step, which complicates automation. In addition, heating the system resulted in a decrease in the extraction efficiency of pesticides, which can be explained by an increase in the solubility of target analytes in the aqueous phase.

An important parameter of the system is pH, which has a significant effect on the extraction of analytes with acidic/basic properties or sensitive to pH [191]. The maximum extraction rates of target analytes were obtained in the pH range of 3 - 7 (Figure 55); hydrolysis of organophosphorus pesticides (malathion and diazinon) was observed in

strongly acidic and strongly alkaline solutions [192,193]. It is known that triadimefon is stable in acidic and basic solutions [194], and imidacloprid is stable in acidic and neutral solutions, but its hydrolysis occurs in an alkaline solution upon heating [195]. Considering that the pH of real samples (juices) varied from 4 to 7, preliminary pH adjustment was not required before the analysis.

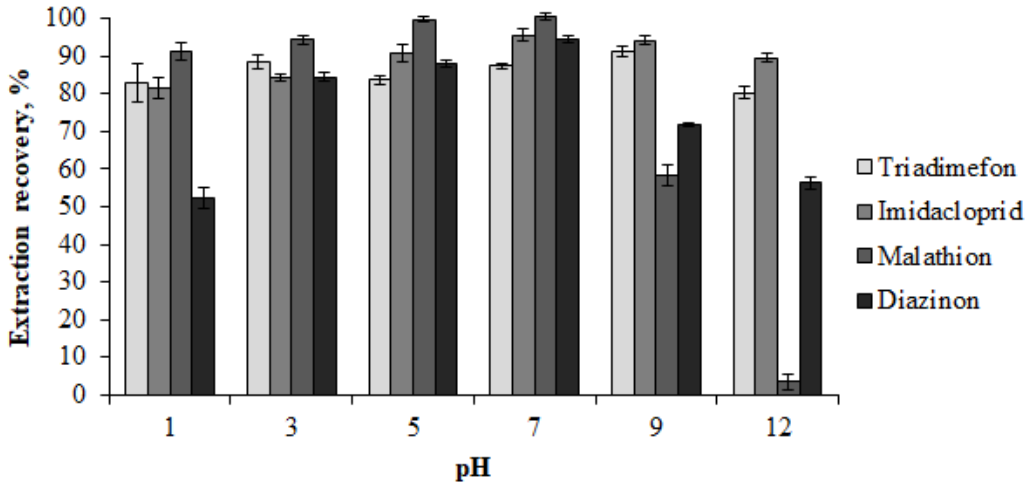

Figure 55. Effect of pH on the extraction recovery of target analytes ($C_{diazinon}$ = 1 µg/L, $C_{malathion}$ = 10 µg/L, $C_{triadimefon}$ = 10 µg/L, $C_{imidacloprid}$ = 50 µg/L, 21 °C, n = 3).

Under the selected parameters, the developed method provided the following linear calibration ranges: for malathion and triadimefon – 10^{-6} - 10^{-2} g/L (LOD = 3×10^{-7} g/L), for diazinon – 10^{-8} - 10^{-3} g/L (LOD = 3×10^{-9} g/L), for imidacloprid – 10^{-5} - 10^{-2} g/L (LOD = 3×10^{-6} g/L).

The proposed method was used to analyze samples of apple, cherry, raspberry, orange, and pineapple juices. All samples were pulp-free, and no pre-treatment or dilution of the samples was required. The accuracy of the results was confirmed by the "add-found" method and reference method (with GC-FID detection) [160]. As can be seen from Table 24, the analytical results obtained using the two methods are in good agreement with each other.

Table 24. Results of determination of malathion, diazinon, triadimefon and imidacloprid obtained using the developed and reference methods (n = 4, P = 0.95; F_{cr} = 9.28; t_{cr} = 3.18).

