Advances in Sample Preparation 6 (2023) 100061

Contents lists available at ScienceDirect

Advances in Sample Preparation

journal homepage: www.elsevier.com/locate/sampre

Development of thin film microextraction method for the multi-residue analysis of selected pesticides

Kübra Kahremanoğlu[a], Yeliz Akpınar[b], Ezel Boyaci[a,*]

[a] Department of Chemistry, Middle East Technical University, Ankara, Turkey, 06800
[b] Department of Chemistry, Kırşehir Ahi Evran University, Kırşehir, Turkey, 40100

ARTICLE INFO

Keywords:
Pesticides
Sample preparation
Thin film microextraction
GC-MS
Multi-residue analysis

ABSTRACT

Pesticides are a class of compounds that are used to protect agricultural products. However, the uncontrolled use of these chemicals increases the risks associated with their overdosing and rises a significant concern about their effect on human health and the ecosystem. Albeit many concerns about their effects, the use of pesticides is inevitable due to the incline in population growth and the presence of limited food resources. As a result, regulatory control of the use of chemicals is critical and the development of methods that provide a reliable determination of pesticide residues in fruits and vegetables is of great importance.

In the present study, a new method based on thin film microextraction was developed for the extraction of pesticides from fruit matrices prior to their gas chromatography-mass spectrometric (GC–MS) determination. As a first step, the thin film extractive devices were prepared by immobilization of hydrophilic-lipophilic balanced (HLB) particles on the surface of carbon mesh. The samplers were optimized in terms of extraction and desorption conditions first in PBS and then in apple juice. The final method was also validated in apple juice samples. Analytical figures of merits of the final method showed acceptable precision for intra- and inter-day reproducibility with ≤20% relative standard deviation (RSD%) and accuracy of ≤15% relative error (RE%), except for trifluralin at 300.0 ng mL^{-1} level. The limits of quantitation (LOQ) for the selected pesticides were found between 1.0 and 5.0 ng mL^{-1} . The samplers developed in this study were also successfully tested in preliminary investigations for the extraction of the pesticides from the surface of fruits and vegetables using agarose gel as a model matrix with the primary aim of showing the step towards the sampling directly on the field.

1. Introduction

Pests are any organisms that are harmful to plants. Insects, plants, rodents, bacteria, and fungi are some examples of pests, and they are controlled by using chemical agents and pesticides. There are different classifications of pesticides based on the target pest (e.g. herbicide, insecticide, fungicide, etc.), hazard (extremely, highly, moderately, slightly, etc.), and chemical nature (e.g. organochlorine, organophosphate, carbamate, etc.).

According to the guidelines published by the World Health Organization (WHO) for using chemical methods to control pests, 17% of global infectious diseases are caused by pests and vectors, which shows the importance of the use of pesticides for public health [1]. In addition, the Food and Agriculture Organization of the United Nations (FAO) reported that 20% to 40% of crops are lost yearly due to pests. Moreover, obtaining high-yield crops became crucial due to the decline in agricultural areas and the increased world population. United Nations (UN) estimated the population to reach 9.2 billion people from 8.0 billion within the next 30 years [2].

In 2019, it was reported that the annual use of pesticides had reached 2 million tons around the world, and it is envisaged to increase further [3]. According to the report of the European Union (EU) published in 2022, the global use of pesticides had reached 4 million tons. Also, it was found that 3.9% of the analyzed samples exceeded the maximum residue level (MRL), which is the highest tolerable concentration of pesticides in/on the crops. Further, 27% of the analyzed samples were contaminated by two or more pesticides [4]. Moreover, another report by EU declared that even in organic crops, organic or synthetic pesticides are found as a result of their use or as cross-contamination from neighborhood fields [1].

* Corresponding author.
E-mail address: ezel@metu.edu.tr (E. Boyaci).

https://doi.org/10.1016/j.sampre.2023.100061
Received 15 January 2023; Received in revised form 15 March 2023; Accepted 19 March 2023

The uncontrolled use of pesticides causes severe problems for the ecosystem and human health. Environmental and medical authorities draw attention to the uncontrolled use of pesticides and their detrimental effects on people in direct or indirect exposure ways. Birth and fetal diseases, asthma, lymphoma, Parkinson's disease, and prostate cancer are some examples of pesticide-induced diseases published by Beyond Pesticides, which is a nonprofit organization to prevent the negative effects of pesticides on public and environmental health [5].

Under the current circumstances, the use of pesticides to produce sufficient and high-quality crops cannot be avoided; however, some specific regulations for their use are accepted by the EU and European Food Safety Authority (EFSA). For instance, for each pesticide and crop, an MRL is specified by the European Union [6]. Yet, the farmers may mix different pesticides in lower concentrations as a pesticide blend to not exceed the MRL or to obtain a stronger effect for controlling the pests. Such mixtures may result even in a higher risk to public health as it is more difficult to perform a realistic risk assessment.

