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ARTICLES

Optimization of Solid Phase Microextraction Procedure for Determination of Paraquat Using Reduction Process[1]

Fateme Ghavidel[a], Seyed Jamaleddin Shahtaheri[b,*], Marzieh Torabbeigi[c], and 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] *Health, Safety and Environment School, 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*

**e-mail: shahtaheri@tums.ac.ir*

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Abstract—Headspace solid phase microextraction (**HS-SPME**) followed by gas chromatography–mass spectrometry was developed for the determination of paraquat in urine. The volatile product resulting from reduction of paraquat by sodium borohydride–nickel chloride was used for HS-SPME. The calibration curve was linear from 10–1000 ng/mL and the limit of detection was 0.1 ng/mL. The optimized methods were validated using 500 and 750 ng/mL concentrations of paraquat in the spiked urine samples. The recoveries obtained in this study were 98.2% for intra-day (n = 6) and 99.2% for inter-day (n = 6), respectively, with RSD lower than 1.1%.

Keywords: paraquat, sodium borohydride–nickel chloride, headspace solid phase microextraction (HC-SPME), gas chromatography–mass spectrometry, urine samples

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Paraquat is one of the most highly toxic herbicides, which has been widely used for the last 60 years. Nowadays, it is used on many crops in about 100 countries. Paraquat can enter the body mainly by swallowing, or through damaged skin, as well as inhalation [1–3]. After skin exposure, small amounts of this herbicide may remain in muscle tissue and then slowly release into the blood [4]. The paraquat chronic effects can include Parkinson's disease and presumably cancer (skin, leukemia, brain, non-Hodgkin's lymphoma, and breast) [5, 6]. Lungs are the main target organ of paraquat poisoning; however, heart, liver, and kidney can also be affected by this compound [7].

The California Environmental Protection Agency considers paraquat as neurotoxicant that can penetrate the nervous system and impact brain functions. The development of brain functions in childhood can be harmfully affected by exposure to paraquat, even in relatively low doses [8, 9]. During last decades, sample preparation procedures have been developed for different organic pollutants including polycyclic aromatic hydrocarbons, pesticides and volatile organic compounds [10–15]. Also, a number of analytical methods have been reported for identification and quantitation of paraquat in biological specimens, which includes radioimmunoassay [16], spectrophotometry [17, 18], gas chromatography [19], HPLC [20, 21], capillary electrophoresis [22, 23] and liquid chromatography–mass spectrometry [24, 25]. Some of these methods are multistage, expensive, laborious, and time-consuming. Modern microextraction techniques such as solid phase microextraction developed by Pawliszyn et al. [26] are rapid, inexpensive, solvent free and quantitative. The development of SPME for pesticide analysis is increasingly interested and has been applied for the determination of pesticides in various environmental and biological samples [27–29]. In SPME procedure the volatile analytes can be adsorbed from a headspace of sample by using a fiber.

The aim of this study was to develop a fast and sensitive method for the determination of paraquat, a non-volatile compound, with HS-SPME by converting it to a volatile compound by reduction procedure. For this purpose, the reduction of paraquat was performed by sodium borohydride–nickel chloride reduction [30]. In this study, after reduction of paraquat with $NiCl_2$–$NaBH_4$, HS-SPME was used as a sample preparation technique and the analyte was

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detected and quantified by gas chromatography–mass spectrometry (**GC–MS**). Through this study, the detection limit was lowered to ng/mL.

EXPERIMENTAL

Reagents and materials. Paraquat dichloride (**PQ**) (1,1'-dimethyl-4,4'-bipyridinium dichloride hydrate) and ethyl paraquat dibromide (**EPQ**) (1,1'-diethyl-4,4'-bipyridinium dibromide, both 99.9%), used as internal standards (**IS**), were purchased from Sigma–Aldrich (Germany). The paraquat standard stock solution (1000 mg/L) was prepared in deionized water. Nickel chloride ($NiCl_2$, analytical grade) sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), and sodium chloride (NaCl) were purchased from Merck (Germany). The working solution of IS, ethyl paraquat dibromide, with concentration 100 μg/mL was prepared. Deionized water was produced by a Milli-Q Reagent Water System. Working standard solutions of paraquat were freshly prepared daily by volume dilution in the HPLC grade water. Nickel chloride (10%, w/v) and $NaBH_4$ (40%) solutions were prepared fresh before every analysis by dissolving 1 and 4 g, respectively, of each material in 10 mL of deionized water.

