

APPLICATION OF FACTORIAL DESIGN TO THE STUDY OF PARAMETERS IN THE DETERMINATION OF PESTICIDE RESIDUES IN APPLE BY HEADSPACE SOLID PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Lukman Bola Abdulra'uf^{1,2*} and Guan Huat Tan^{1*}

¹ Department of Chemistry, Faculty of Science, University of Malaya, Lembah Pantai, 50603, Kuala Lumpur, Malaysia

² School of Basic and Remedial Studies, Kwara State College of Education, P.M.B 1527, Ilorin, 240244, Kwara State, Nigeria

* Author for correspondence; E-mail: abdulraflukman@siswa.um.edu.my, Tel. +60102343957

Abstract: Solid-phase microextraction (SPME) is a solvent-less sample preparation method which combines sample preparation, isolation, concentration and enrichment into one step. SPME process involves two basic steps which are the partitioning of analytes between the polymeric fiber coating and the sample matrix, and desorption of the concentrated extracts into the analytical instrument, without any clean-up step. In this study, factors affecting the solid phase microextraction of pesticide residues (fenobucarb, diazinon, chlorothalonil and chlorpyrifos) were studied using a factorial design. The interactions and effects of temperature, time and salt addition on the efficiency of the extraction of the pesticides were evaluated using 2^3 factorial designs. A 10 g sample was weighed into 20 mL amber glass vial, fortified with the standard mixture at three concentration levels (10, 50 and 100 $\mu\text{g.L}^{-1}$) and allowed to rest for 2 hrs. Optimum dilution was made with 10 mL of distilled water containing 10% NaCl and the mixture was shaken ultrasonically for 3 min. The analytes were then extracted with 100 μm PDMS fibers according to the factorial design matrix and desorbed into gas chromatography-mass spectrometry detector. The developed method was applied for the analysis of apple samples and the limit of detections was between 0.01 and 0.2 $\mu\text{g.L}^{-1}$, which were lower than the MRLs for apples. The relative standard deviations were between 0.1 and 13.37% with average recovery of 80 to 105%. The linearity ranges from 0.5 – 50 $\mu\text{g.L}^{-1}$ with RSD greater than 0.99.

1. Introduction

Sample preparation is the most crucial and critical steps in the analysis of pesticide residues from complex fruit and vegetable matrices [1]. Solid phase microextraction is a solvent free sample preparation and extraction technique developed by Pawliszyn and his co-workers in 1990 [2]. It is an efficient, simple, versatile and effective adsorption/absorption and desorption technique with minimum matrix interference. It eliminates the use of toxic solvents and combines sampling, isolation, concentration and enrichment in one step [3, 4]. It was developed to overcome the problems associated with solvent-based, time consuming techniques, such as, liquid- liquid extraction (LLE), solid phase extraction (SPE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) [5]. The traditional techniques also required a large volume of sample and

solvents which impose environmental pollution and health hazards [6]. The SPME technique is based on the use of a fused silica or metal alloy that is coated on the outside with an appropriate polymerized stationary phase, attached to a stainless steel, mounted on a fiber holder housed in a modified syringe [7, 8]. The SPME process involves two basic steps which are the partitioning of analytes between the coating and the sample matrix, and the desorption of the concentrated extracts into analytical instruments [3]. There is no need for any clean-up step [9], thus preventing lost and contamination of target analytes.

Previous studies in the development of SPME methods have used univariate approach, in which each factor is optimized once at a time and involve many experiments [10]. The univariate approach is time consuming and does not allow the determination of interactions between the extraction conditions. For this experiment, extraction temperature, time and salt addition were varied at two levels selected for the factorial design: 30 $^{\circ}\text{C}$ and 60 $^{\circ}\text{C}$, 30 and 60 min, and 5 and 10% of NaCl respectively (Table 1).

2. Materials and Methods

2.1 Standard Reagents and Solutions

Pesticide standards (fenobucarb, diazinon, chlorothalonil and chlorpyrifos) were obtained from Accustandard Inc., New Haven CT, USA with percentage purity greater than 97%. All the solvents used for this study were pesticide grade and were purchased from Fisher Scientific, Loughborough, UK. Stock standard solutions of 100 mg.L^{-1} of each compound was prepared in pesticide grade methanol and stored at -18°C . Working standard solutions containing the pesticides were prepared weekly by diluting the stock solution in methanol to concentration of 10 mg.L^{-1} .