Sample	Analyte	Added, µg/L	Found, µg/L		t-test	F-test	R, %
			This method	Reference methos			
	malathion	0	< LOD	< LOD			-
		10	9.8 ± 0.3	9.87 ± 0.022	2.62	2.83	2
	diazinon	0	< LOD	< LOD			-
		10	10.0 ± 0.6	10.1 ± 0.8	1.22	1.41	0
	imidacloprid	0	< LOD	< LOD			-
		10	9.9 ± 0.5	10.03 ± 0.27	1.69	8.30	1
	triadimefon	0	< LOD	< LOD			-
		10	10.2 ± 0.5	10.4 ± 0.5	2.14	1.11	
Raspberry juice	malathion	0	< LOD	< LOD			-
		10	10.5 ± 0.5	9.9 ± 0.6	2.49	7.6	5
	diazinon	0	< LOD	< LOD			-
		10	9.82 ± 0.34	10.06 ± 0.31	2.33	1.5	2
	imidacloprid	0	< LOD	< LOD			-
		10	10.01 ± 0.24	9.7 ± 0.5	1.91	4.4	0
	triadimefon	0	< LOD	< LOD			-
		10	10.3 ± 0.5	10.2 ± 0.4	2.11	1.51	3
Cherry juice	malathion	0	< LOD	< LOD			-
		10	9.6 ± 0.6	9.9 ± 0.5	1.52	9.10	4
	diazinon	0	< LOD	< LOD			-
		10	10.01 ± 0.29	10.04 ± 0.3	1.42	2.91	0
	imidacloprid	0	< LOD	< LOD			-
		10	10.01 ± 0.03	9.6 ± 0.5	2.56	10.45	1
	triadimefon	0	< LOD	< LOD			-
		10	10.2 ± 0.5	10.1 ± 0.4	1.02	2.33	2
Apple juice	malathion	0	< LOD	< LOD			-
		10	9.82 ± 0.29	10.0 ± 0.3	3.02	1.14	2

	diazinon	0	< LOD	< LOD			-
		10	10.2 ± 0.5	10.6 ± 0.6	2.64	8.70	2
	imidacloprid	0	< LOD	< LOD			-
		10	10.2 ± 0.3	10.1 ± 0.4	1.47	10.15	2
	triadimefon	0	< LOD	< LOD			-
		10	9.7 ± 0.5	9.9 ± 0.4	1.34	1.65	3
Orange juice	malathion	0	< LOD	< LOD			-
		10	9.6 ± 0.4	9.9 ± 0.4	3.16	1.02	4
	diazinon	0	< LOD	< LOD			-
		10	10.0 ± 0.1	10.1 ± 0.4	1.11	6.39	0
	imidacloprid	0	< LOD	< LOD			-
		10	9.9 ± 0.5	9.6 ± 0.6	2.43	5.22	1
	triadimefon	0	< LOD	< LOD			-
		10	10.2 ± 0.3	10.4	2.17	4.82	2

The automated method can be considered as a universal tool for simple and express extraction of target analytes into the organic phase in order to eliminate the interfering effect of a complex sample matrix for subsequent analysis by liquid chromatography methods. The sugaring-out HLME was automated based on in-a-syringe concept (section 1.3) for the first time. Despite the listed *advantages* of the developed approach, it involves the use of relatively large volumes of acetonitrile as extraction solvent, which, despite its complementarity with the chromatographic system, is a toxic extractant. Nevertheless, due to the extraction being carried out in a closed flow system, the analyst's contact with the organic solvent is minimized.

The research results were published in the journal Talanta [8].

6.3. Automated micellar microextraction

One of the latest developments successfully implemented in the practice of using DLLME is the use of surfactants as extraction and dispersion solvents, which allows to eliminate the main drawback of DLLME: the use of toxic extraction solvents and a decrease in the extraction efficiency due to an increase in the solubility of analytes in the aqueous phase by classical dispersive solvents (acetonitrile, methanol). It is known that

both medium-chain carboxylic acids [196] and their salts [197] can act as anionic surfactants and form micellar phases. For example, the CMC of hexanoic acid is 0.1 mol/L [196], and that of sodium hexanoate is about 0.4 mol/L [198].

A new scheme for the micellar microextraction is proposed: at the preliminary stage, medium-chain fatty acid was mixed with a sodium hydroxide solution in a certain ratio and the formation of a concentrated emulsion was observed. When the obtained emulsion was added to an aqueous sample, microdroplets of excess acid were dispersed throughout the entire volume of the aqueous sample by the anionic surfactant (salt of fatty acid), followed by redistribution of the components of the extraction mixture and separation of the supramolecular solvent phase (Figure 56).