Biological samples. After obtaining approval from Tehran University of Medical Sciences (Vice-Chancellor for Research), all participants signed related consent form for the voluntary participation. A limited number of healthy and non-exposed persons were randomly selected for this study. Urine samples were collected in the mornings under the documented consent and stored at 4°C until use.

Reduction of paraquat in volatile product. The chemical reduction of paraquat including $NaBH_4$–$NiCl_2$ was done according to the Kanno et al. report [31]: 400 μL of 10% (w/v) $NiCl_2$, 200 μL of 40% (w/v) $NaBH_4$, 25 mL of EPQ (100 μg/mL) and paraquat solution were added to a 15 mL plastic tube. The tube was vortexed to mix the solution, and the reduction was done for 1.5 h. The solution was centrifuged for 10 min at 2000 rpm, 5 μL of sodium hydroxide (5 M) was added to terminate the liberation of hydrogen. The resulted volatile product from the reduction of paraquat was isolated from the gas mixture by solid phase microextraction.

HS–SPME procedure. The sample was extracted via the HS-SPME procedure using a 100 μm PDMS-coated fiber mounted in a manual syringe holder (Supelco, Bellefonte, PA, USA). The fiber was conditioned by inserting it in the injection port of the gas chromatograph for 0.5 h at 250°C, according to the instructions by the manufacturer. SPME procedure was performed in biological samples by placing 500 μL of urine sample and appropriate volumes of pesticide solution at two-level concentrations of 500 and 750 ng/mL, these levels were selected as spiked samples in order to optimize variables of interest.

Five milliliters of the solution resulted from the reduction procedure was transferred in to a 10-mL vial. The vial was rapidly sealed with a silicone septum and aluminum cap and heated up to 70°C in aluminum block heater. Samples were magnetically stirred during extraction (400 rpm). SPME needle was passed through the vial septum in order to expose the fiber (100 μm PDMS) on the headspace of the sample. After 20 min, the needle was removed from the vial and inserted into the injection port of the GC–MS for thermal desorption of the sample for 5 min.

Gas chromatography. GC–MS analyses for paraquat were performed using a gas chromatograph model CP 3800 coupled with a mass selective detector model Saturn 2200. Chromatographic separation was achieved on a VF-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min in a constant flow rate mode. Mass selective detector was operated by electronic impact (70 eV) in selected ion monitoring (SIM) mode. The injector port and interface temperature were 280°C and ion source temperature was 220°C. The injector was operated in splitless mode. The oven temperature was maintained at 80°C for 1 min; programmed at 10 grad/min to 200°C and 20 grad/min to 280°C with a hold at 280°C for 10 min.

Data analysis. The statistical calculations and analysis were performed using SPSS version 16. Some tests such as paired *T*-test and Scheffe analysis of variance were used for data analyzing. The level of significance was taken as $p < 0.05$. In order to optimize the method, a constant concentration was used for each pesticide. For each level of intended variables, three samples with defined concentration were prepared and the mean of extraction efficiencies calculated after analyzing the analytes. Then, according to the obtained data, the optimum level was selected for each variable.

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometery. The obtained chromatogram in this study is shown in Fig. 1. The resulted fragmentation pattern by electron impact mass spectrometry of perhydrogenated paraquat is shown in Fig. 2. The spectrum is similar to that reported by Kanno et al. [31] showing the parent ion (*m/z* 196) and two predominant mass fragments (*m/z* 181, 96). The list of target and qualifier ions of perhydrogenated paraquat is shown in Table 1. For the quantitative determination, the calibration curve of paraquat was established by the peak area of mass fragment *m/z* 196.