2.2 Sample Preparation

A 10 g chopped sample was weighed into 20 mL amber glass vial, fortified with the standard mixture at three concentration levels and allowed to rest for 2 hrs. Optimum dilution was made with 10 mL of distilled water containing 10 % NaCl and the mixture was shaken ultrasonically for 3 min. The analytes were then extracted with 100 μm PDMS fibers (obtained

from Supelco, Bellefonte, PA, USA) in the headspace mode [11], according to the factorial experimental design (Table 2). To study the effect of these factors and their possible interactions, a 2^3 randomized-block experimental design was conducted. Repeatability study was performed using a standard solution at 10, 50 and 100 $\mu\text{g.L}^{-1}$ with two replicates.

2.3 GC-MS Analysis

The extracted analytes were analyzed by GC/MS. The capillary column used was DM-5MS of 30 m x 0.25 mm x 0.25 μm i.d. The oven temperature program was as follows: initial oven temperature 60 $^{\circ}\text{C}$, held for 2 min then ramped to 180 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C.min}^{-1}$ and finally increased to 280 $^{\circ}\text{C}$ (12 min) at 5 $^{\circ}\text{C.min}^{-1}$. The MS transfer line temperature was 260 $^{\circ}\text{C}$, ion source 230 $^{\circ}\text{C}$ and ionization model 70eV. The analysis of target analytes were carried out in selected ion monitoring (SIM) mode. One target and two qualifier ions were monitored (Table 3). The first ion is the target and other two are reference ions

Table 1: Factors and levels of the variables

Variables	Low	High
Extraction time (min)	30	60
Extraction temperature ($^{\circ}\text{C}$)	30	60
Salt concentration (% w/v)	5	10

Table 2: Factorial design matrix ^a

Run Order	Std Order	Block	Time (min)	Temp. ($^{\circ}\text{C}$)	Salt (%)
1	3	1	30	60	5
2	2	1	60	30	5
3	1	1	30	30	5
4	8	1	60	60	10
5	7	1	30	60	10
6	5	1	30	30	10
7	4	1	60	60	5
8	6	1	60	30	10
9	10	2	60	30	5
10	12	2	60	60	5
11	14	2	60	30	10
12	13	2	30	30	10
13	16	2	60	60	10
14	15	2	30	60	10
15	9	2	30	30	5
16	11	2	30	60	5

^a Generated using Minitab statistical software

Table 3: Fragment ions of pesticides used for quantification

Pesticides	Mol Wt	Ret. time(min)	Ion (m/z)
Fenobucarb	207.3	24.25	121, 91, 150
Diazinon	304.35	28.40	179, 137, 152
Cholorothalonil	265.91	29.64	266, 263, 268
Thiobencarb	213.66	32.37	97, 144, 197

3. Results and Discussion

3.1 Optimization of Parameters

The extraction of pesticides by SPME technique depends on the type and volume of fiber coating and the distribution constant between the stationary phase and the sample matrix. The matrix related condition is varied by salt addition, the extraction temperature and time. The three factors were chosen based on the thermodynamic and kinetic theories of SPME. The thermodynamic theory is used to evaluate the amount of analyte extracted, while the kinetic theory estimates the rate of extraction. The extraction is a dynamic partitioning process between the target analytes and the fiber, and there is a maximum sensitivity at equilibrium [12]. To study the effect of extraction temperature, time and salt addition (NaCl) on the extraction of pesticide residues, a factorial 2^3 randomized-block experimental design was applied. The design matrix is shown in Table 2. The design allows the assessment of the main effect and interactions between the selected conditions (Table 4). Temperature showed positive effect, salt addition was also found to have positive effect on all the pesticides investigated, while time showed a positive effect on all except fenobucarb, which is attributed to its lower boiling point.

