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Optimization of Hollow Fiber Liquid-Phase Microextraction Combined with GC-ECD for Determination of Organochlorine Pesticides in Orange Juice

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The hollow fiber protected liquid phase microextraction (HF-LPME) technique was evaluated for the extraction of organochlorine pesticides in orange juice prior to gas chromatography-electron capture detection (GC-ECD) analysis. At first, the effects of the HF-LPME experimental parameters on the enrichment factor were studied simultaneously using a 2^{5-1} fractional factorial design. The HF-LPME variables of interest were extraction time, stirring speed, acceptor phase, salt (NaCl, w/v %) and pH. In the next step, a two-level plus centre point fractional factorial experiment was performed to evaluate the effects of three parameters, including the extraction time, stirring speed and pH of donor phase. The optimized HF-LPME conditions were as follows: an extraction time of 20 min, a stirring speed of 800 rpm, an acceptor phase of toluene, no salt and a source pH of 6. The extraction calibration plots were linear over the range of 0.25-10 ng mL^{-1} (r^2: 0.9863-0.9995) and the limits of detection for ten pesticides studied were from 0.012 to 0.456 ng mL^{-1}. Good enrichment factors were achieved (66-251-fold) with this method and good repeatabilities were obtained, with relative standard deviations below 19.9 %.

Keywords: Chlorinated pesticides, Experimental design, GC-ECD, Hollow fiber microextraction, Juice.

INTRODUCTION

Pesticides are used on a large scale for agriculture purposes and their adverse effect on both human health and the environment are a matter of public concern[1]. Although most of the chlorinated pesticides have been banned decades ago, they can be still found in the environment due to their lipophility and their resistance to metabolism processes. The organochlorine pesticides are included in the group of persistent of organic pollutants and linked to the carcinogenicity and endocrine disruption in mammals and concerns over the toxicity are exacerbated by pollutant hydrophobicity, which results in bioaccumulation in fatty tissues and biomagnifications through food chains[2].

Consumption of plants or plants products such as fruit and juice is a major part of the food consumption of human beings. Orange is one of the most widely grown fruit in Mediterrian area of Turkey and is the most exported product in Turkey. Hence, there is an increasing demand for developing rapid, reliable and inexpensive method for the determination of such contaminants in fruit and juice. Many methods have been developed in the last years for the determination of organochlorine pesticides. The most widely used methods are based on gas chromatography and high-performance liquid chromatography[3-6]. For the analysis of organochlorine pesticides in food, sample preparation are required in order to isolate the analytes from the complex matrices, remove interfering compounds and achieve a sufficient sensitivity. A wide variety of conventional sample preparation techniques have been used to extract and purify pesticides from fruits and vegetables, including liquid-liquid extraction[7,8], solid-phase extraction[9-11] and supercritical fluid extraction[12]. These techniques, which are easy to carry out and provide excellent recovery, are, however, time-consuming, tedious and hazardous to analysts' health due to large volumes of organic solvents involved.

More recently, microextraction techniques have emerged as novel sample preparation technique in matrices such as food[13-16]. The commonly used microextraction techniques involve solid-phase microextraction (SPME)[5,6,17], liquid-phase micro-extraction (LPME)[18-20]. Solid-phase microextraction is a solvent-free technique developed by Pawliszyn in 1990s, however, the fiber required is fragile and expensive and its lifetime is limited. During the past few years several techniques have been developed for LPME, including single-drop micro-extraction[13] (SDME), hollow fiber LPME (HF-LPME)[21,22] and stir bar microextraction[23] (SBME). Because of its minimization of hazardous solvent usage, LPME is of interest to many analysts. Single-drop microextraction combines extraction, concentration and sample introduction in a single step and

overcomes a few disadvantages of conventional liquid-liquid extraction (LLE), such as time-consuming, use of large amounts of toxic organic solvent[24]. However, the stability of the suspended organic drop is easily affected by temperature, stirring rate and air bubbles[18]. To improve the solvent stability, HF-LPME was thus developed. Since the organic phase is protected by the hollow fiber, the stability is greatly improved and higher stirring rates can be applied to reduce the equilibrium and extraction times. HF-LPME can be performed a small length of porous hollow fiber-protected solvent. This technique is inexpensive and there is considerable freedom in selecting appropriate solvents for extraction of different analytes. Since very little solvent is used, there is minimal exposure to toxic organic solvent for the operator. At the same time, it combines extraction, concentration and sample introduction in one step.