Due to the significant number of new studies evidencing the adverse effect of pesticides, there is a need for more sensitive and reliable methods that are suitable to determine these agrochemicals in a multi-residue approach. Several sample preparation methods using classical extraction techniques (liquid-liquid extraction, LLE), and Soxhlet extraction are accepted by Environmental Protection Agency (EPA) for the pesticides. However, these methods are developed long ago, and usually, they do not fulfill the current analytical methods requirements for being green. For instance, in Soxhlet extraction, several hundreds of milliliters of solvents are required with overnight refluxing of the solvent for the extraction of pesticides from solid samples, which makes the method solvent- and time-consuming as well as labor-intensive [7–10]. Another sample preparation technique that is commonly used in pesticide determination is solid-phase extraction (SPE). The main drawback of this approach is requirement of extra sample preparation steps such as precipitation and or filtration prior to SPE to remove macromolecules and other particles from the matrix to avoid the clogging of the SPE cartridge during the extraction. Moreover, the breakthrough volume of SPE may vary from analyte to analyte which limits the sample volume (so the sensitivity of more retained analytes) to the breakthrough volume of the less retained analyte. Additionally, the sorbents used in SPE are single use, which makes them economically unfavorable. An alternative and prevalent method obtained by the combination of LLE and SPE is QuEChERS which stands for quick, easy, cheap, effective, rugged, and safe method. This approach has gained significant attention as addresses some of the limitations stated above. However, in this method, even more sample preparation steps are required, and each step is prone to introduce errors to the analytical method. Still, this technique is labor-intensive, and relatively large volumes of solvents are consumed. Solid phase microextraction (SPME), as a widely used technique for sampling and sample preparation, provides a platform that can address the need for reliable determination of multi-residue in complex matrices with minimal solvent use. Moreover, its thin film microextraction (TFME) geometry enhances the sensitivity of analysis by the use of a larger volume of extractive phase. Because the larger volume of the extractive phase is spread as a thin film on a relatively large surface, TFME improves the sensitivity of the method without scarifying the sampling time. Due to the many advantages of SPME over classical extraction techniques, there are various methods developed for pesticides involving SPME as a sample preparation approach in diverse matrices such as water [11–15], different vegetables and fruits [16–22], and soil [15,23,24]. The most commonly used extractive phases in these studies are polypropylene (PP), polyacrylate (PA), poly(dimethylsiloxane)–divinylbenzene (PDMS/DVB), PDMS, and molecularly imprinted polymers (MIPs). Recently, covalent organic framework (COF) and carbon nanomaterial-based coatings have gained popularity as well. Among various extractive phases, when multiresidue analysis is essential, hydrophilic-lipophilic balanced (HLB) particles which enable the extraction of a wide range of analytes with different physicochemical properties become imperative. Moreover, when HLB particles were immobilized in polytetrafluoroethylene amorphous fluoropolymer (PTFE-AF) in a fiber geometry, the resulting sampler became suitable for direct thermal desorption in GC and solvent desorption for LC analyses [25]. Although this coating has significant advantages, it has not been tested yet in TFME format for sensitive determination of pesticide.

In this study, HLB/PTFE AF coated TFME devices were prepared, and an analytical method was optimized for the extraction of pesticides from apple juice followed by gas chromatography-mass spectrometric (GC–MS) analysis. Based on the limit of quantitation (LOQ) of the developed TFME-GC–MS method (between 1.0 and 5.0 ng mL^{-1}), it can be concluded that the final method is capable to monitor the pesticides at an order of magnitude lower concentration levels than their maximum permissible levels.

2. Material and methods

2.1. Chemicals and apparatus

Analyte standards, trifluralin, methyl-parathion, carbaryl, chlorpyrifos-methyl, malathion, and diazinon were purchased from Sigma-Aldrich (Germany). LC-grade methanol was obtained from Merck (Germany). A stock solution of each pesticide (1.0 mg mL^{-1}) was prepared in methanol and stored at 4 °C in the fridge. Working and calibration solutions were prepared before each analysis freshly from the stock solution of each pesticide. pH 7.4 phosphate-buffered saline (PBS) salts; including KCl, NaCl, and KH_2PO_4, were purchased from Isolab (Germany), while Na_2HPO_4 was purchased from Sigma-Aldrich (Germany). Two different brands of apple juices were obtained from a local market, apple drink (which contains at least 10% apple juice) and 100% apple juice. PTFE AF 2400 used as a polymeric binder during the preparation of TFME devices and it was obtained from Sigma-Aldrich (Germany). For the dissolution of PFTE AF 2400, perfluorohexane (FC-72) was used and purchased from ABCR (Germany).

Ultra-pure water (18.2 MΩ.cm at 25 °C) was obtained from a Milli-Q water purification system from Millipore (USA). Samples were agitated in a mechanical shaker CAT AEK-SH10 PAS Technology Deutschland GmbH. The pH of the buffers was measured using HANNA HI 2002 Edge pH meter. Conductivity of solutions were measured using AZ8361 conductivity meter. Apple juice samples were centrifugated using Nuve NF 200 bench-top centrifuge. Isolab blue band filter with 110 mm diameter and 2.5 μm pore size was used for sample filtration.

2.2. GC–MS analysis

The separation and quantitation of pesticides were performed in Agilent 6890A gas chromatography system equipped with a 5973 quadrupole mass selective detector (Agilent Technologies, USA). The fragments of analytes were obtained using an electron impact (EI) ion source with 70 eV. For the separation of six pesticides, an ultra-inert (5%-phenyl)-methylpolysiloxane (HP-5MS) column with 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness was used (Agilent Technologies, USA). Helium was used as a carrier gas at a 1.2 mL/min flow rate. The injection volume was 1.0 μL, while during the injection injector port was kept at 250 °C (with a split ratio of 1:1). The temperature gradient used during the separation was as follows. For the first 5 min the column was kept at 60 °C and then heated up to 200 °C at 80 °C/min rate and kept at 200 °C for 2 min. Then, with 20 °C/min rate it was increased to 220 °C and kept for 1 min at this temperature. Finally, with 20 °C/min rate was increased to 240 °C and kept for 1 min. The total analysis time was 10.75 min. A selected ion monitoring (SIM) method was used for quantifications with m/z of 93 (173), 109 (155), 137 (179), 144 (115), 286 (288), and 306 (355) for malathion, methyl-parathion, diazinon, carbaryl, chlorpyrifos-methyl, and trifluralin, respectively. The qualification ions used in the method are given in the

parenthesis. The physicochemical properties of the selected pesticides are summarized in Table S1 (Supporting Information Section)

2.3. Preparation of TFME devices

For the preparation of TFME devices first, a coating slurry consisting of homemade HLB particles in PTFE AF 2400 solution was prepared as described by Gionfriddo et al. [25]. The slurry was spread on the surface of carbon mesh as a film using a thin film applicator with a gap of 30 μm. The resulting extractive phase was measured using a digital caliper from different points of the thin film and was found as 28 ± 2 μm. The resulting material was dried at 80 °C in an oven overnight and then cut with bistoury to have TFME samplers with 1.5 cm length and 0.5 cm width. Another set of TFME samplers was prepared with 0.5 cm x 0.5 cm dimensions.

