Marmara Fen Bilimleri Dergisi 2018, 4: 398-402
DOI: 10.7240/marufbd.398657

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Determination of Chlorpyrifos Residues in Cherry Samples by Direct Immersion Solid Phase Microextraction Coupled with Gas Chromatography Mass Spectrometry

Direkt Daldırma Katı Faz Mikroekstraksiyon Yöntemi ile Kiraz Örneklerinde Klorpirifos Kalıntılarının Gaz Kromatografisi Kütle Spektrometresinde Tayini

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Abstract

A method based on a direct immersion solid phase microextraction gas chromatography – mass spectrometry (DI-SPME-GC-MS) was developed for the quantitative determination of chlorpyrifos (CRF) residues in cherry samples. The performance of three kinds of commercial fiber coatings: PA (polyacrylate, 85µm), PDMS–DVB (polydimethylsiloxane–divinylbenzene 65 mm) and PDMS (polydimethylsiloxane, 100 mm) were compared. Various parameters affecting SPME procedures such as temperature, extraction and desorption times, salinity, stirring rate were investigated. The analytes from all sample extracts were preconcentrated for 30 minutes in the SPME fiber at 60 °C and then the fiber was desorbed by heating at 250 °C for 10 minutes in the gas chromatography injection port. Limits of detection (LOD) and quantitation (LOQ) values were 0.03 µg/L and 0.1 µg/L, respectively. An average recovery range was 93-98% with relative standard deviation (*RSD) < 10 %.*

Keywords*:* Fiber, Pesticides, DI-SPME, GC-MS

Öz

Direkt daldırma katı fazlı mikroekstraksiyon ile kiraz örneklerinde klorpirifos kalıntılarının gaz kromatografisinde tayini (DI-SPME-GC-MS) için bir yöntem geliştirilmiştir. Üç çeşit ticari fiber kaplamanın, PA (poliakrilat, 85 um), PDMS-DVB (polidimetilsiloksan-divinilbenzen 65 mm) ve PDMS (polidimetilsiloksan, 100 mm), performansı karşılaştırıldı. Katı Fazlı Mikroekstraksiyon prosedürlerini etkileyen sıcaklık, ekstraksiyon ve desorpsiyon süreleri, tuzluluk, karıştırma oranı gibi çeşitli parametreler incelendi. Tüm numune ekstraktlarından alınan analitler, 30 dakika boyunca 60 ° C' de SPME fiberine adsorbe edilmiş ve daha sonra fiber, gaz kromatografi enjeksiyon portunda 10 dakika boyunca 250 ° C'de ısıtılarak desorpsiyon sağlanmıştır. Yöntemin tespit sınırı (LOD) ve tayin sınırı (LOQ) değerleri sırasıyla 0,03 µg / L ve 0,1 µg / L dir. Yöntemin ortalama geri kazanım aralığı % 93-98 iken bağıl standart sapma (RSD) <% 10 dur.

Anahtar Kelimeler: Fiber, Pestisit, DI-SPME, GC-MS

I. INTRODUCTION

With a growing population, producers rather than obtaining fewer products via natural processes started using various chemicals (pesticides) to increase production. The number of used pesticides and their complexity has increased significantly over time. After the application pesticides may exhibit various different behaviors. While some of them may evaporate into the atmosphere, some can mix with surface waters via water flow and erosion or may undergo photodegradation due to sunlight. Generally, 0.015% – 6% of the amount of pesticides used reach the target living organism, while the rest gets mixed into the environment. Pesticides have been found to be carcinogens and mutagens and affect nervous system. Chlorpyrifos is the kind of pesticide organophosphorus (OPP). This compound is used for controlling various harmful pests

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Gönderilme/Submitted: 26.02.2018, **Düzenleme/Revised:** 06.11.2018, **Kabul/Accepted:** 07.11.2018

of mosquitoes, flies, soil and greens. It is difficult to measure small amounts of pesticides found in natural foods, because foreign ions and matrix effect may interfere with these measurements [1-3]. Chromatography is one of the most common methods used for this purpose, but an extraction step is needed prior to the measurement [4-9]. Among the extraction techniques used for analysis of pesticides are liquid – liquid extraction [10], solid phase extraction [11], QuEChERS method [9] and solid phase microextraction (SPME) [12-16]. The SPME is easy to use, feasible, rapid and solventless. The SPME also features unique preparation procedure, which is based on a length of fused silica fiber coated with a polymeric phase.