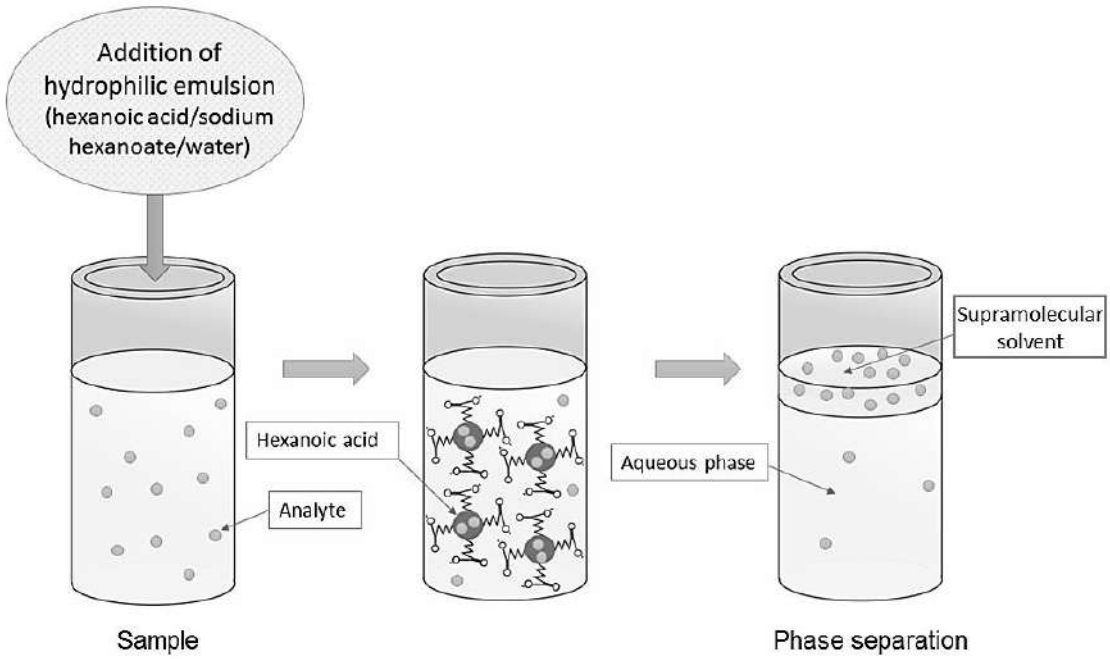

Figure 56. Scheme of DLLME using salt of medium-chain fatty acid as dispersive anionic surfactant.

The proposed approach of micellar microextraction was applied for the determination of thirteen PAHs in tea infusion. It is known that plant crops may contain PAHs if they were grown on soil contaminated with these substances as a result of industrial emissions, oil and gasoline spills, forest fires, or irrigation with water containing PAHs.

To ensure the efficiency of the extraction process, mixtures of carboxylic acids (2,2-dimethylpropanoic, hexanoic, heptanoic, octanoic, and nonanoic) and 1 mol/L sodium hydroxide in ratios from 1:2 to 1:10 (vol./vol.), respectively, were studied. It was found that the formation of the micellar phase is possible in the following systems: hexanoic acid and 1 mol/L NaOH in ratios from 1:2 to 1:4 (vol./vol.); heptanoic acid and 1 mol/L NaOH in ratios of 1:2 and 1:3 (vol./vol.); octanoic acid and 1 mol/L NaOH in a ratio of 1:2 (vol./vol.). Mixtures of heptanoic and octanoic acids and 1 mol/L NaOH in ratios of 1:3 and 1:2 (vol./vol.), respectively, were not considered in further studies due to their high viscosity, which made further automation of sample preparation impossible. As expected, no formation of micellar phase was observed using 2,2-dimethylpropanoic acid-based emulsions, since it does not have surfactant properties. Based on the studied enrichment factors of PAHs for the extraction systems based on hexanoic and heptanoic acids and 1 mol/L NaOH in ratios of 1:4 and 1:2 (vol./vol.), respectively (Figure 57), a conclusion was made about a higher extraction efficiency in the case of a mixture of hexanoic acid and 1 mol/L NaOH (1:4, vol./vol.).

The proposed version of micellar microextraction using medium-chain fatty acid and its salt was automated based on flow method (“in-a-syringe” analysis [116]). The automated system included a syringe pump, a 5 mL glass syringe, a valve, and a magnetic stirring device with a motor. According to the proposed scheme (Figure 58), a portion of the sample was fed into the syringe using a syringe pump and a multi-way valve, and then a portion of the hydrophilic emulsion was sent there through another channel. The solution was stirred using a magnetic stirrer and motor, after which the motor was stopped to achieve the phase separation with the extraction of the analytes into the micellar phase. A portion of the micellar phase released from the top was collected in an automated mode for subsequent HPLC-FLD analysis. At the final stage, the system communications were washed with acetonitrile.