2.4. Optimization of TFME procedure

The TFME protocol consisted of several steps including preconditioning of the TFME device, rinsing, extraction, second rinsing, and desorption. In a typical study, as a first step, the TFME samplers were preconditioned in methanol. As a second step, the excess methanol present on the surface of the sampler was removed by dipping it into distilled water for 3 s. Then, the residual water on the TFME device was removed gently with a paper towel. As a next step extraction was performed. Following the extraction, the TFME devices were washed quickly with water to remove any matrix components and dried gently with a paper towel. As the final step, desorption was performed. All experiments were conducted at 20 °C and the agitation rate for the extraction and desorption was set to 1000 rpm. Experimental details used in each investigated extraction parameter are given below.

To ensure the complete desorption of extracted pesticides from TFME films, desorption time was investigated as the first parameter. For this purpose, PBS (pH 7.4) buffer was spiked with a pesticide mixture and extractions were performed from this matrix using 0.5 cm x 0.5 cm TFME samplers. The experimental parameters used during the extraction were as follows; sample volume: 4.0 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 60 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.5 mL, desorption time: 5, 15, 30, 60, and 120 min.

Following the desorption studies, the extraction time profile of each analyte was investigated. For this purpose, PBS buffer was spiked with a pesticide mixture, and extractions were performed using 0.5 cm x 0.5 cm TFME samplers. The experimental parameters used during the extraction were as follows; sample volume: 4.0 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 5, 15, 30, 60, 120 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.5 mL, desorption time: 60 min.

The effect of sample pH on extracted amount of pesticides was also investigated. To show the effect of pH, phosphate buffer solutions with pH of 3.0, 5.0, 7.0, 10.0, and 12.0 were prepared and spiked with pesticides and then equilibrated for 1 hour. Extractions were performed using 0.5 cm x 0.5 cm TFME samplers. The experimental parameters used during the extraction were as follows; sample volume: 4.0 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 60 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.5 mL, desorption time: 60 min.

2.5. Evaluations in real samples

The effect of salt addition on extraction performance of the TFME devices was studied in detail in the real samples as the real matrix is more complex (high dissolved solid, ions, presence of binding components, etc.) compared to water. For this purpose, apple juices with different juice contents (commercial 10% apple juice, commercial 100% apple juice, and commercial 100% apple juice diluted with water (50/50) (v/v)) were used.

Initially, the effect of salt was investigated with a commercial apple drink for which the label indicates that 10% apple juice is present in it. For this experiment, the apple drink was spiked with pesticides and samples were equilibrated for 1 hour. Then, NaCl was added to have 0%, 5%, 10%, and 20% NaCl in final solutions. TFME samplers were preconditioned and washed as described in Section 2.4. Extractions were performed using 1.5 cm x 0.5 cm TFME samplers. The experimental parameters used during the extraction were as follows; sample volume: 1.5 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 60 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.0 mL, desorption time: 60 min.

In a separate experiment, the salt effect was investigated using 100% apple juice as a sample matrix. For this purpose, the apple juice was spiked with pesticides and samples were equilibrated for 3 h. Following the equilibration, NaCl was added to the samples to have 0%, 5%, 10%, and 20% NaCl in final solutions. Extractions were performed using 1.5 cm x 0.5 cm TFME samplers. TFME samplers were preconditioned and washed as described in Section 2.4. The experimental parameters used during the extraction were as follows; sample volume: 40.0 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 60 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 0.6 mL, desorption time: 60 min.

Also, the effect of dilution with water in the presence of salt was investigated. Extractions were performed using 1.5 cm x 0.5 cm TFME samplers. For this experiment, first, 100% apple juice was spiked with pesticides to contain 250.0 ng mL^{-1} of each pesticide in the final samples, and samples were equilibrated for 3 h. Then samples were diluted with water in half. Finally, NaCl was added to diluted samples to have 0%, 5%, 10%, and 20% NaCl solutions in the final samples. The extraction conditions described for 100% apple juice was used for the evaluations.

Finally, the effect of sample centrifugation and filtration on extracted amounts of pesticides from apple juice was investigated. For this experiment, pesticides were spiked to the apple juice before or after the centrifugation and filtration steps. Experimental details and schematic representation of the performed experiment (Figure S1) are provided in Supplementary Information Section.

2.6. Extraction from agarose gel as solid matrix representative

This study was performed as a preliminary investigation to show the possibility of using the samplers for on-site sampling directly from the surface of the fruit and vegetables.

2.6.1. Investigation of extraction time profile

To mimic solid samples, agarose gel was prepared and spiked to contain the selected pesticides at 250.0 ng mL^{-1} final concentration. For this purpose, first 2% agarose (w/v) solution was prepared by bringing to boil. Then the solution was cooled to approximately 60 ^{0}C and the selected pesticide were added to the solution. Before solidification of the solution to a gel, 50.0 mL of mixture was poured in separate Petry dishes and cooled to room temperature. Before the extraction, TFME samplers (1.5 cm x 0.5 cm) were preconditioned and washed as described in Section 2.4. The experimental parameters used during the extraction were as follows; sample volume: 50.0 mL, analyte concentration: 250.0 ng mL^{-1}, extraction time: 5, 15, 30, 60 min, agitation: static. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.5 mL, desorption time: 60 min. Desorption solvents were kept at −20 °C untill their analysis in GC–MS.