The fiber in the SPME is fused silica optical fiber. SPME applications have two types: gas sampling (headspace) or solution sampling (direct immersion). In both cases, the SPME's needle is brought to the appropriate position and the fiber is subjected to the action of the sample. Analytes partition between the coating and the sample. After equilibrium is reached, the metal needle is withdrawn and the next step is transferring of the substance from the fiber to the chromatograph [11, 2]. The goal of this study was to obtain reliable, sensitive, environmental friendly and quick extraction and analysis method for confirmation and quantification of chlorpyrifos in cherry samples.

II. MATERIAL AND METHOD

2.1 Apparatus

An Agilent 5975C (Agilent Technologies, Santa Clara, CA, USA) 6890 gas chromatographic system with split/splitless injector, and an HP 5973 mass detector were used to identify and quantify chlorpyrifos in samples. HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm) was used for separation of pesticide. Helium was used as the carrier gas (1.0 mL/min). The injection port was held at 250 ºC in splitless mode. The oven temperature program was shown in Table 1. A SPME fiber holder for manual use and fibers of PA (polyacrylate, 85μm), PDMS (polydimethylsiloxane, 100 mm) and PDMS–DVB (polydimethylsiloxane–divinylbenzene 65 mm) from Supelco (Bellefonte, PA, USA) were used.

Table 1: Oven temperature program of GC-MS for detection of chlorpyrifos

Ramp rate (°C /min)	Oven Temperature (°C)	Hold time (min)
----	100	2
15	250	5

2.2 Sample Preparation of Cherry Samples

Firstly, pits were removed from cherry samples and then the samples were passed through the blender. After weighing out 50 grams of each cherry species they were put into sample cups. Then all samples were treated with the same volume of 0, 0.1 mg/L, 0.01 mg/L and 0.005 mg/L intermediate solution of chlorpyrifos for the matrix-matched and standard addition procedures. The mixtures were vortexed for homogenization and incubated at 25 ºC for 1 hour.

Water: methanol mixture was added to all samples and the volumes of all samples were brought up to the same level. The water: methanol ratio in the final sample was 80%, while the salt content was at 8%. The samples were placed in separate vials (4 mL). For direct immersion (DI)-SPME, the PDMS fiber (100 μm) was directly immersed into sample and exposed to the solution stirred at 350 rpm. After an adsorption time of 30 min at 60 °C, the fiber was immediately injected into the GC injection port (250 °C). PDMS fiber was desorbed in the splitless mode for 10 min.

2.3 Comparison of Fiber Coatings

There are many types of SPME coatings available commercially. The most widely used coatings for definition of pesticide residues in fruit samples are polydimethylsiloxane–divinylbenzene (PDMS–DVB), polydimethylsiloxane (PDMS) and polyacrylate (PA). A SPME procedure using these three commercialized fibers was used for the detection of selected group of organophosphate pesticides. The detector response (peak area) of the chlorpyrifos samples was used for the comparison of extraction performance of PDMS, PA and PDMS-DVB. The PDMS coated fiber showed best extraction efficiency and therefore PDMS was chosen further experimental processes (Figure 1).