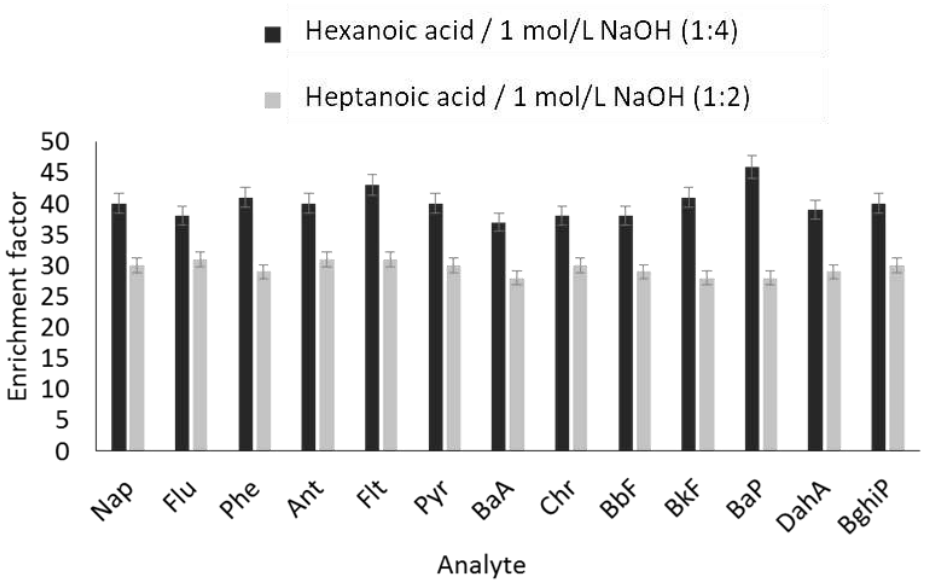

Figure 57. Enrichment factors of PAHs (BaA – benz[a]anthracene, Chr – chrysene, BbF – benzo[b]fluoranthene, BaP – benzo[a]pyrene, BkF – benzo(k)fluoranthene, DahA – dibenz[a,h]anthracene, BghiP – benzo[ghi]perylene, Nap – naphthalene, Flu – fluorene, Phe – phenanthrene, Ant – anthracene, Flt – fluoranthene, Pyr – pyrene) for the extraction systems based on hexanoic and heptanoic acids and 1 mol/L NaOH in ratios of 1:4 and 1:2 (vol./vol.) ($C_{analyte}$ = 5 μg/L, n = 3).

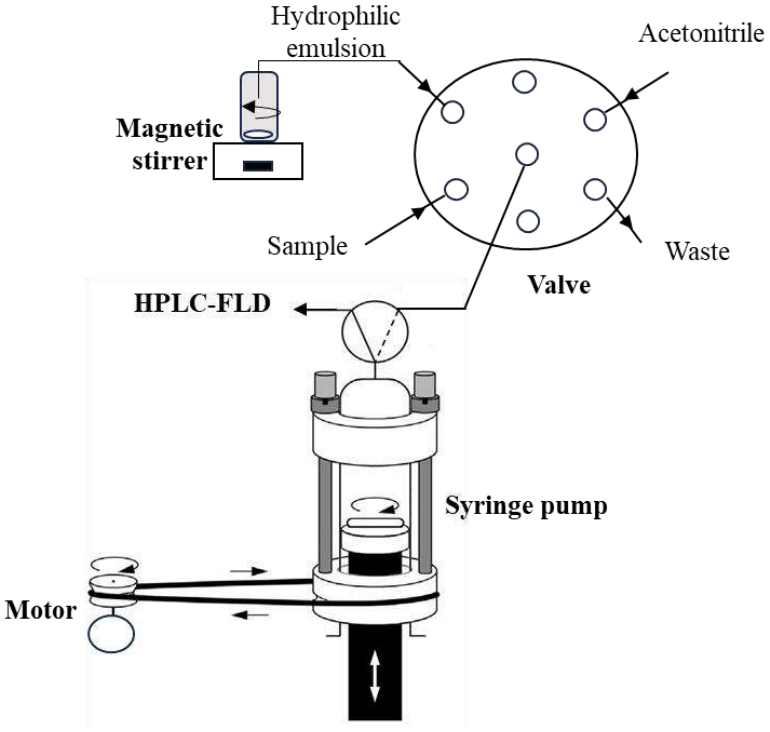

Figure 58. Automated scheme of the DLLME using salt of medium-chain fatty acid as dispersive anionic surfactant.

To ensure optimal conditions for performing new approach of micellar microextraction, various parameters were studied. The ratios of the components of the extraction mixture were studied first. Three ratios of hexanoic acid and 1 mol/L NaOH, which provided the separation of the micellar phase were studied: 1:2, 1:3, and 1:4 (vol./vol.). The highest analytical signal was achieved at the ratio of 1:4 (vol./vol.), so it was selected for further experiments. The effect of the sodium hydroxide solution concentration in the range from 0.25 to 2.0 mol/L on the extraction efficiency of the target analytes was also studied. It was found that the sodium hydroxide concentration of 1 mol/L provided the highest analytical signal among the others.

Next, the volumes of aqueous samples in the range from 3.0 to 3.8 mL (with a step of 0.1 mL) and volumes of extraction emulsion from 1.0 to 1.2 mL (with a step of 0.1 mL) were studied. The maximum analytical signal was achieved with a sample volume of 3.8 mL and an emulsion volume of 1.2 mL. It should be noted that a further increase in the sample volume led to 1) a significant decrease in the volume of the released micellar phase, which complicated the automation, as well as to 2) a decrease in the analytical signal and high RSD values.