2.6.2. Pesticide distribution analysis

For this purpose, firstly the pesticide mixture was spiked to 2% agarose solution as described above to have gels with 0.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, and 750.0 ng mL^{-1} analyte concentrations. In a 96-well plate, 2.0 mL of each gel was randomly placed in the different wells and a pesticide distribution map was generated. These gels were used for extractions with the samplers to show the spatial resolution ability of the TFME sampler on the solid surface. For this study, TFME samplers with 0.5 cm x 0.5 cm dimensions were used. The experimental parameters utilized during the extraction were as follows; extraction time: 60 min, agitation: static, extraction temperature: 20 ˚C. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 0.6 mL, desorption time: 60 min. Desorption solvents were kept at −20 ˚C untill their analysis.

2.7. Validation of analytical method

The developed analytical method was validated to show its reliability, reproducibility, and sensitivity using apple juice as a sample. During validation, linear dynamic range (LDR), the limit of quantitation (LOQ), accuracy, and reproducibility (intra- and inter-day precision), were determined using matrix-matched external standard calibration.

For the determination of LDR, 100% apple juice (with pulp) was spiked with pesticides to have 0.1, 0.25, 0.50. 1.0, 5.0, 10.0, 50.0, 100.0, 250.0, and 500.0 ng mL^{-1} of each pesticide in the final samples and equilibrated for 3 h. Each sample was diluted with water in half. Before extraction, the required amount of NaCl was added to the samples to have 10% NaCl (w/v) in the final samples. Prior to the extraction, TFME samplers (0.5 cm x 0.5 cm) were preconditioned and washed as described in Section 2.4. The experimental parameters used during the extraction were as follows; sample volume: 40.0 mL (50/50 diluted sample), extraction time: 60 min. The experimental parameters used during the desorption were as follows; desorption solvent: methanol, desorption volume: 1.5 mL, desorption time: 60 min. Desorption solvents were kept at −20 ˚C untill their analysis.

The LOQ of each pesticide was calculated from the back calculation of nominal concentration using the linear regression equation of matrix matched TFME calibration. In these studies, the LOQ was defined as the lowest concentration that provides maximum of 20% relative error in back calculations.

To show the accuracy of the developed TFME-GC–MS method, three different concentrations of pesticides representing quality control (QC) points over LDR of the matrix matched calibration were spiked (blind to analyst) to apple juice. The spike levels were 5.0 ng mL^{-1}, 30.0 ng mL^{-1}, and 300.0 ng mL^{-1} as low, medium and high concentrations, respectively. Following the extraction/desorption conditions described above the unknown concentrations of pesticides were determined by matrix-matched external standard calibration and then their relative errors (RE%) were calculated.

For intra-day reproducibility, three different sets of extractions were performed three times a day while for inter-day reproducibility, three different sets of extractions were performed on three consecutive days. Each experiment was performed in triplicate. The same QC levels described for method's accuracy were used in repeatability studies. Following the extraction/desorption conditions described above the unknown concentrations of pesticides were determined by matrix-matched external standard calibration and then the relative standard deviations (RSD%) were calculated.

3. Results and discussion

3.1. Optimization of SPME parameters

3.1.1. Desorption time profiles

Because complete desorption of analytes is required for the quantitative analysis with high sensitivity, as a first experiment, the desorption time profile was investigated. The results of this experiment are shown in Supplementary Information Section in Figure S3. As can be seen from the figure, methyl-parathion, trifluralin, carbaryl, malathion, and diazinon were completely desorbed within 15 min, but the desorption of chlorpyrifos-methyl required 30 min. A second desorption was also performed under the same desorption conditions with the same TFME samplers to investigate if there is a carryover of analytes on the sampler or not. No analytes were found in the second desorption, indicating that 30 min of desorption time using methanol as a desorption solvent result in quantitative desorption conditions and can be used for further experiment.

3.1.2. Extraction time profile

The extraction time profiles of the pesticides were investigated to find the shortest time that provides the best sensitivity for the final method. The Student's *t*-test at 95% confidence level (CL) was used for the comparison of two means to decide if there is a significant difference on extracted amounts between two extraction time points. The extraction results are depicted in Fig. 1. As can be seen from the figure, there is significant increase in extracted amounts of all pesticides when 5 min sampling was compared to 15 min sampling. Moreover, under studied conditions all the analytes except methyl-parathion reached equilibrium extraction in 30 min. On the other hand, the extracted amount of methyl-parathion at 120 min was still significantly different than 60 min. For further studies 60 min was selected as extraction time as provides reasonable balance for method sensitivity versus sampling time.

3.1.3. Effect of sample pH on the extraction

As stated in several studies [13,17,26], pesticides are more stable in weakly acidic media while degrade in alkali media. Therefore, it is critical to study the sample where the analytes of interest are stable and represent the real state of the system under investigation. Besides, one of the ways to enhance the method's sensitivity is to find extraction conditions where the extraction is maximum. Moreover, it is critical to know if the sample pH has a significant effect on the sorption amount as external TFME calibration is applied in a different matrix than the sample itself, albeit of matching the matrix. For these reasons, the effect of the sample pH on extracted amount of analytes was evaluated using pH 3.0, 5.0, 7.0, 10.0 and 12.0 buffers as sample matrices. The results obtained from this study are shown in Fig. 2. The extraction profile obtained for different pHs was almost the same for all analytes showing better extractions under acidic conditions. As the pH of solution increased the extraction of the selected analytes gradually decreased. These findings are in agreement with the results obtained in different studies [17,27–29]. The pH of the apple juice used in this study was 4.5. Because sufficient method sensitivity was obtained at this pH, no pH adjustment was performed in further experiments. However, for the analysis of real samples with a pH value higher than 5.0, especially when better sensitivity is required, pH adjustment to 5.0 can be performed to enhance the extraction efficiency.