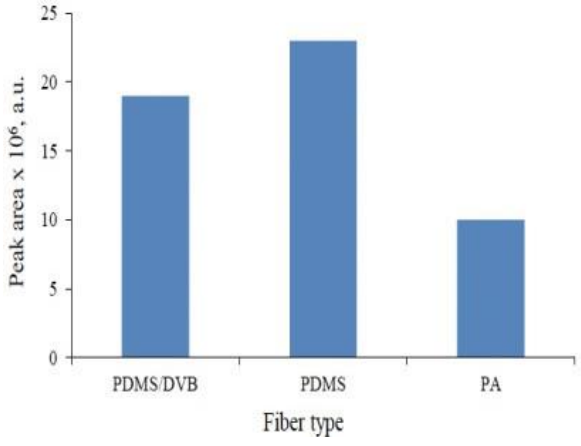

Figure 1. Comparison of fiber coatings (10 μg/L chlorpyrifos, 30 min, 60°C, 300 rpm, n = 3, a.u: arbitrary unit)

III. RESULTS AND DISCUSSION

The main factors that influence the absorption and desorption processes in direct immersion solid phase micro extraction process were optimized.

3.1 Effect of Desorption Temperature

The injection port temperature should be high, consistent with the thermal stability of the sample, to give the fastest rate of evaporation and small sample volume into the column. For this reason, the injection port temperature was tested with the standard chlorpyrifos solution at the following temperature values 230ºC, 250ºC and 270ºC, while the other parameters were held constant. As shown in Figure 2, the highest peak area was obtained at 250ºC, therefore, 250ºC was chosen as the optimum temperature for our experiments.

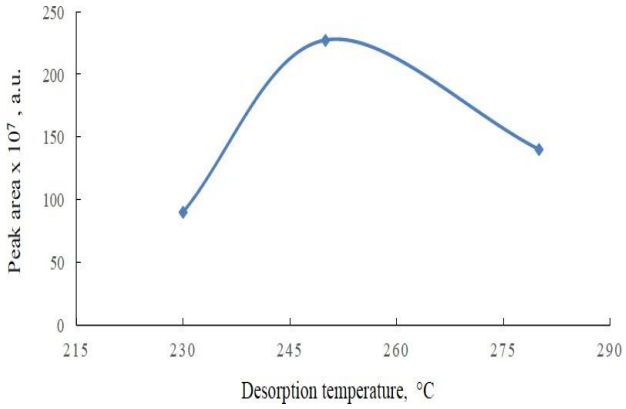

Figure 2. Influence of desorption temperature (10 µg/L chlorpyrifos, 30 min, 60°C, 300 rpm, n = 3, a.u: arbitrary unit)

3.2 The Effect of Organic Solvent on Sample Extraction

Firstly, we aimed to determine the optimal water: methanol mixture ratio to be used in the SPME method as an extraction solution in the analyses of chlorpyrifos in the cherry samples. For this purpose, we used ratios ranging from 90% (v/v) to 50% (v/v). The rate at which extraction was the highest was determined as 80% (v/v). (Figure 3)

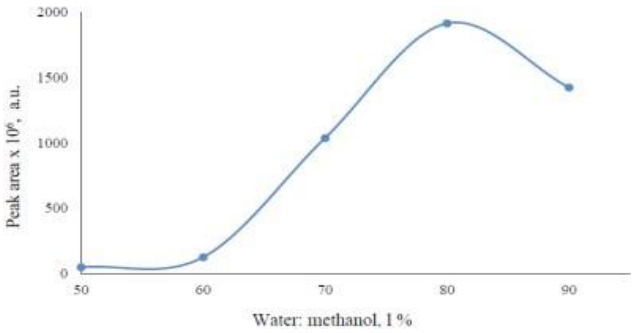

Figure 3. Influence of organic solvent mixture ratio (10 µg/L chlorpyrifos, 30 min, 60°C, 300 rpm, n = 3, a.u: arbitrary unit)

3.3 Effect of Extraction Temperature

While high temperatures provide rapid evaporation, they also play a role in increasing the rate of diffusion of substances. This in turn, ensures that more substance is adsorbed on the fiber. Furthermore, as the temperatures increase the SPME fiber coating's ability to adsorb organic matter decreases. Moreover, high temperatures increase the degradation rate of pesticides undergoing hydrolysis and thus decrease the yield. Therefore, it is more suitable to use low temperatures for analysis of unstable compounds. In our study, a range of temperatures from 30 to 80°C were used in order to determine the highest optimal extraction temperature for analysis of substances. 60°C was selected as the optimal extraction temperature (Figure 4).