The goal of this study to optimize and validate a simple, rapid and inexpensive, HF-LPME-gas chromatography with electron capture detection methodology for the determination of ten organochlorine pesticides in orange juice. To out best of knowledge, no previous works dealing with the HF-LPME extraction of these organochlorine pesticides from orange juice.

In the first step, the experimental parameters of HF-LPME, such as extraction time, stirring speed, salt concentration, organic solvent and pH, were optimized using a fractional factorial design (FFD) based on 2^{n-1}. In the next step, the parameters that were found to affect the HF-LPME were optimized. Optimization was performed using orange juice spiked organochlorine pesticides. Under the optimal conditions, precision, linearity, limits of detection (LOD) and accuracy of the proposed method were further evaluated.

The proposed procedure was successfully applied for extraction and quantification of organochlorine pesticides in orange juice.

EXPERIMENTAL

Pesticides standards each at a concentration of 0.2 mg mL^{-1} in dichloromethane or methyl alcohol, were purchased from AccuStandard, Inc. (New Haven, CT, USA). NaCl, NaOH and HCl (37 %, analytical grade) were purchased from Merck (Darmstadt, Germany). The extraction solvents 1-octanol and toluene were purchased from Merck (Darmstadt, Germany) and deionised water was obtained using a Millipore ELIX 3 (USA) water purification system.

The working standard solutions of the organochlorine pesticides were prepared from stock solutions (0.2 mg mL^{-1}) and were stored in a freezer at 4 °C. Working standard solutions were prepared at a concentration of 10 μg mL^{-1}. Orange juice samples were purchased from the local market.

The polypropylene hollow fiber (600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) was a gift from membrane GmbH (Wuppertal, Germany). The Bandelin Sonorex ultrasonic bath (RK 51OH, Germany) was used for hollow fiber pre-washing.

The pH meter was Mettler Toledo (MP 220, USA). A magnetic stirrer (MS-H-Pro Magnetic Stirrer, Dragon Lab, USA) with a magnetic bar (10 mm length and 3 mm diameter, Supelco, USA) was used for mixing the orange juice samples. Sample vials with PTFE-silicone septa (15 mL) were obtained from Supelco (Bellefonte, PA, USA).

Chromatographic conditions: Analyses of pesticides were performed using GC (Thermo Quest, USA) equipped with an electron-capture detector at 300 °C. The analytical capillary column ZB-5 (30 m × 0.32 i.d × 1.0 μm film thickness) was obtained from Phenomenex (USA). The oven temperature was programmed as follows: Initial temperature, 150 °C held for 0.5 min, then at rate of 20 °C min^{-1} to 230 °C (hold 2 min), 20 °C min^{-1} to 260 °C (hold 4 min) and 20 °C min^{-1} to 280 °C maintaining this temperature 8 min. The injection temperature was 250 °C, N_2 was used as the carrier gas at 1.5 mL min^{-1} and make-up gas at 30 mL min^{-1} (purity ≤ 99.995 %).

HF-LPME procedure: A 5 mL orange juice sample of analyte-spiked which was centrifuged at 5000 rpm for 3 min was placed in 15 mL glass vial and 5 mL deionized water was added. The hollow fiber was cut into a 15 mm length and ultrasoniced in acetone to remove the contaminant and dried in the air. A 10 μL microsyringe was first filled with the acceptor phase (toluene or 1-octanol) and a 15 mm fiber was mounted on it. The fiber was impregnated by immersing in the acceptor phase for 10 s. Then, the fiber lumen was filled the acceptor phase by pushing the plunger while the fiber was being held in the acceptor. The bottom of end of the fiber was closed by squeezing with a warm pincers to prevent leakage. Then, the fiber was immersed in orange juice sample. After an extraction period (10 or 40 min) 2 μL of the acceptor phase was withdrawn into the syringe and injected into the GC. The used fiber was discarded and a fresh one was used for the next extraction.

