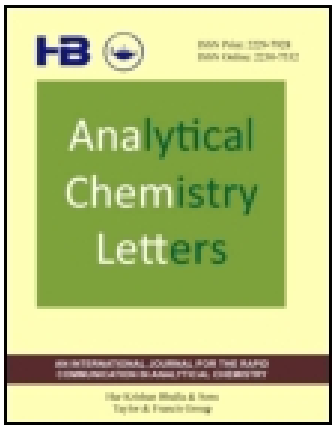

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Microwave Assisted Head Space Solid Phase Microextraction for Analysis of Butachlor and Chlorpyrifos Pesticides in Urine

Fateme Ghavidel[a], Seyed Jamaleddin Shahtaheri[b], Marzieh Torabbeigi[c] & Abbas Rahimi Froushani[d]

[a] Department of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran 14155-6446, Iran

[b] Department of Occupational Health Engineering, School of Public Health, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran 14155-6446, Iran

[c] Faculty of Health and Environment, Shahid Beheshti University of Medical Sciences, P.O. Box 16858-116, Tehran, Iran

[d] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran 14155-6446, Iran

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Fateme Ghavidel [1], Seyed Jamaleddin Shahtaheri [2] *, Marzieh Torabbeigi [3], Abbas Rahimi Froushani [4]

[1] Department of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran 14155-6446, Iran
[2] Department of Occupational Health Engineering, School of Public Health, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran 14155-6446, Iran
[3] Faculty of Health and Environment, Shahid Beheshti University of Medical Sciences, P.O. Box 16858-116, Tehran, Iran
[4] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran 14155-6446, Iran

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Abstract: Microwave assisted head-space solid phase microextraction (MA-HS-SPME) followed by gas chromatography using electron capture detection system (GC-ECD) were developed for determination of chloraacetanilide (butachlor) and chlorpyrifos present in biological samples. The different parameters including microwave irradiation power and microwave irradiation time, which affected on the extraction rate, were optimized. The optimum conditions for extraction of both pesticides were as following: irradiation power of 600 W, microwave irradiation time 10 min, sample volume: 10 ml, sample pH=2 and added salt 10 %. The limit of detection (LOD) for butachlor and chlorpyrifos were 0.0864 and 0.0832 ng/ml, respectively. The optimized methods for both compounds were validated for 6 consequtive days and 6 inter-day using two concentrations of 10 and 100 ng/ml in spiked urine samples that the ranges of obtained recoveries were 91-104 %.

Key words: MA- HS-SPME, GC-ECD, biological samples, chlorpyrifos, butachlor.

Introduction

Pesticides are widely used for agricultural purposes world-wide. Pesticides can be harmful to the variety of non-target organisms including human. Therefore, toxic effects of pesticides on humans and the environment are of great concern [1-4]. Among the various pesticides available, butachlor and chlorpyrifos are the ones most widely used in developing countries including Iran by the relevant plant protection organization. Butachlor is structurally belonging to the chloroacetanilide family and chlorpyrifos is in the organophosphorus pesticides category. It is worth mentioning that butachlor is a suspected carcinogen [5]. Butachlor toxicology studies in laboratory animals indicate low toxicity following acute oral, dermal, and inhalation exposure; however, chronic exposure may lead to liver and kidney toxicity [6]. Chlorpyrifos as organophosphorus pesticides is very toxic when absorbed by human organisms because of its property of acetyl-cholinesterase inhibition. Chlorpyrifos exposure may lead to acute toxicity at higher doses, persistent health effects following

*Corresponding author (Seyed Jamaleddin Shahtaheri)
E-mail: < shahtaheri@tums.ac.ir >

acute poisoning or from long-term exposure to low doses, and developmental effects in fetuses and children even at very small doses [7]. Therefore, evaluation of the risk associated to such pesticides to human is of great importance. Measurement of contaminant levels in body tissues and fluids is of essential approach [8,9]. Therefore, a rapid, accurate, and sensitive method is required for biological monitoring of these pesticides. Several analytical techniques have been developed for sample preparation to facilitate identification of pesticides from biological and environmental samples including Liquid–liquid extraction (LLE) [10,13], solid-phase extraction (SPE) [14], supercritical fluid extraction (SFE) [15] and matrix solid-phase dispersion (MSPD) [16]. Generally, these methods are multistage, expensive, laborious, and take long time to be performed and require large volume of solvents [17]. High performance liquid chromatography (HPLC) [18,19] and gas chromatography (GC) [20,21] as Chromatographic methods were widely used for analysis of pesticides.