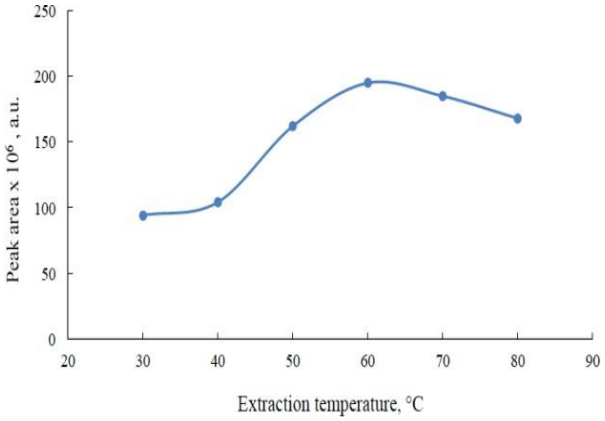

Figure 4. Influence of extraction temperature (10 µg/L chlorpyrifos, 30 min, 300 rpm, n = 3, a.u: arbitrary unit)

3.4 Influence of extraction time

The effect of extraction time plays a considerable role in extraction procedure. Since the adsorption of substances with large molecules will take longer, the extraction

time may need to be prolonged as well. In this study, the influence of the extraction time on the efficiency of extraction procedure was obtained by testing the extraction times between 20 min to 40 min. 30 minute was found optimum time for the PDMS fiber extraction. (Figure 5).

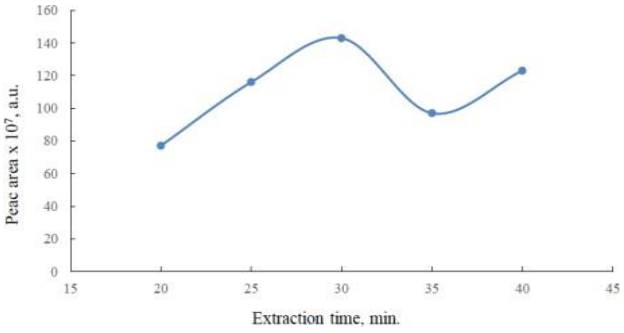

Figure 5. Influence of extraction time (10 µg/L chlorpyrifos, 60°C, 300 rpm, n = 3, a.u: arbitrary unit)

3.5 Speed and stirring

During the stirring step the adsorption of samples onto fiber material is activated and accelerated. The speed of extraction is dependent on dimensions of the vial and a magnetic stirrer. During a very fast extraction, the quantities of adsorbed substance are reduced. In our study, stirring rates was tested between 200 to 500 rpm. The ideal stirring rate was determined as a 300 rpm (Figure 6).

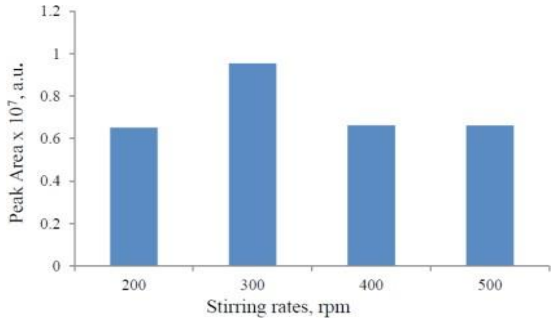

Figure 6. Influence of stirring rates (10 µg/L chlorpyrifos, 60°C, n = 3, a.u: arbitrary unit)

3.6 Salt concentration

Addition of salt to samples changes the solubility of desired substance in the sample and thus enables more efficient extraction. Hence, we investigated the influence of adding salt on our extraction procedure as well. The samples were prepared that had 1 ppm chlorpyrifos and NaCl:water solutions ranging from 0.2% (w/v)-10% (w/v), while all other conditions were kept constant. The highest peak area was achieved with 8% salt, thus this concentration was chosen to use in other experiments (Figure 7).