3.2. Evaluations in real samples

The effect of salt addition on extraction was investigated in real samples. For this purpose, a commercial drink that contains 10% apple juice (apple drink) and 100% apple juice were bought from the supermarket and used in the investigations. The pH and conductivity of matrices used in the study are summarized in Table S2 (Supporting Information Section).

The effect of salt addition on extraction of pesticides from apple drink (10% apple juice) is shown in Supplementary Information Section in Figure S4. As can be seen from the figure the extracted amounts of pesticides are affected significantly from the presence of salt in the sample and reached to their maximum value around 5% NaCl. When the salt concentration exceeds 10% NaCl (w/v), the extracted amount of analytes started to decrease for all compounds. However, statistically

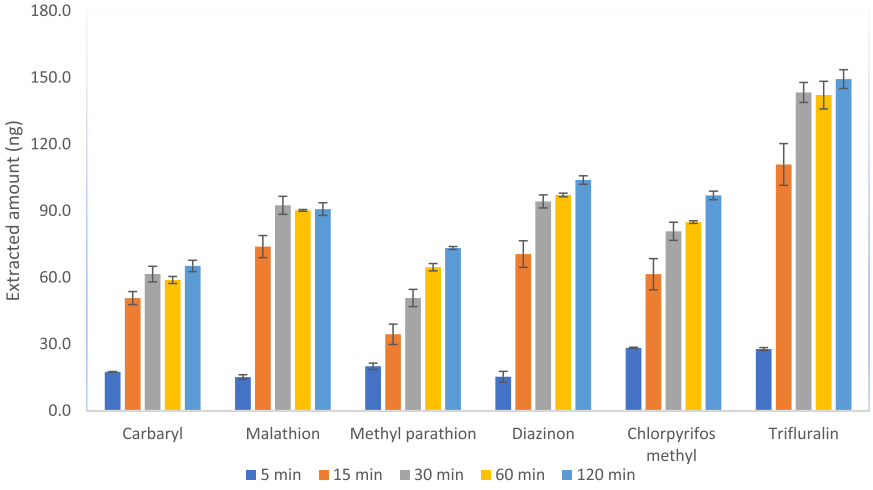

Fig. 1. Extraction time profiles of pesticides with TFME devices from PBS (n = 3). Extraction conditions: 4.0 mL sample volume, 250.0 ng mL^{-1} analyte concentration, 1000 rpm agitation rate. Desorption conditions: 1.5 mL methanol, 60 min desorption at 1000 rpm agitation rate.

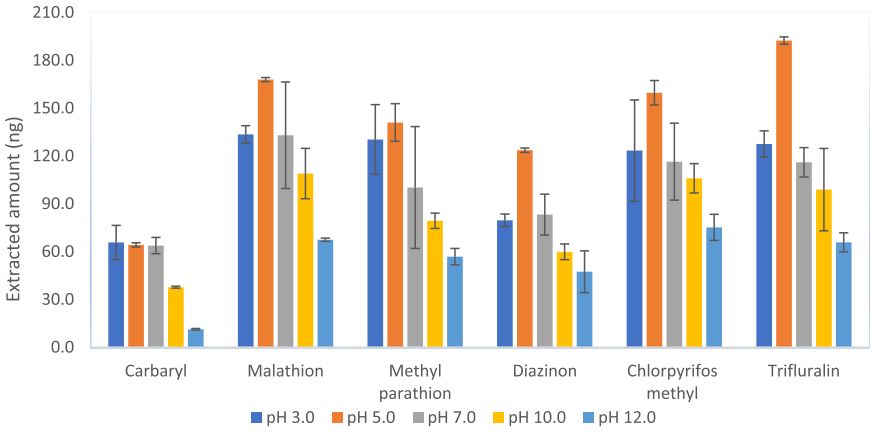

Fig. 2. Effect of sample pH on extraction of pesticides with TFME samplers (n = 3). Extraction conditions: 4.0 mL sample volume, 250.0 ng mL^{-1} analyte concentration, 1000 rpm agitation rate. Desorption conditions: 1.5 mL methanol, 60 min desorption at 1000 rpm agitation rate.

significant difference for this decrease (at 95% CL) was observed only for trifluralin, malathion, diazinon and chlorpyrifos-methyl. This phenomenon can be explained by the decrease of the diffusion coefficients (which results in lower extracted amounts) as sample viscosity increases by added salt [18]. Similar findings were reported in literature for diazinon, in a study conducted by Maddah et al. for the extraction of diazinon from environmental water and they concluded that the extraction recovery of diazinon increases up to 10% NaCl and then starts to decrease [30]. This decrease was explained by Schelling et al. as the formation of a layer around the extractive phase which was preventing the sorption of analytes by the extractive phase [26].

The results obtained in 100% apple juice are shown in Fig. 3. As can be seen from these results the amounts of pesticides extracted from this matrix is significantly less compared to 10% apple juice. Similarly to previous case, the first addition of salt enhanced the extracted amounts. Contrary to our results for 10% apple juice for which 5% added salt enhanced the extraction directly to a peak value for all compounds, in this study a gradual increase of extracted amount was observed by increase in amount of salt added. For methyl-parathion, carbaryl and diazinon the maximum sorption was obtained at 10% NaCl while for malathion was 5%. In the case of trifluralin and chlorpyrifos methyl, a gradual increase was observed up to 20% of salt which was the highest concentration tested. Moreover, for the extraction of trifluralin (Log P 5.3) and chlorpyrifos-methyl (Log P 4.3), the overall effect of salt addition on extracted amount was less significant. In fact, it has been reported that the effect of salt addition becomes critical for the compounds with a Log P value lower than 3 as the presence of ions decreases the solubility of compounds in their aqueous matrix, thus enhancing their affinity towards the extractive phase [31]. Therefore, the minimal effect of salt addition on the extraction of the most lipophilic compounds observed here is reasonable behavior. However, the results obtained in apple drink containing 10% apple juice did not have the same trend, indicating that other phenomena are also critically involved Two major reasons might have contributed to this observation. The first one is the presence of higher amount of binding components in the pure apple juice which decreases the concentration of free form of the lipophilic compounds, thus the extraction. The second reason could be associated