It is known that the pH value of the sample solution can influence the stability of PAHs [199]. Therefore, the effect of sample pH on the analytical signal value was studied in the range from 3.0 to 9.0. It was found that at sample pH less than 7.0, the analytical signals decreased due to protonation of sodium hexanoate and formation of hexanoic acid. In this case, the volume of hexanoic acid in the micellar phase increased, which led to dilution. In alkaline solutions, ionization of hexanoic acid (pKa = 4.88) and a decrease in the analytical signal were observed. Thus, the optimal pH value of the sample solution was chosen to be 7, eliminating the need for special preliminary sample treatment, since all the studied tea infusion samples had a neutral pH.

Spontaneous dispersion of droplets at the moment of introducing the emulsion into the sample was observed immediately, however, the use of mechanical stirring resulted in an increase in the signal by approximately 2 times, while the stirring time was not important. This can be explained by the fact that stirring the mixture helped to form an even finer emulsion, and therefore, to increase the contact area of the phases. It should be

noted that the longer the stirring time, the longer the phase separation process: 10 s / 3 min - 60 s / 12 min, therefore, a stirring time of 10 s was chosen.

Higher efficiency of the proposed approach based on using sodium hexanoate as a dispersive agent in comparison with its conventional version, where polar organic solvents – acetonitrile and methanol – were used as dispersive solvents, was proven (Figure 59).

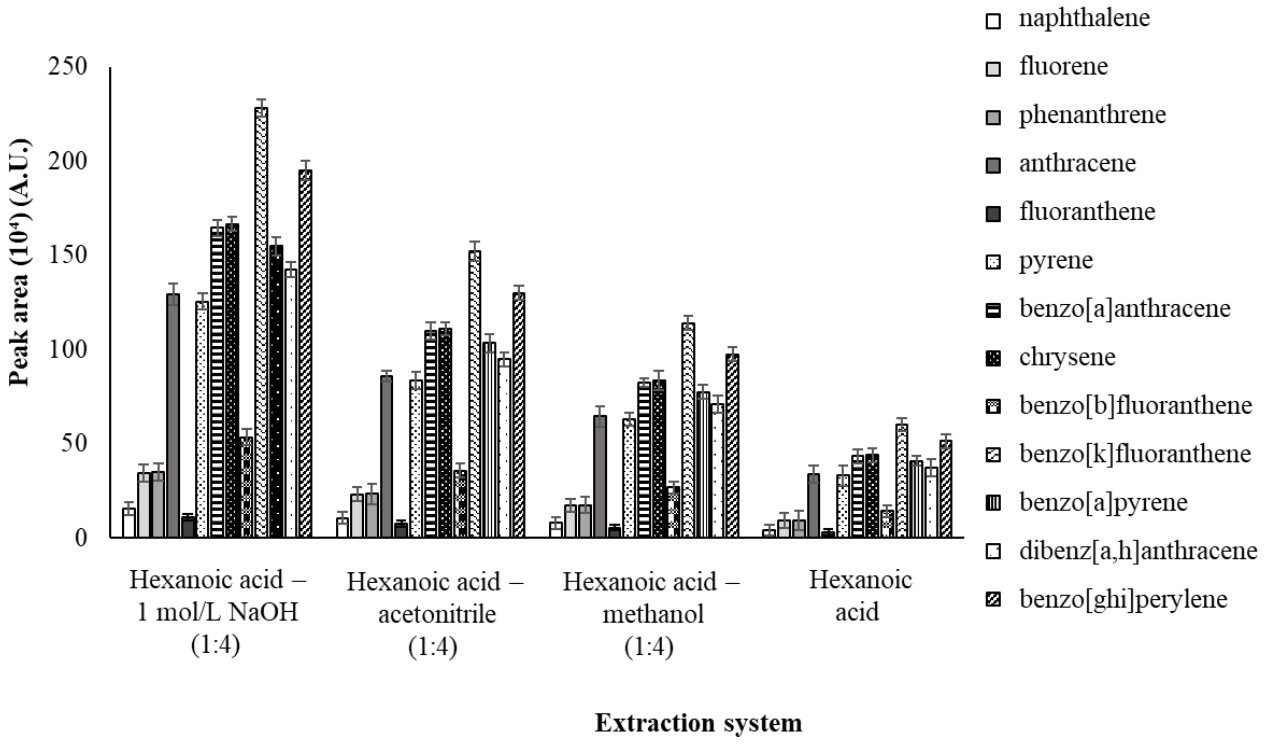

Figure 59. Comparison of the developed DLLME approach with the conventional DLLME and LLE ($C_{analyte}$ = 5 μg/L, n = 3).