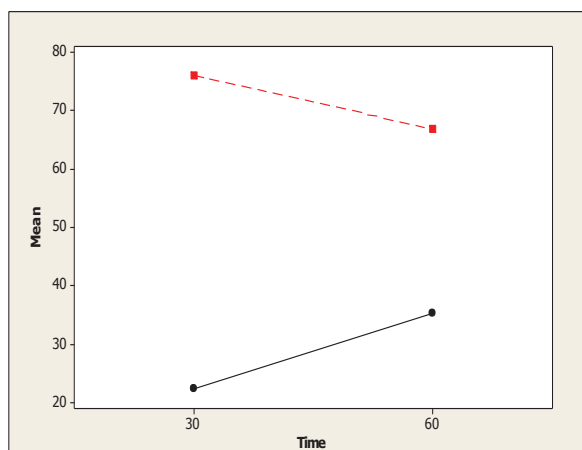
As shown in Table 4, temperature, time and NaCl addition were significant for all analytes. Moreover most interactions were also significant. Temperature showed a positive interaction with time for all analytes, it also showed a positive interaction with salt addition and positive interaction between time and salt addition for diazinon and chlorpyrifos, while salt addition showed negative interaction with temperature and time in fenobucarb and chlorothalonil. This different interactions is as a result of the pesticides different solubility in water with diazinon and chlorpyrifos been more soluble, because they are triprotic. As shown in Table 4, positive effect implies that the factors enhance extraction efficiency and gave better peak areas, while negative effect showed that the interaction reduced extraction efficiency. The time – temperature interaction plots (Fig 1) showed the impact of change in extraction temperature on time (average peak area vs extraction time)

Table 4: Main effect, interactions between factors for pesticide residues ^a

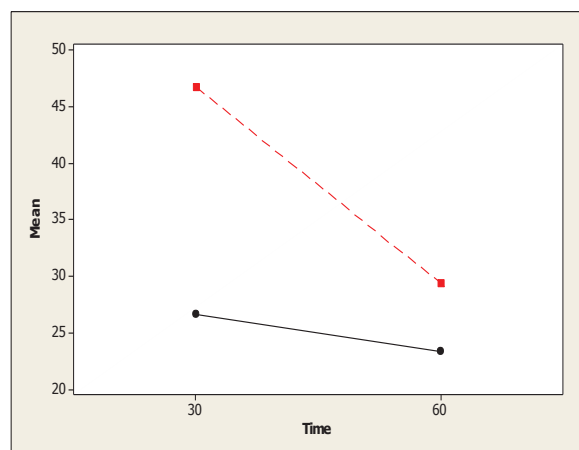
Pesticides	T	t	S	T, t	T, S	t, S	T, t, S
Fenobucarb	+	+	+	+	-	-	-
Diazinon	+	+	+	+	+	+	+
Cholorothalonil	+	+	+	+	-	-	-
Chlorpyrifos	+	+	+	+	+	+	+

^a Factors: T, temperature; t, time; S, salt addition (NaCl), -, negative effect; +, positive effect

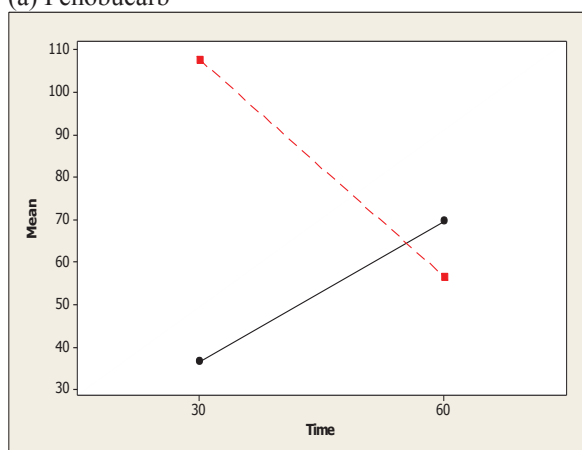
The plots showed that more analytes were extracted at higher extraction temperature and lower time and at higher percentage of salt addition. The factors considered and their interaction at different levels led to limits of detection and quantification below the MRL values for the sample analyzed.