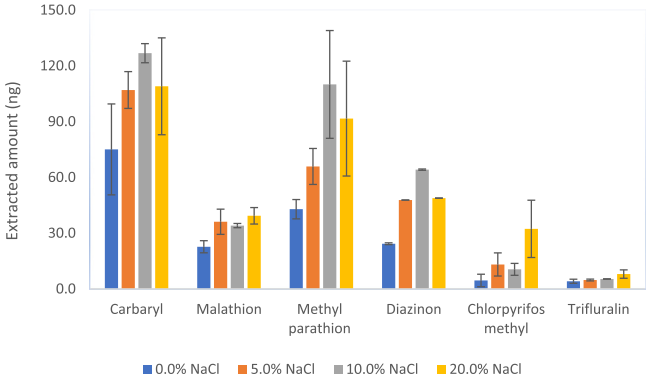

Fig. 3. Effect of salt addition on extraction of pesticides from 100% apple juice (n = 3). Extraction conditions: 40.0 mL sample volume, 250.0 ng mL^{-1} analyte concentration, 1000 rpm agitation rate. Desorption conditions: 0.6 mL methanol, 60 min desorption at 1000 rpm agitation rate.

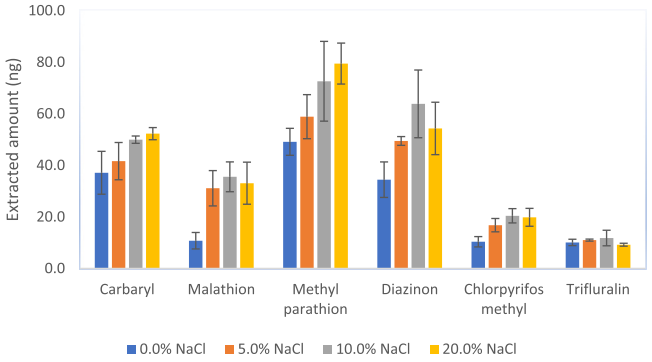

Fig. 4. Effect of salt addition on extraction of pesticides in diluted apple juice (50:50) (n = 3). Extraction conditions: 40.0 mL sample volume, 250.0 ng mL^{-1} analyte concentration, 1000 rpm agitation rate. Desorption conditions: 0.6 mL methanol, 60 min desorption at 1000 rpm agitation rate.

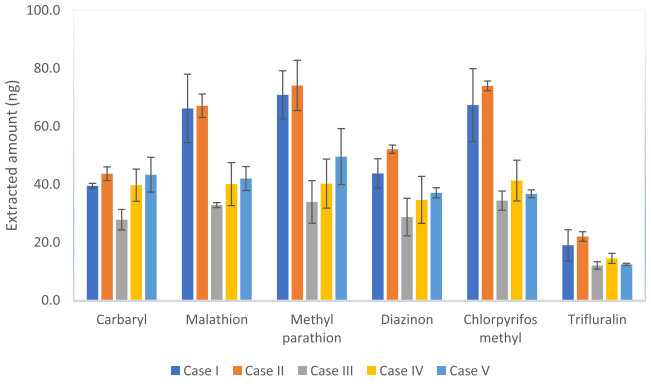

Fig. 5. Effect of sample centrifugation and filtration on the extraction of pesticides with TFME samplers (n = 3). Extraction conditions: 40.0 mL sample volume (diluted apple juice (50:50)), 250.0 ng mL^{-1} analyte concentration, 1000 rpm agitation rate. Desorption conditions: 0.6 mL methanol, 60 min desorption at 1000 rpm agitation rate.

with the decrease in the solubility of the lipophilic compounds by added salt to a level at which analyte precipitation from the sample starts.

To eliminate the negative effect of the pulpy and dense nature of apple juice, the effect of salt addition was studied by diluting the samples with water in half. The results of this experiment are shown in Fig. 4. During the assessment of the results, it should be kept in mind that the volume of juice samples before dilution was 20.0 mL; therefore, they had 50% less absolute amount of pesticides compared to 40.0 mL pure apple juice samples. As can be seen from the results, similar to pure apple juice samples, a gradual increase of analyte extraction with increase of added salt is present. However, the gradual decrease of sorption at 20% salt was not observed for all analytes. Yet, the best sorption was obtained in samples containing 10% NaCl which was in agreement with pure apple juice and apple drink results. The comparison of the sorption amounts of chlorpyrifos-methyl and trifluralin from pure and diluted apple juice at 10% NaCl concentrations, revealed that the absolute extracted amounts approximately were doubled in diluted samples, albeit of less amount of analyte present. This indicates the advantage of sample dilution to decrease the matrix effect and increase the absolute recoveries. Interestingly, no significant difference was observed for extracted absolute amounts of malathion and diazinon in pure and diluted samples. Still indicating the advantage of the dilution of the sample. Contrary to the other four compounds, carbaryl and methyl-parathion, the compounds with the lowest molecular weights, were extracted in higher absolute amounts from pure juice compared to diluted juice. This further proves that for very polar analytes viscosity of the sample and presence of binding molecules is less critical compared to the most nonpolar analytes.