Method optimization. The conditions of chemical reduction of paraquat in the sample medium were optimized using one affecting parameter at the time

approach. The effects of reaction time (30, 60 and 90 min), sodium borohydride (40%, w/v) quantification (100, 200, 400 and 600 μL) and pH (7–9) on the reduction reaction were investigated by 15 mL of paraquat solution (250 ng/mL) [2, 30, 32]. The final chromatographic performance showed that 0.2 μL of 40% $NaBH_4$, reaction time of 90 min, and pH 8 were the optimum conditions for the chemical reduction process.

The parameters that affect the HS-SPME procedure, including time of extraction and temperature, were investigated. The effect of extraction time was evaluated by exposing the fiber to the sample headspace at different extraction times, including 5, 10, 20, and 30 min (Fig. 3). The extraction time higher than 20 min showed just a slight increase in the signal, therefore, 20 min was selected as optimum time. The effect of temperature on the extraction efficiency was examined by varying temperature from 50 to 80°C. The efficiency increased by increasing temperature from 40 to 70°C. Thereafter, the temperature had no significant effect ($P < 0.001$, $n = 6$) on the extraction rate (Fig. 4). Therefore, 70°C was selected as an optimum extraction temperature.

Method validation. The method validation was performed under the optimized conditions. The limits of detection (**LOD**) and quantification (**LOQ**), the inter- and intra-day precision, linearity, and the recoveries were calculated: linear standard curve in the range of

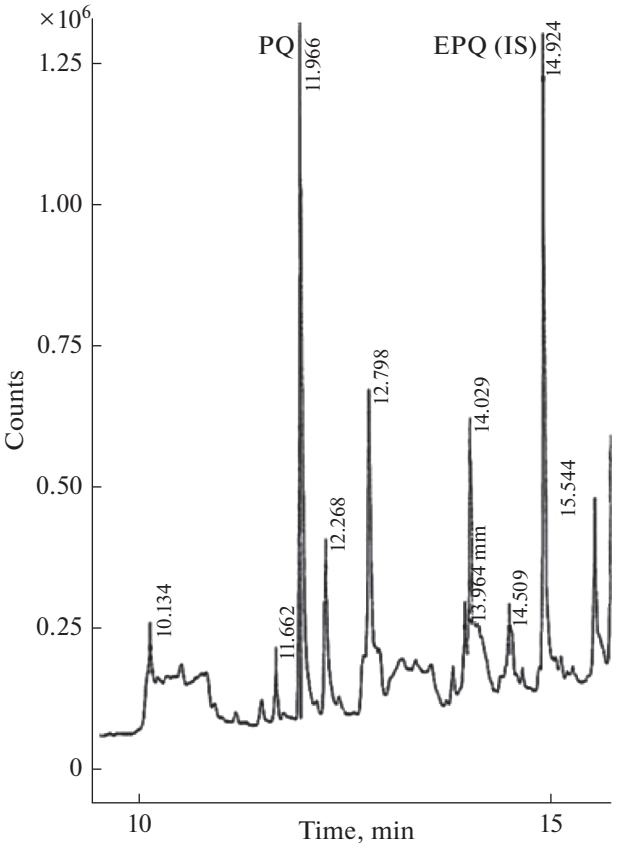

Fig. 1. Chromatogram of spiked sample with paraquat (0.5 μg/mL) and ethyl paraquat dibromide (IS) (0.5 μg/mL).