Modern microextraction techniques such as solid-phase microextraction (SPME), that is rapid, simple, inexpensive, and quantitative analytical technique was developed by Pawliszyn and co-workers in the early 1990s [22]. SPME has been employed as a sample preparation method for the analysis of pesticides in various matrices.[23] In SPME, headspace (HS) approach is preferred for extracting analytes from biological samples, including blood and urine [24-28]. HS-SPME procedure has been applied for pesticides purification in biological samples such as chloraacetanilide[27] and organophosphorus pesticides [30,31].

Microwave assisted extraction (MAE) is a rather new extraction method which widely applied to the extraction of organic compounds from various matrices [32,33]. Generally, Microwave assisted extraction decrease organic solvent consumotion [34]. Under the microwave irradiation, the temperature rises rapidly. Therefore, microwave heating improve SPME sampling efficiency for organic compounds. In recent years, many studies can be found using MA-SPME for analysis of pesticides in various samples [35,36].

Optimized MA-HS-SPME procedure coupled with gas chromatography (GC) with electron capture detection (ECD) for simultaneous determination of butachlor and chlorpyrifos in urine samples was done in this study. The various parameters, which affected on the extraction procedure, including efficiency microwave irradiation power and irradiation time were optimized. Also optimum conditions were achieved for the real samples. Limit of detection, limit of quantification, and linearity range of the method were established for evaluating the pesticides.

Materials and methods

Reagent and materials

Pesticides were purchased from Sigma-Aldrich (Germany) including butachlor (97.7 %) and chlorpyrifos (99.9 %). Stock solutions of butachlor (1000 mgL^{-1}) and chlorpyrifos (2500 mgL^{-1}) were prepared in acetone and methanol, respectively and stored at 4ºC. All solvents (methanol, acetone, and water) were HPLC grade (Merck-Germany). Other reagents with analytical grade were obtained from Merck (Germany). Biological samples were prepared by adding the appropriate volume of the methanol standard solution and stored at 4°C. Working standard solutions of both pesticides were freshly prepared daily by volume dilution in the HPLC grade water.

Biological samples

Urine samples used for optimization studies were collected from healthy and ‘’non-exposed” subject.

Gas chromatography-electron capture detector (GC-ECD)

Chromatographic analysis was carried out by a Varian CP 3800 gas chromatograph equipped with an electron capture detector (ECD) and a split/splitless injector. Pesticides were separated in a HP-5 (30 m × 0.32 mm, 0.25 μm film thickness) column. Nitrogen (99.999 %) with a flow rate of 2 ml/min was used as a make-up and carrier gas. The detector and injector temperatures were held constant at 300 and 280°C, respectively. The column oven temperature was programmed as the initial temperature was 80°C held for 1 min, 30°C/

min to 200°C for 2 min, 10°C/min to 250°C for 3 min, and 20°C/min to 280°C for 4 min.

Microwave assisted head-space solid phase microextraction (MA-HS-SPME)

The microwave oven used in this study was modified from the home-used Samsung GE4020W/GE4020 (2450 MHz, Korea) with a maximum power of 900 W, equipped with a cooling condenser connecting to tap water. A glass tube was used to seal and guide the vapor through the SPME fiber. MA-HS-SPME system was set-up as shown in Figure 1. The SPME device for manual extraction purchased from Supelco (Bellefonte, PA, USA). Polydimethylsiloxane (PDMS 100 µm) fiber was used as extraction phase. Before using, the fiber was conditioned according to the recommended instructions by the manufacturer. SPME procedure was performed for biological samples by placing 1000 µl of urine sample and appropriate volumes of pesticides solution at two level concentrations of 10 and 100 ng/ml in 50 ml glass round flask. Hydrochloric acid and sodium chloride was added for adjusting the pH at 2. NaCl was added for adjusting salt concentration to 10 %. Finally HPLC grade water was added, so that, the total sample volume was 10 ml. The flask was placed in the microwave oven and connected to the water condenser through a Y type glass tube. SPME needle pierced the glass tube septum in order to expose the fiber (100 µm PDMS) on the headspace of the solution. PDMS fiber was exposed to the headspace of glass tube at 600 W for 10 min. After 10 min, needle was removed from the glass tube and inserted immediately into the heated injection port of the GC-ECD for thermal desorption of the sample for 5 min.

Figure 1. Assembly of the MA-HS-SPME

Results and discussion

Method optimization

Microwave heating time and microwave heating power affecting extraction efficiency in MA-HS-SPME technique, were studied and optimized.

MA-HS-SPME was done in different microwave irradiation power (100, 300, 600, and 900 W) for 5 min. The obtained results shown in Figure 2 indicated that, the extraction rate was increased by arising microwave power from 100 to 600 W for both pesticides, and then was decreased in power of 900 W. It is indicated that, both pesticides might be lost due to their volatilities in high irradiation power [37]. Therefore, irradiation power of 600 W was chosen as an optimum irradiation power.