Design of the experiments: In this work, the effects of five factors on the experimental response (*e.g.*, the peak areas of the studied organochlorine pesticides) were investigated using 2^{5-1} fractional factorial design. These five factors were the following: Extraction time, stirring speed, acceptor solvent, NaCl % and pH. The values investigated in these experiments and their coded levels are listed in Table-1. The statistical significance of each individual factor and their combinations were evaluated at the 95 % confidence level using Minitab 15 software (Minitab Inc., State College, PA, USA). After obtaining these results, a new fractional factorial design with medium points was applied to optimize three selected factors: extraction time, stirring speed and pH. The values of each the factors examined in this study as well as the design matrix are given in Table-2.

RESULTS AND DISCUSSION

Screening design: The screening of experimental factors is the first step in an efficient assessment of any analytical system. If large numbers of factors are involved, reduced factorial designs, such as 2^{n-1} fractional factorial designs, are applied. By applying such schemes, it is possible to detect the most significant factors or variables with only a few experiments.

In this study, a 2^{5-1} fractional factorial design was performed to study the influence of five factors on the HF-LPME procedure and to find out the optimal experimental conditions. The experimental sequences were randomized to minimize the effects of the uncontrolled factors. The results obtained from the evaluation of the significant factors by fractional factorial design are shown in Fig. 1 using Pareto charts. The

TABLE-1
DESIGN MATRIX FOR THE SCREENING DESIGN (2^{5-1})

Factor	Code	Level	
		Low	High
Extraction time (min)	A	10	40
Stirring speed	B	400	1000
NaCl (%, w/v)	C	0	20
pH	D	2	8
Acceptor phase	E	Toluene	1-Octanol

Run	A	B	C	D	E
1	40	400	20	2	1-Octanol
2	10	1000	0	8	1-Octanol
3	40	1000	0	8	Toluene
4	40	400	0	2	Toluene
5	10	400	20	2	Toluene
6	10	400	0	8	Toluene
7	10	1000	20	2	1-Octanol
8	40	400	20	8	Toluene
9	40	1000	20	2	Toluene
10	10	400	20	8	1-Octanol
11	40	1000	20	8	1-Octanol
12	40	1000	0	2	1-Octanol
13	10	1000	0	2	Toluene
14	10	1000	20	8	Toluene
15	40	400	0	8	1-Octanol
16	10	400	0	2	1-Octanol

TABLE-2
FACTORS AND LEVELS APPLIED IN
2^{3-1} FRACTIONAL FACTORIAL DESIGN

Factor	Code	Level		
		Lower	Central	Upper
Extraction time (min)	A	10	20	30
Stirring speed	B	600	800	1000
pH	D	4	6	8

Run	A	B	D
1	30	600	4
2 (C)	20	800	6
3	10	1000	4
4	30	1000	8
5	10	600	8
6 (C)	20	800	6
7 (C)	20	800	6

Pareto charts in Fig. 1 illustrate the influence that each factor has on the response of some of the studied and total pesticides. In the Pareto charts, the bar lengths are proportional to the absolute values of the estimated main effects.

In all cases, the presence of NaCl (%, w/v) had a negative effect on the efficiency of pesticides extraction, *e.g.*, pesticides recovery is highest when no salt is present. Addition of salt to the samples may have several effects on the extraction process as it increases the ionic strength of the solution. Generally, addition of a certain amount of salt can decrease the solubility of analytes in the aqueous phase and enhance their partitioning into the organic phase. For HF-LPME, however, the presence of salt was found to restrict the extraction of analytes. Addition of salt increases the viscosity of the bulk solution, which reduced the rate of diffusion of analytes over fiber[21,25]. Therefore, further experiments were performed with out salt addition. According to Fig. 1 acceptor phase was the most significant factor in this study and the extraction efficiency was high when toluene was used. To further fine-tune the optimization, two