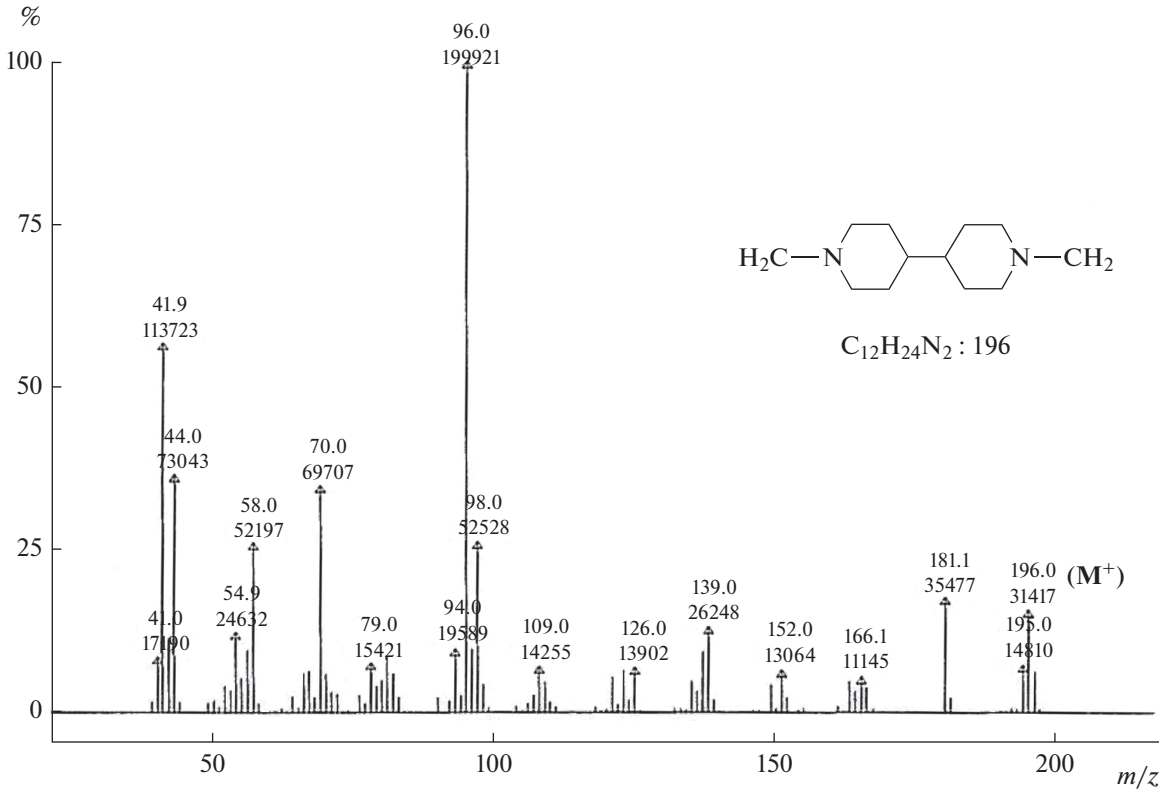

Fig. 2. Mass spectra and molecular structure of perhydrogenated paraquat.

Table 1. Target and qualifier ions AND retention times for the GC–MS assay of perhydrogenated paraquat in urine

Target compound	Target ion (*m/z*)	Qualifier ion (*m/z*)	Retention time, min
Perhydrogenated paraquat	96	196.181	11.96

Table 2. Inter- and intra-day reproducibility of the optimized SPME method for spiked urine samples ($n = 6$)

Concentration, ng/mL	Inter-day		Intra-day	
	recovery, %	RSD, %	recovery, %	RSD, %
500	98.62	1.057	98.19	1.103
750	99.22	0.742	99.04	0.791

10–1000 ng/mL was obtained each day for 6 consecutive days; the obtained correlation coefficients (r^2) were 0.995, LOD and LOQ—0.107 and 0.325, respectively. The intra- and inter-day precisions of the method were determined by two spiked extracted samples at concentrations of 500 and 750 ng/mL (Table 2). Inter-day reproducibility was studied by analysis of spiked samples once in six consecutive days, and intra-day reproducibility was done by analyzing spiked samples 6 times on each day. Figure 1 shows the chromatogram of urine sample with spiked pesticides. The optimized extraction protocol was followed along with optimum chromatographic conditions. On each day of analysis paraquat was determined by the calibration curve generated on that day. Intra-day reproducibility of the procedure was evaluated by calculating the relative standard deviation (**RSD**) (Table 2). The resulting RSDs illustrate that this method is much more precise at two concentrations. The recoveries obtained by the SPME–GC–MS method are between 98.2–99.2%, as shown in Table 2.