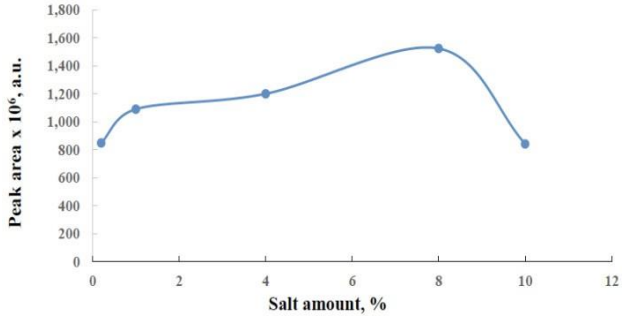

Figure 7. Influence of salt addition (10 µg/L chlorpyrifos, 30 min, 60°C, 300 rpm, n = 3, a.u: arbitrary unit)

3.7 Analytical performance of method

The dynamic linear range of GC-MS combined with the solid phase microextraction method was obtained by assay of different concentration levels. The linear range was determined 0.04 –50 µg/L (y = 13377x – 30.232) with the correlation coefficient (R^2) exceeding 0.9886. The LOD and LOQ for chlorpyrifos were determined as 0.03µg/L and 0.1 µg/L with the lowest concentration that genera- ted minimum signal-to-noise ratio of 3:1 and 10:1, respectively. Meanwhile, the relative standard deviation (RSD %) was determined to be 9%, 5%, 2% for concentrations such as, 0.05 µg/L, 0.1 µg/L and 0.3 µg/L, respectively. GC-MS chromatogram of chlorpyrifos in a cherry sample was given in Figure 8. A standard mixture of commonly used organophosphate pesticides (ametryn, azinphos-methyl, azinphos-ethyl, simazine, atrazine, diazinon, molinate, parathion-methyl, pendimethalin, prometryn, propazine, alachlor, metolachlor, malathion, terbuthylazine, pirimicarb, terbutryn, trifluralin, parathion-ethyl) were analysed to determine the specificity for chlorpyrifos in carry samples. According to our results, all samples at a concentration of 1.0 µg/L none of the tested pesticides were interfered with the accurate detection of chlorpyrifos in the mixed solution.

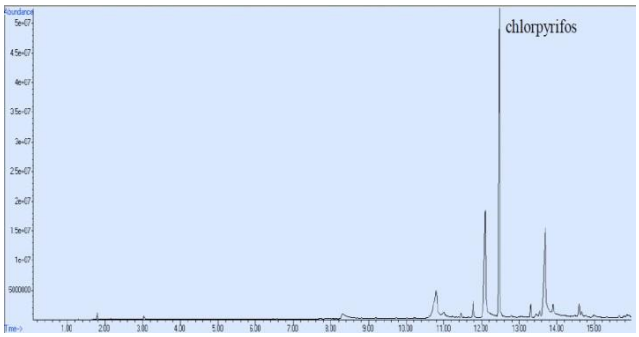

Figure 8. GC-MS Chromatogram of chlorpyrifos (m/z : 199 (99%), retention time: 12.53 min.)

CONCLUSIONS

A sensitive, specific and simple GC-MS-SPME method was developed for detection of chlorpyrifos in cherry samples. SPME is an attractive solvent free extraction method, reduces disposal costs as well as extraction time. The accuracy of this procedure was assessed using recovery assays and reference materials. In this study limits of detection (LOD) and quantitation (LOQ) values of method were determined as 0.03 µg/L and 0.1 µg/L, respectively and average recovery range was 93-98% with relative standard deviation (RSD) < 10 %. We believe that this procedure is a useful alternative for the accurate detection of chlorpyrifos and cherry samples.

ACKNOWLEDGMENT

This procedure is supported by the Scientific Research Project Unit of Marmara University. [FEN-C-YLP-090414-0101].

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