Microwave irradiation time was evaluated from 5 to 15 min at power of 600 W. Figure 3 shows the recovery of both two pesticides on PDMS fiber during different irradiation times. Results showed that, recovery increases from 5 to 10 min, however, extraction rate not shown considerable changes. Therefore, further experiments were done for 10 min.

Method validation

The method was validated using obtained optimized conditions. The detection limits (LODs), quantification limits (LOQs), the inter- and intra-day precisions (RSD), the linearity, and the recovery was calculated. The results were tabulated in Table 1. The linear standard curves in the range of 0.1-250 ng/ml and 0.1-500 ng/ml for butachlor and chlorpyrifos were obtained each day for 6 consecutive days, respectively. The correlation coefficients (R^2) of calibration curves were 0.995 and 0.998 for butachlor and chlorpyrifos, respectively (Table 1). Intra- and inter-day precisions of the method were determined by two spiked extracted samples at

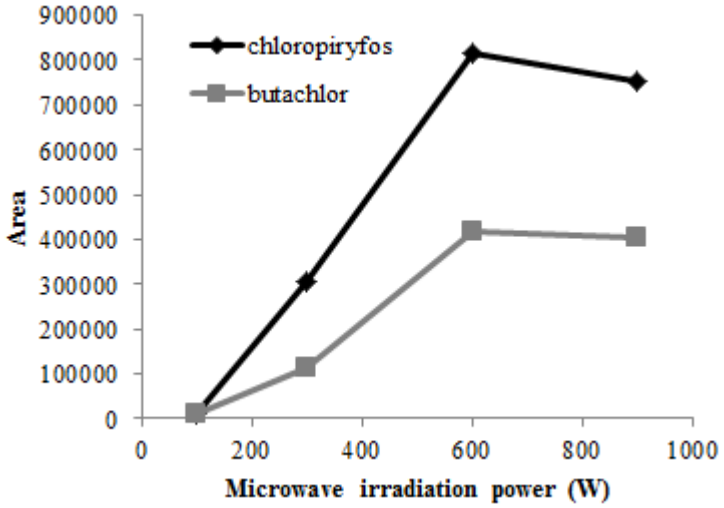

Figure 2. Influence of microwave irradiation power on extraction efficiency

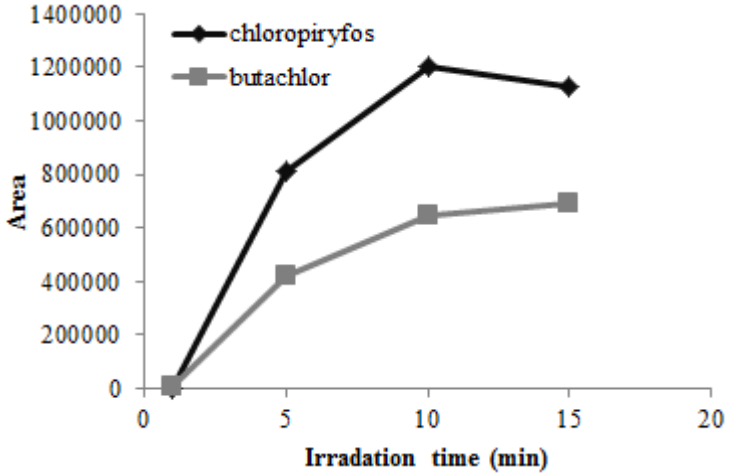

Figure 3. Influence of microwave irradiation time on extraction efficiency

Table 1. Analytical performance of the established MA-HS-SPME method for two pesticides

Analyte	Linear rang (ng/ml)	R^2	LOD (ng/ml)	LOQ (ng/ml)
Butachlor	0.1 - 250	0.995	0.0864	0.2620
Chlorpyrifos	0.1 - 500	0.998	0.0832	0.2522

concentrations of 10 and 100 ng/ml followed by analysis each day for 6 consecutive days to estimate intra-day reproducibility as well as six times a day to estimate inter-day reproducibility. Figure 4 shows the chromatograms of urine sample with and without spiked pesticides. Intra-day relative standard deviations (RSD) were different for butachlor and chlorpyrifos. The results indicate that, the method was much more precise at higher concentrations. The recoveries obtained by MA-SPME-GC-ECD method were between 91-104 % that shown in Table 2.