Addition of the methanol-hexanoic acid extraction mixture (1.2 mL) to the aqueous sample (3.8 mL) resulted in the formation of a stable emulsion, which required centrifugation to separate the phases, making it impossible to automate the extraction process. Nevertheless, after centrifugation, the separated upper phase was analyzed, but the low peak resolution did not allow comparison of the obtained results with data from other systems. The use of acetonitrile as a dispersive solvent resulted in spontaneous formation of an organic phase during the extraction process, however, the analytical signals were significantly lower than those obtained using the developed micellar

approach. This can be explained by the fact that in the case of acetonitrile, a much larger volume of organic phase was formed during the extraction process under the same conditions than in the case of developed approach, and, therefore, a dilution was observed.

The composition of the micellar phase released during the extraction process was determined. The water content in the released micellar phase was determined using coulometric titration by the Karl Fischer method. It was found (n = 3) that the phase contained (12.0 ± 0.4) % water. The hexanoic acid content in the micellar phase was determined by acid-base titration using 0.1 mol/L NaOH solution and phenolphthalein indicator. It was found that the released phase consisted of (72.1 ± 1.0) % hexanoic acid. The sodium hexanoate content was estimated by the difference in the total mass of the micellar phase and the sum of the masses of hexanoic acid and water. Its percentage content was found to be (15.9 ± 0.7) %. Thus, the molar ratio of sodium hexanoate, water and hexanoic acid in the released micellar phase was determined to be 1:6:6, respectively. Cryo-SEM images got for the obtained micellar phase are presented in Figure 60. It can be seen the formation of supramolecular structures, which can presumably be McBain micelles (cylindrical or lamellar) [200] or, according to [197], vesicles. From reference data [196], it is known that the CMC of hexanoic acid is 0.1 mol/L, which is consistent with our experimental data.

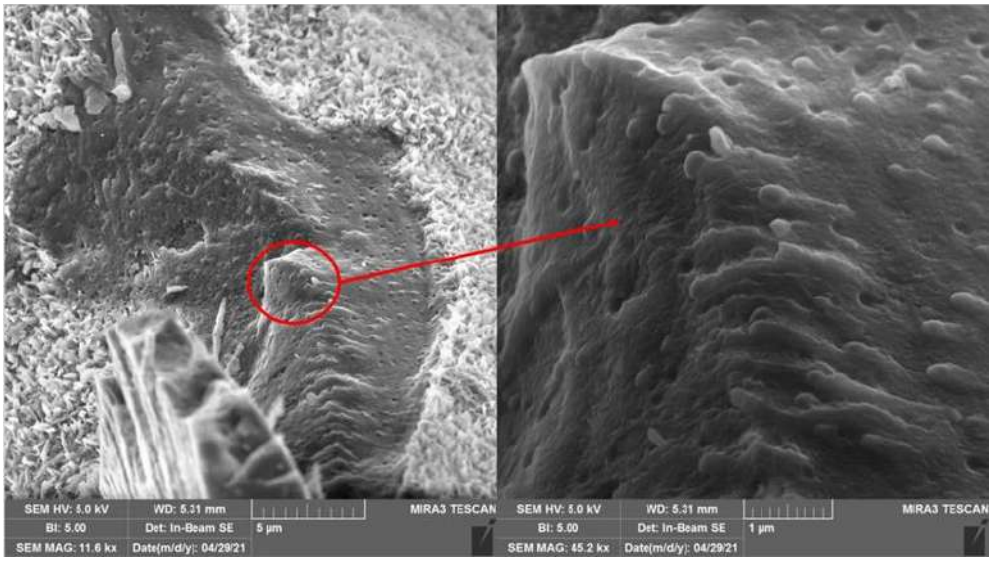

Figure 60. Cryo-SEM images for the obtained micellar phase.

An important feature of the developed procedure is the low viscosity of the released micellar phase. The measured value of the kinematic viscosity was (8.8 ± 0.3) mm^2/s at 20 °C, which opens up the opportunities for further analysis using the HPLC-FLD system without dilution, and therefore allows maintaining the sensitivity of the method.

The analytical characteristics of the developed approach combined with HPLC-FLD were established. The linear calibration ranges of 0.05–50.00 μg/L with correlation coefficients greater than 0.995 were obtained for 13 PAHs. The LOD values calculated as 3 σ were in the range of 0.02–0.04 μg/L. According to EU regulatory requirements for PAH determination, the sensitivity achieved in this work is sufficient for the determination of trace amounts of PAHs in food products [201]. The repeatability, characterized by the RSD values, did not exceed 9 %.