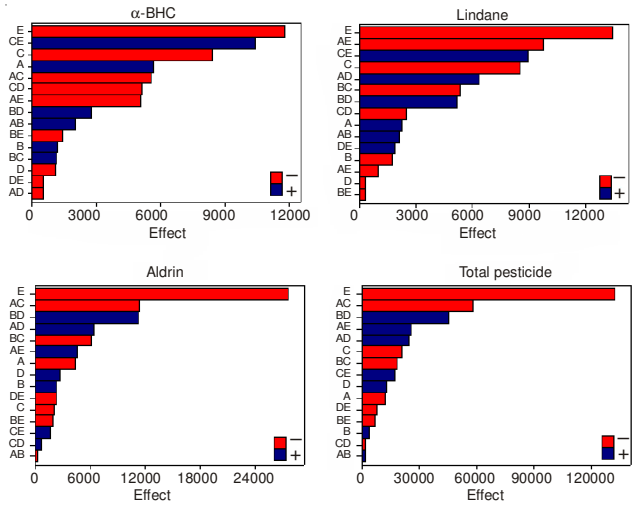

Fig. 1. Pareto charts for some selected pesticides; (A) extraction time; (B) stirring speed; (C) NaCl (%, w/v); (D) pH; (E) acceptor phase

of the five variables were fixed at appropriate values: toluene as acceptor phase and no added NaCl.

Stirring speed showed positive effects on the extraction efficiency for some pesticides while showing negative effects for some pesticides (Fig. 2). At high stirring speed, a vortex formed in the donor phase and the formation air bubbles on the surface of the fiber that caused a decrease on the extraction efficiency Thus, in order to investigate the influence of stirring speed on extraction efficiency, it was considered in the next step of design.

As shown in Fig. 1, the donor phase pH is the next most significant factor and exerts a positive effect on the extraction of the most of the pesticides. Although it is expected that there is no effect of pH on the extraction efficiency of organochlorine pesticides, pH adjustment yields better selectivity for organochlorine pesticides extraction efficiency as it alters the coefficient of dissociable species. In order to investigate the influence of pH on extraction efficiency, pH was considered in the next step of design.

According to Fig. 1, the extraction time also has a negative effect on the extraction efficiency in this study. At longer extraction times decreases on the extraction efficiency was observed. This decrease was due to the loss of acceptor phase from the fiber over the extraction process.

In conclusion, the parameters that exerted the most significant effects on the HF-LPME of selected organochlorine pesticides were extraction time, stirring speed and pH. These parameters were considered for further optimization using fractional factorial design with medium point.

Optimization design: In the next step, a 2-level fractional factorial design (2^{3-1} = 4 experiments + 3 centre points = 7 experiments) was employed to optimize the three factors selected using screening design. A three replicate centre points were included in the design estimate the experimental variance and check the loss of linearity between the levels chosen for each variable. The experiments were run in a random manner in order to minimize the effect of uncontrolled variables.

The examined levels of the factors are given in Table-2. These three variables and their low, central and high levels are as follows: Extraction time (10-20-30 min), stirring speed (600-