A microwave assisted headspace solid phase microextraction (MA-HS-SPME) method in combination with gas chromatography-electron capture detector (GC-ECD) was used for the extraction and quantification of butachlor and chlorpyrifos. This technique is simple, inexpensive and fast in compare with the other conventional methods. The various parameters

Table 2. Inter-day and intra-day reproducibility of method for spiked urine sample

Analyte	Spiked Concentration (ng/ml)	Inter-day (N = 6) Recovery (%)	Inter-day (N = 6) RSD (%)	Intra-day (N = 6) Recovery (%)	Intra-day (N = 6) RSD (%)
Butachlor	10	103	10.56	101	11.43
	100	91	1.622	91	1.736
Chlorpyrifos	10	104	6.766	102	8.451
	100	99	0.763	99	0.974

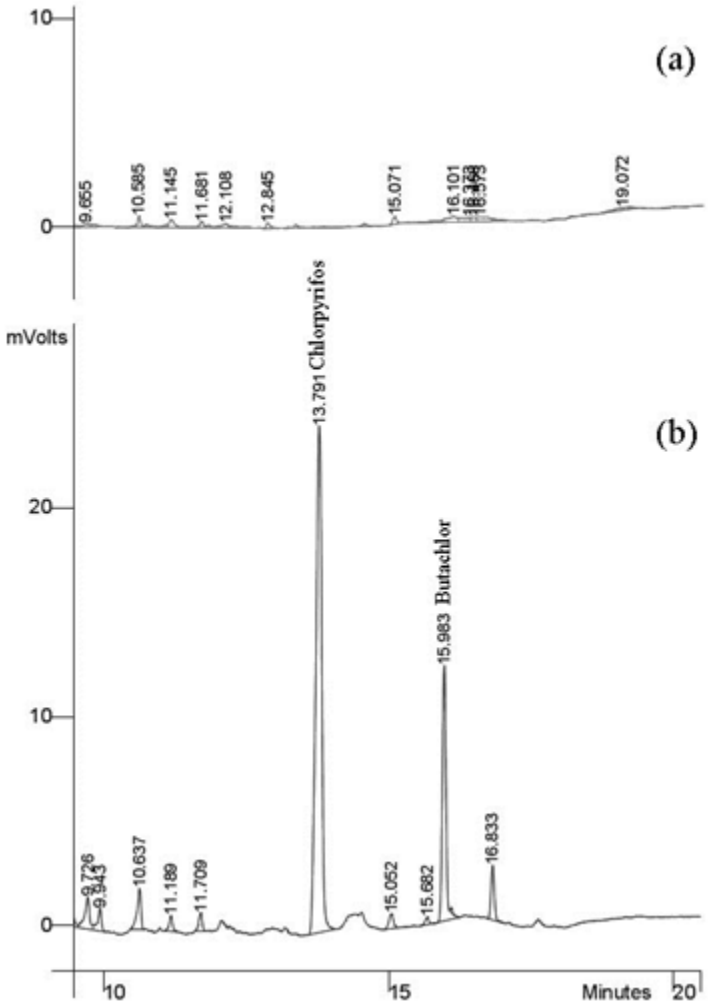

Figure 4. Chromatograms of urine sample (a) without and (b) with spiked sample of chloroprifos and butachlor

including microwave irradiation power and microwave irradiation time, which affected on the extraction rate, were optimized. To investigate the role of sample microwave irradiation power on extraction of butachlor and chlorpyrofos by MA-HS-SPME, a range of irradiation power from 100 to 900 W was tested. Up to irradiation power 600 W was increased the extraction of analyte and then decreased by arising irradiation power. This indicates that higher power (900 W) would cause

the loss of butachlor and chlorpyrifos based on their volatilities. Irradiation time is another parameter can mainly affect on the extraction of analyte in MA-HS-SPME. It can be seen that, recovery increases with time and reaches an optimum 10 min, and then not shown considerable changes. This indicates that, butachlor and chlorpyrifos might be lost due to their volatilities with increasing microwave irradiation time. Finally, to validate the optimized method, a preliminary validation of the possible use of the optimized method for determining butachlor and chlorpyrifos in urine was performed using spiked samples and standards. The intra-day and inter-day relative standard deviation of the method was investigated by spiking urine sample with butachlor and chlorpyrifos. Linear standard curves (extracted) over the range of 10 and 100 ng/ml were obtained each day (n = 6) with a correlation coefficient of 0.991 or higher. Relative standard deviation of low concentration (10 ng/ml) was greater than high concentration (100 ng/ml) in all days. However, the optimized method is enough sensitive for both range of applied concentrations. Different concentration of 0.1-250 ng/ml was applied for butachlor and a linear calibration curve was obtained. Also, a similar linearity was obtained for chlorpyrifos, using a range concentration of 0.1-500 ng/ml. The limit of detection (LOD) was obtained for butachlor and chlorpyrifos were 0.086 and 0.083 ng/ml respectively. Further experiments of reproducibility of the method were carried out on spiked urine samples to validate the possibility of using the optimized MA-HS-SPME for measuring butachlor and chlorpyrifos when an environmental study and biological monitoring of worker exposed to such pollutant are required. The results indicated that, the developed MA-HS-SPME-GC-ECD method was simple, fast, selective, suitable and reliable for environmental and biological monitoring of butachlor and chlorpyrifos pesticides.

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