The developed automated procedure was applied for the determination of 13 PAHs (benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, benzo[k]fluoranthene, dibenz[a,h]anthracene, benzo[ghi]perylene, naphthalene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene) in four tea samples. According to literary data, they can be found in products of plant origin. The results were verified using the "add-found" method (Table 25). The specificity of 13 PAH determination in real sample analysis was estimated by comparing the retention time of each analyte in the real sample and the standard solution; the retention time difference did not exceed 2 %. The sample preparation time according to the developed scheme was 4 min. HPLC-FLD analysis time for the determination of 13 PAHs was 20 min.

Table 25. Results of PAHs determination tea infusion (n = 3, P = 0.95; F_{cr} = 19.00; t_{cr} = 2.78).

Sample	**Analyte**	**Found, μg/L**	**Added 0.5 μg/L**	**Added 5 μg/L**
			R, %	
Green tea	Naphthalene	<LOD	1	4
	Fluorene	<LOD	11	1
	Phenanthrene	<LOD	12	0
	Anthracene	<LOD	10	2

	Fluoranthene	<LOD	3	3
	Pyrene	<LOD	14	3
	Benz[a]anthracene	<LOD	11	7
	Chrysene	<LOD	1	4
	Benzo[b]fluoranthene	<LOD	1	1
	Benzo[k]fluoranthene	<LOD	12	6
	Benzo[a]pyrene	0.062 ± 0.011	5	3
	Dibenze[a,h]anthracene	<LOD	5	1
	Benzo[ghi]perylene	<LOD	0	8
Tea with jasmine	Naphthalene	<LOD	15	6
	Fluorene	<LOD	4	1
	Phenanthrene	<LOD	1	2
	Anthracene	<LOD	8	5
	Fluoranthene	<LOD	4	1
	Pyrene	<LOD	0	5
	Benz[a]anthracene	<LOD	1	10
	Chrysene	0.054 ± 0.009	5	1
	Benzo[b]fluoranthene	<LOD	15	1
	Benzo[k]fluoranthene	<LOD	4	2
	Benzo[a]pyrene	<LOD	3	5
	Dibenze[a,h]anthracene	<LOD	12	4
	Benzo[ghi]perylene	<LOD	9	3
White tea	Naphthalene	<LOD	7	1
	Fluorene	<LOD	2	11
	Phenanthrene	<LOD	13	0
	Anthracene	<LOD	3	7
	Fluoranthene	<LOD	2	11
	Pyrene	<LOD	12	8
	Benz[a]anthracene	<LOD	10	1
	Chrysene	<LOD	1	4
	Benzo[b]fluoranthene	<LOD	13	1
	Benzo[k]fluoranthene	<LOD	14	5

	Benzo[a]pyrene	<LOD	5	2
	Dibenze[a,h]anthracene	<LOD	1	3
	Benzo[ghi]perylene	<LOD	2	11
Black tea	Naphthalene	<LOD	8	1
	Fluorene	<LOD	14	7
	Phenanthrene	<LOD	15	2
	Anthracene	<LOD	6	9
	Fluoranthene	<LOD	12	0
	Pyrene	<LOD	9	4
	Benz[a]anthracene	<LOD	1	6
	Chrysene	<LOD	7	2
	Benzo[b]fluoranthene	<LOD	12	5
	Benzo[k]fluoranthene	<LOD	3	3
	Benzo[a]pyrene	<LOD	1	2
	Dibenze[a,h]anthracene	<LOD	1	5
	Benzo[ghi]perylene	<LOD	1	3

The developed automated techniques for the determination of PAHs in tea are presented in Table 26. The *advantages* of the proposed approach are its rapidity (sampling throughput is 15 samples/hour) and simplicity of the sample preparation procedure due to automation. Unlike the previously developed DLLME approach for the determination of PAHs in water [202], which involves extraction into trichloroethylene (a toxic narcotic substance), the proposed method is oriented toward green analytical chemistry.

Table 26. The techniques developed for the determination of PAHs in tea.