According to the review published by Shalini et al., apple pulp contains about 3.6% sugars, 4.0% proteins, and 9.5 to 22.0% carbohydrates [32]. The presence of macromolecules in the sample affects the diffusion of analytes; therefore, it may affect the extraction process. Moreover, the analytes might bind to macromolecules and their free concentration within the matrix may decrease, resulting in lower recoveries. In order to further investigate the effect of the matrix components on extraction of the pesticides, the effect of sample centrifugation and filtration on extracted amounts was evaluated. For this purpose, five experimental conditions were tested. In Case I the apple juice was centrifuged, and the supernatant was spiked with the pesticides. In Case II the apple juice was centrifuged and then filtered, and the filtrate was spiked with pesticides. In Case III, the apple juice was spiked with the pesticides without involving any filtration and centrifugation steps. In Cases IV and V, the apple juice was spiked with the pesticides and then the matrix removal methods described in Case I and Case II (respectively) were applied and then the extractions were performed under the same conditions. The results of this experiment are given in Fig. 5. As can be seen from the results, except for carbaryl, there is significant effect of the matrix. Comparison of Case I and Case II, which only differs by addition of filtration step, suggest no significant difference between the extracted amounts. However, comparison of Case I and II to Case III in which matrix components were not separated, shows a clear effect of the matrix. Yet, it is not clear if this is because of sample viscosity or binding to macromolecules. Comparison of Case I and II (post-spiked) to Case IV and V (pre-spiked), clearly shows that there is enhancement in the extracted amount from the samples which were post spiked, albeit the matrix viscosity is the same in both groups. This observation suggests that some portions of the analytes are retained by macromolecules. Interestingly, only carbaryl did not show difference for extracted amounts between four cases (Case I, II, IV and V), suggesting that this compound does not show significant binding to matrix components. Yet, the comparison of Case III to the other cases suggests a slight effect of the sample viscosity on the extracted amount of carbaryl.

Consequently, no filtration or centrifugation step was implemented to the final method as dilution of the apple juice with water in half then addition of NaCl to have a 10% concentration (w/v) in the final samples provides reasonable balance for the sensitivity of all analytes.

3.3. Extraction from agarose gel as solid matrix representative

To show the suitability of the developed TFME samplers for the on-site multi-residue analysis of pesticides, preliminary studies were conducted on agarose gel.

3.3.1. Extraction time profile

To show the suitability of the developed thin films for potential on-site sampling directly from the surface of fruit and vegetables, agarose gel was used as surrogate matrix spiked with the analytes. Although the pesticides were distributed homogeneously through the gel layer, the extractions were performed from the surface of the gel. Therefore, the sampling does not only represent the surface of the potential crop but also extraction from a semi solid (fruit/vegetable puree) sample. The extraction time profile obtained in the gel model under static extraction conditions are illustrated in Fig. 6. Although none of the analytes reached equilibrium under the tested sampling times, they were quantitatively determined even with 5 min sampling. It should be noted that the extractions were performed under static conditions for which the boundary layer between the extractive phase and sample is thick and has its largest value for this system. Since the analytes reach the extractive phase via passive diffusion through this boundary layer, the sorption of analytes is slowed under static extraction condition. In fact, according to Fick's law, the diffusion of an analyte in a certain system can be predicted. The-mean squared displacement of a molecule can be estimated using the equation given below. Where D denotes diffusion

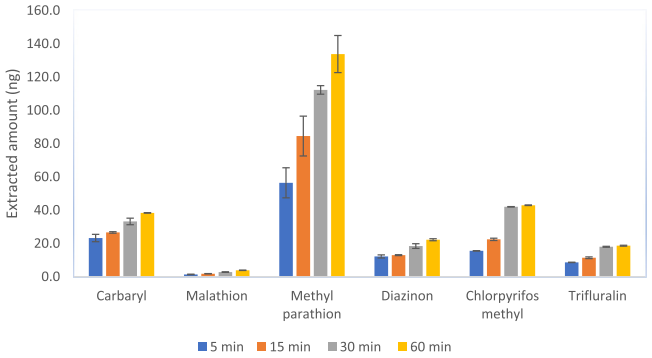

Fig. 6. Extraction time profile of pesticides in 2% agarose gel ($n = 3$). Extraction conditions: 50.0 mL gel volume, 250.0 ng mL^{-1} analyte concentration, sample agitation is static. Desorption conditions: 1.0 mL methanol, 60 min desorption at 1000 rpm agitation rate.

constant of the molecule and has an average value of $5.8x10^{-6}cm^2/s$ for small molecules in aqueous systems and decreases only by 5% in the 2% agarose gel [33]. The term d in the equation is the dimensionality of the system, and t is time, which in this case is the extraction time [34].

$$\langle x^2 \rangle = 2d\,Dt$$

Based on the calculations, for 60 min of sampling which was the longest extraction time (so the contact time between the agarose gel and the extractive phase) in this study, the molecular displacement was only 2 mm. This indicates that only the analytes present within 2 mm distance from the TFME device were extracted on the extractive phase and the extraction was restricted almost to the surface of the gel. This clearly explains why the extraction did not reach to equilibrium conditions. As sufficient sensitivity was obtained for all analytes, the optimum extraction time was chosen as 60 min for further experiments.

3.3.2. Pesticide distribution analysis

In view of the fact that pesticide concentration may vary through the crop surface, space-resolved sampling becomes critical to decide whether the level of pesticides exceed certain levels. Therefore, developed TFME samplers (with surface area of 0.25 cm^2) were evaluated in terms of their applicability for sampling from smaller surface area (approximately 1 cm^2) of agarose gel with varying concentrations (0.0, 10.0, 25.0, and 50.0, 100.0, 250.0, 500.0, and 750.0 ng mL^{-1}) of pesticides. The obtained results from this study are shown in Figure S5. As can be seen from the figure, even at 10.0 ng mL^{-1} all pesticides could be detected from the gel surface. The only exception was for methyl-parathion which was quantified at minimum of 25.0 ng mL^{-1} .