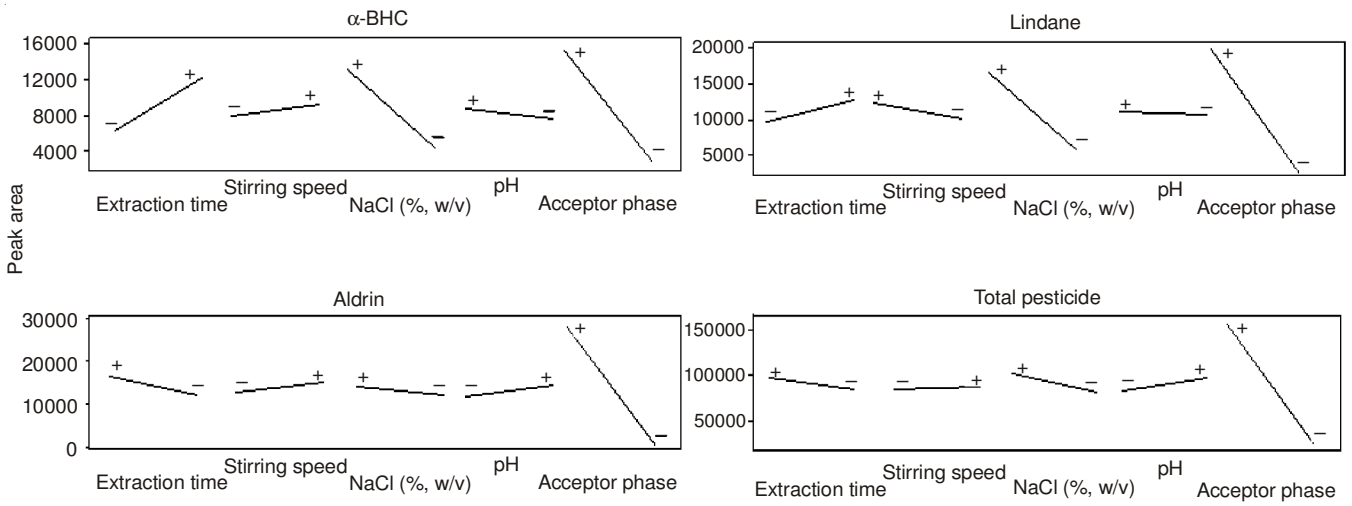

Fig. 2. Main effect plots of some selected pesticides

800-1000) and pH (4-6-8). The curvature effect value is high and positive, which means that there is a maximum analytical signal (peak area) around the centre point. Hence, the conditions of the centre points have been used for the following experiments (Fig. 3). To investigate the interactive effects of two factors on the experimental response (peak area) values, the three-dimensional surface was drawn by varying two of the process variables. Fig. 4 shows the Lindane 3-D surface plot.

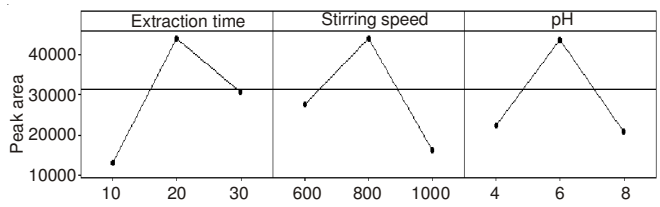

Fig. 3. Main effect plots of lindane

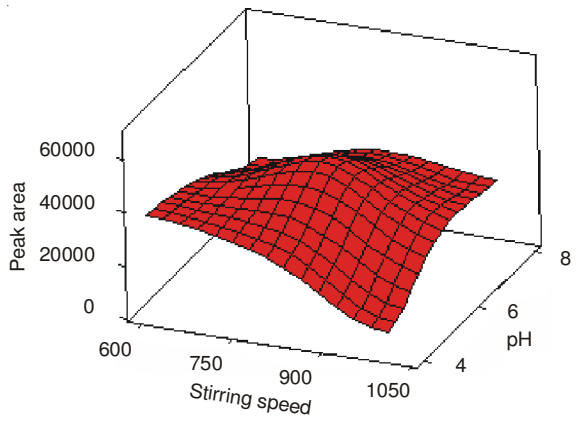

Fig. 4. Response surface for lindane by plotting of stirring speed *vs.* pH

According to the overall results of the optimization study, the following experimental conditions were chosen for subsequent experiments: 20 min extraction time, 800 rpm stirring speed, 0 % NaCl, toluene acceptor solvent and pH 6 (Table-3).

TABLE-3
OPTIMIZED VALUES OF THE VARIABLES

Optimized conditions	
Extraction time (min)	20
Stirring speed	800
NaCl (%, w/v)	0
pH	6
Acceptor phase	Toluene

TABLE-4
LINEAR RANGES, CORRELATION COEFFICIENTS, ENRICHMENT FACTORS, DETECTION LIMITS, REPEATABILITY AND REPRODUCIBILITY OF THE HF-LPME-GC-ECD METHOD FOR TEN OCPs IN THE ORANGE JUICE SAMPLES