For further comparison, LODs and linearity ranges of some previous similar studies have been tabulated in Table 3. The obtained results indicated that the proposed method is easy, fast, precise, and reproducible with a wide linear range and does not require any toxic solvent for extraction. In addition, a successful application of the HS-SPME technique was optimized to determine paraquat at a low concentration level in urine samples. Finally, validation of methods for determining paraquat in the spiked urine sample was performed. Inter-day and intra-day RSDs of the method were evaluated by spiking urine sample with paraquat. As Table 2 shows, the developed SPME–GC–MS method is enough efficient, precise, and accurate for quantification of paraquat in biological samples.

The HS-SPME–GC–MS procedure seems to be a useful and convenient method for qualitative and quantitative evaluation of paraquat in biological samples. This technique is simple, inexpensive, and fast compared to the more conventional methods. Through this study, the optimum conditions have been established. Also, validation of method for determining paraquat in urine sample was performed. Inter- and intra-day RSD of the method was evaluated by spiked urine sample with paraquat. The results indicated that the developed HS-SPME–GC–MS method was suitable for monitoring paraquat in biological medium.

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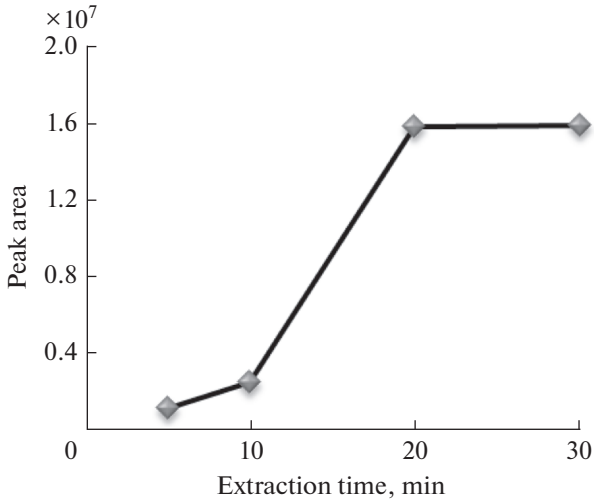

Fig. 3. Influence of extraction time on the extraction efficiency.

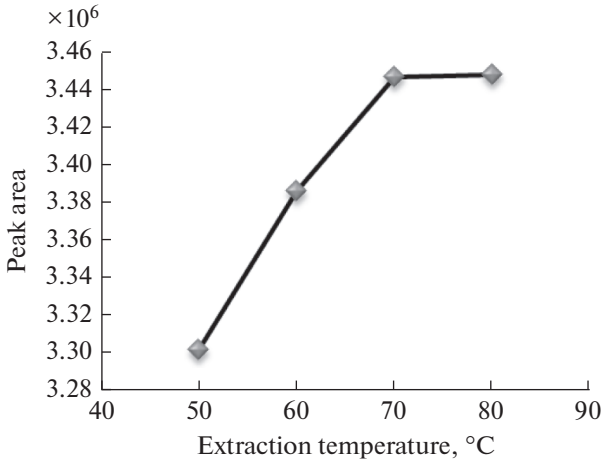

Fig. 4. Influence of extraction temperature on the extraction efficiency.

Table 3. Limit of detection and linearity range of some previous similar studies

Method of extraction	LOD	Linearity range	Reference
LLE–GC–MS	0.016 μg/g	0.0156–0.5 μg/g	[32]
SPE–GC–MS	50 ng/mL	100–50000 ng/mL	[2]
SPME–GC–MS	10 ng/mL	100–50000 ng/mL	[30]
SPME–GC–MS	0.1 ng/mL	10–1000 ng/mL	Present work

LLE—liquid–liquid extraction, SPE—solid phase extraction

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