Detection method	**Sample preparation**	**Time of analysis, min**	**Analytes**	**LOD**	**Reference**
GC-MS	SPE	25	16 PAHs: Nap, Ace, BaA, Chr, BbF, BaP, BkF, IP, DahA, BghiP, Flu, Phe, Ant, Flt, Pyr, Ap	2×10^{-5} - 6×10^{-5} μg/L	[203]

HPLC-FLD	LLE (acetonitrile)	40	4 PAHs: BaA, Chr, BbF, BaP	0.10 - 0.25 µg/kg	[204]
GC-FID	LLE (dichloromethane), SPE	-	16 PAHs: Nap, Ace, BaA, Chr, BbF, BaP, BkF, IP, DahA, BghiP, Flu, Phe, Ant, Flt, Pyr, Ap	0.1 - 0.8 µg/kg	[205]
GC-MS/MS	QuEChERS (water:acetonitrile)	25	18 PAHs: Nap, Ace, BaA, Chr, BbF, BaP, BkF, IP, DahA, BghiP, Flu, Phe, Ant, Flt, Pyr, Ap, 1-methylnaphthalene, 2-methylnaphthalene	0.01 - 0.20 µg/L	[206]
HPLC-FLD	LLE (hexane), solvent exchange	90	18 PAHs: Nap, Ace, BaA, Chr, BbF, BaP, BkF, IP, DahA, BghiP, Flu, Phe, Ant, Flt, Pyr, Ap, 1-methylnaphthalene, 2-methylnaphthalene	0.01 - 0.21 µg/kg	[207]
HPLC-FLD	DLLME in-a-syringe (micellar phase)	4	13 PAHs: Nap, Phe, Ant, Pyr, Flu, Flt, BaA, Chr, BbF, BkF, BaP, BghiP, DahA	0.02 - 0.04 µg/L	This work
TGF – tetrahydrofuran; BaA – benzo[a]anthracene, Chr – chrysene, BbF – benzo[b]fluoranthene, BaP – benzo[a]pyrene, BkF – benzo[k]fluoranthene, DahA – dibenz[a,h]anthracene, BghiP – benzo[ghi]perylene, Nap – naphthalene, Flu – fluorene, Phe – phenanthrene, Ant – anthracene, Flt – fluoranthene, Pyr – pyrene.					

The results obtained were published in the journal Talanta [19].

CONCLUSION

1. New extraction systems and materials have been developed. Based on them a set of new approaches to microextraction of xenobiotics from food samples, providing the opportunity to increase the efficiency of interphase distribution of target analytes with a focus on the features of the chemical composition of analyzed samples and their aggregation state, has been proposed.

2. Methods of static and dynamic headspace microextraction on ferromagnetic nanoparticles for selective extraction and pre-concentration of volatile substances from liquid and solid-phase food samples have been developed; the adsorption ability of ferromagnetic nanoparticles towards volatile phenols and hydrogen selenide in static and dynamic headspace microextraction conditions, respectively, was studied.

3. A fundamentally new approach to membrane microextraction of organic substances (capable of ionization) from suspended samples based on the use of carboxylic acids as switchable hydrophilicity solvents has been proposed; the main regularities of mass transfer of fluoroquinolones from suspended samples to impregnated membranes have been established.

4. The method of microextraction of analytes capable of ionization (fluoroquinolones) into a deep eutectic solvent based on medium-chain fatty acid and terpenoid was realized; the possibility of dispersing the extraction solvent by carbon dioxide, which is formed *in-situ* during microextraction, was shown; the proposed approach provided the possibility of automation of dispersive liquid-liquid microextraction into deep eutectic solvent based on stepwise injection analysis.

5. The possibility of microextraction of polar organic analytes (fluoroquinolones) from solid-phase samples into deep eutectic solvent based on quaternary ammonium salt and alcohol formed *in-situ* during sample preparation was studied and substantiated; the approach provided an increase in the extraction efficiency of target analytes from the solid-phase matrices.

6. New extraction systems based on carboxylic acid and primary amine as well as carboxylic acid and its salt for micellar microextraction of polar and nonpolar organic analytes from liquid and solid phase samples have been proposed; it has been shown that the phases separated are compatible with liquid and gas chromatography systems.

7. The possibility of using natural terpenoid (menthol) as a green extraction solvent to perform dispersive liquid-liquid microextraction without dispersive solvent due to its phase transitions has been experimentally substantiated; the extraction efficiency of menthol towards benzoic and sorbic acids has been established.

8. The dispersive liquid-liquid microextraction based on phase transition of dispersive solvent (liquid – gas) has been developed; it has been experimentally confirmed that the developed method allows efficient separation and pre-concentration of organophosphorus pesticides from liquid food samples.

9. New automated schemes of micellar, homogeneous and dispersive liquid-liquid microextraction of xenobiotics, providing the possibility of increasing the precision and sample throughput of chemical food analysis, have been developed; as a consequence, new highly sensitive techniques based on the combination of automated microextraction and chromatographic analysis have been developed.

10. On the basis of the conclusions made and the results obtained, *14 original techniques for the determination of xenobiotics* (antibiotics, pesticides, preservatives, polycyclic aromatic hydrocarbons, phenols, Se (IV)) in various food samples (products of plant and animal origin, baby food, alcoholic and soft drinks, biologically active food additives) by chromatographic and spectral methods were developed.

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