Since the pesticides concentrations were randomly distributed in the 96 well plate, a heat map (Figure S6) showing a color palette for each concentration was generated from the extraction results. As can be seen from the heat maps, random distribution of concentrations on the 96 well plate could be differentiated correctly by 0.5 × 0.5 cm HLB/PTFE AF devices, indicating the capability of TFME for monitoring the spatial resolution of chemical changes on a surface.

Table 2
Intra-day precision of the TFME-GC–MS method.

	RSD% ($n = 3$)		
	5.0 ng mL^{-1}	30.0 ng mL^{-1}	300.0 ng mL^{-1}
Trifluralin	19	18	**21**
Chlorpyrifos-methyl	18	14	17
Malathion	18	10	8
Methyl-parathion	15	8	10
Diazinon	17	12	6
Carbaryl	3	6	6

3.4. Validation of analytical method

For the validation of the method in 100% apple juice, analytical figures of merits were determined using matrix-matched external standard calibrations. For this purpose, the apple juice free of targeted pesticides was spiked in a range of 0.10–500.0 ng mL^{-1} concentrations of pesticides. Then the developed method was used for the extraction, desorption and GC–MS analysis. The representative matrix-matched TFME calibration curves are given in Supplementary Information Section as Figure S7. The linear dynamic range (LDR) was between 1.0–500.0 ng mL^{-1} for trifluralin and malathion, and 5.0–500.0 ng mL^{-1} for chlorpyrifos-methyl, methyl-parathion, carbaryl, and diazinon. The limit of quantitation (LOQ) was 1.0 ng mL^{-1} for trifluralin and malathion while for chlorpyrifos-methyl, malathion, methyl-parathion, carbaryl, and diazinon was 5.0 ng mL^{-1} . It should be mentioned that the LOQ obtained in this study are mainly restricted by the solvent volume used to desorb the analytes as only 1.0 µL of 600.0 µL of desorption solution was injected to the GC–MS system. In the presence of large volume thermal desorbed, direct thermal desorption from the TFME device to GC–MS would be feasible which could further improve the method's LOQs. The accuracy of the TFME-GC–MS method was evaluated as an analyst blind test by spiking three concentrations from the linear range to apple juice free of pesticides. The relative error (RE%) for each analysis is given in Table 1. As can be seen from the table the methods accuracy is acceptable as provides less than 15% RE for all of the analytes at all tested levels. Only for trifluralin at 300.0 ng mL^{-1} level more than 20% of RE% was observed.

The intra-day reproducibility of the developed TFME-GC–MS method was evaluated by repeating three times in a day the protocol in spiked samples with low (5.0 ng mL^{-1}), mid (30.0 ng mL^{-1}), and high (300.0 ng mL^{-1}) concentrations. Besides, inter-day reproducibility was evaluated for three consecutive days with the same spike levels used in intra-day evaluations. The percent relative standard deviation (RSD%) for intra-day and intra-day reproducibility are shown in Table 2 and Table 3, respectively. The reproducibility of the method varied between 6 and 21% RSD and 3–20% RSD for intra-day and intra-day studies, respectively. Similarly, to methods accuracy only trifluralin was above 20% at 300.0 ng mL^{-1} level.

In overall, the results show acceptable reproducibility and accuracy for the determination of pesticides in 100% apple juice which could be further improved by implementing internal standards in the method.

Table 1
The LOQ and the accuracy of the developed method ($n = 3$).

Analyte	LOQ (ng mL^{-1})	Relative Error (RE%)		
		5.0 ng mL^{-1}	30.0 ng mL^{-1}	300.0 ng mL^{-1}
Trifluralin	1.0	0.3%	13.8%	**24.5%**
Chlorpyrifos-methyl	5.0	1.4%	3.0%	8.2%
Malathion	1.0	5.7%	6.2%	0.8%
Methyl-parathion	5.0	6.0%	12.9%	5.4%
Diazinon	5.0	1.4%	0.3%	4.1%
Carbaryl	5.0	7.3%	7.8%	1.6%

Table 3
Inter-day precision of the TFME-GC–MS method.

	RSD% (n = 3)		
	5.0 ng mL^{-1}	30.0 ng mL^{-1}	300.0 ng mL^{-1}
Trifluralin	13	19	19
Chlorpyrifos-methyl	17	16	19
Malathion	15	3	12
Methyl-parathion	20	9	11
Diazinon	17	10	7
Carbaryl	6	4	6

4. Conclusions

In the current study, a new, reliable TFME-GC–MS method was proposed for the simultaneous determination of six pesticides, namely, trifluralin, methyl-parathion, chlorpyrifos-methyl, diazinon, carbaryl, and malathion in apple juice and on the surface of fruit/vegetables which was mimicked with agarose gel. The use of HLB particles ensured the efficient extraction of pesticides with different physicochemical properties while thermally stable PTFE AF acted as an ideal immobilizer for the extractive particles and made the resulting samplers suitable for both solvent and direct thermal desorption. Because real samples vary in terms of their pH, ionic strength, and matrix complexity, these parameters become critical during the method development in real samples. In this study, it was shown that the sample pH has a significant effect on the adsorption of pesticides on the new sampler, and extracted amount increases under slightly acidic conditions with maximum sorption at pH 5.0. Besides, the results revealed a strong positive effect of salt addition on extracted amounts of pesticides. The amount of salt necessary to reach optimum sorption conditions was also correlated with sample complexity. The applicability of the samplers for chemical distribution mapping directly from the surface of fruit/vegetable was also shown successfully by performing extraction in an agarose gel model. The results obtained in this study revealed that the samplers can be used reliably both in collected samples and on field sampling directly on the fruit/vegetable surface.

Declaration of Competing Interest

The authors declare no competing interest.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the Office of Scientific Research Projects Coordination of Middle East Technical University. The project number is GAP-10316. The authors thank Sılanur Sevgen for her help in sketching the graphical abstract.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2023.100061.

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