Pesticides	r^2	Lineer range ng mL^{-1}	LOD ng mL^{-1}	EF[a]	RSD %[b] repeat-ability (n = 5)	RSD %[b] repro-ducibility (n = 3)
α-BHC	0.9995	0.50-10	0.019	217	9.0	9.7
Lindane	0.9995	0.25-10	0.040	72	10.6	14.8
Heptachlor	0.9903	0.25-10	0.031	128	6.7	13.2
Aldrin	0.9950	0.25-10	0.012	144	7.0	5.9
α-Endosulfan	0.9977	0.25-10	0.015	174	6.0	9.4
p,p'-DDE	0.9991	0.25-10	0.045	161	15.6	8.6
Dieldrin	0.9990	0.50-10	0.030	174	4.4	14.0
Endrin	0.9923	0.25-10	0.037	251	3.3	2.2
p,p'-DDD	0.9990	0.25-10	0.046	115	19.9	23.2
p,p'-DDT	0.9863	0.50-10	0.456	66	10.5	11.3

[a]Enrichment factor; [b]orange juice spiked with 10 ng mL^{-1}

Method of evaluation: Linear dynamic ranges (LDRs), coefficients of determination (r^2), relative standard deviations (RSDs), enrichment factor (EF) and limits of detection (LODs) based on a signal-to-noise ratio (S/N) of 3 were calculated using optimal conditions. The results are summarized in Table-4.

The enrichment factors were calculated by the following equation:

$$E = 1/(V_o/V_a + 1/K)$$

where K = distribution coefficient, V_o = volume of organic (acceptor) solvent and V_a = volume of aqueous sample (donor). K is calculated based on the two phase equilibrium condition.

$$K = C_{o,eq}/C_{a,eq}$$

where $C_{o,eq}$ = concentration of analyte in the acceptor phase and $C_{a,eq}$ = concentration of analyte in the donor phase.

The precision of the method was evaluated by calculating relative standard deviations, which were based on the peak areas of the replicate runs for orange juice sample. The relative standard deviations and enrichment factors ranged from 3.3 to 19.9 % and from 66 to 251, respectively. Fig. 5 shows the chromatograms of spiked and unspiked orange juices.

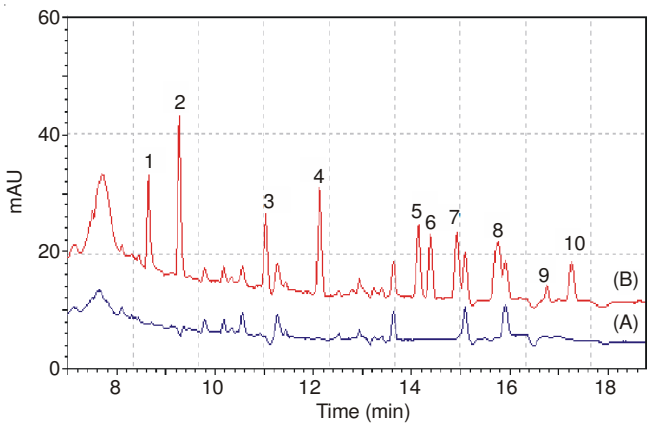

Fig. 5. Chromatograms of organochlorine pesticides obtained by HF-LPME under optimized conditions; (A) blank orange juice sample (B) orange juice sample spiked at 10 ng mL^{-1}. Peak identification: (1) α-BHC; (2) Lindane; (3) Heptachlor; (4) Aldrin; (5) α-Endosulfan; (6) *p,p'*-DDE; (7) Dieldrin; (8) Endrin; (9) *p,p'*-DDD; (10) *p,p'*-DDT

Conclusion

In this study the HF-LPME technique has been successfully developed and optimized for the determination of organochlorine pesticides in orange juice. To optimize the HF-LPME conditions, a fractional factorial design (FFD) based on 2^{5-1} and a 2-level fractional factorial design (2^{3-1}) plus three centre points were employed. The optimal experimental conditions found from this statistical evaluation included: extraction time of 20 min, stirring speed of 800 rpm, percent NaCl of 0, acceptor solvent of toluene and pH of 6. The method provides a good enrichment factor, good repeatability and reproducibility and low detection limit for the spiked orange juice samples. Compared to the traditional methods such as LLE, this method provides minimal consumption of organic solvent, which is in agreement with green chemistry. The proposed method is simple, inexpensive, fast and can be successfully applied for preconcentration and determination of pesticides in different fruit juice samples.

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