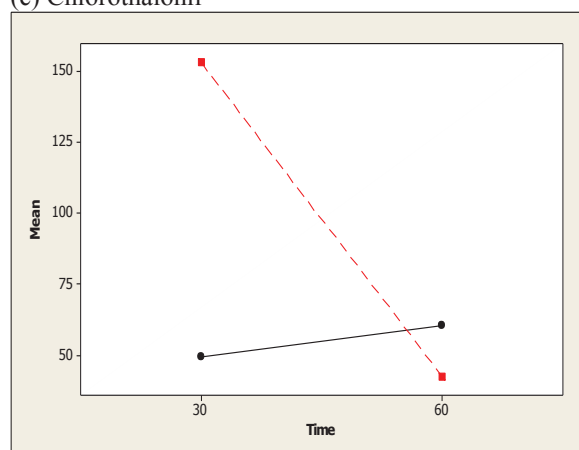
(a) Fenobucarb



(c) Chlorothalonil



(b) Diazinon



(d) Chlorpyrifos

Figure 1: Time-temperature interaction plots.

Table 5: Method Validation

Pesticides	Linearity ($\mu\text{g.L}^{-1}$)	LOD ($\mu\text{g.L}^{-1}$)	LOQ ($\mu\text{g.L}^{-1}$)	R^2	Recovery (%)	RSD (%)
1. Fenobucarb	1 – 50	0.2	1.0	0.998	105.5	13.7
2. Diazinon	0.5 – 50	0.01	0.5	0.999	89.48	1.7
3. Chlorothalonil	0.5 – 50	0.01	0.5	0.999	83.78	0.1
4. Chlorpyrifos	0.5 – 50	0.01	0.5	0.999	80.20	3.33

Temperature: 30 °C 60 °C

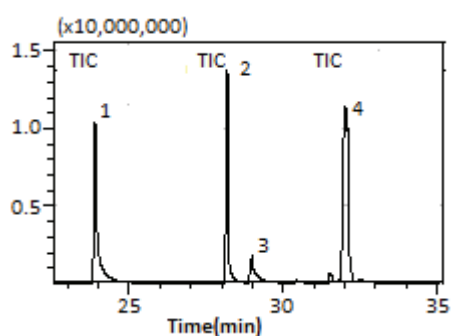


Figure 2. GC-MS (TIC) Chromatogram of a distilled water spiked at $10 \mu\text{g.L}^{-1}$ and extracted by HS-SPME at at 30 min, 60°C and 10 % NaCl. Peak identification as in Table 5.

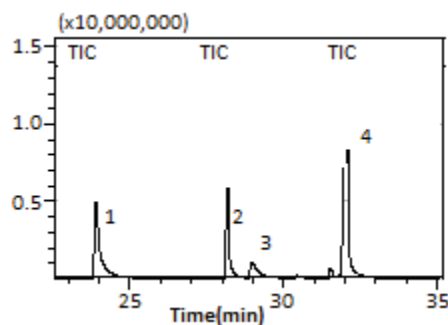


Figure 3. GC-MS (TIC) Chromatogram of an apple sample spiked at $10 \mu\text{g.L}^{-1}$ and extracted by HS-SPME at 30 min, 60°C and 10 % NaCl. Peak identification as in Table 5.

Consequently, a single factor was used for all the pesticides at 60°C for 30 min in the presence of 10 % NaCl for the extraction of target pesticides in apple.

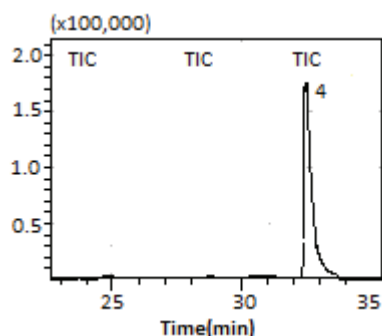


Figure 4. GC-MS (TIC) Chromatogram of an apple sample obtained after application of HS-SPME at 30 min, 60 °C, 10 % NaCl. Peak identification as in Table 5.

3.2 Validation of Analytical Figure of Merit

The analytical figure of merit of the developed method was validated under the best conditions (30 min, 60 °C, 10 % NaCl) by determining the repeatability, recoveries and linearity at 10 µg.L⁻¹. The external standard calibration curve was constructed by a five point concentration level, each analyzed in triplicate. The method linearity ranged from 0.5 to 50 µg.L⁻¹, with coefficients greater than 0.99. The limit of detections ranged from 0.01 to 0.2 and quantifications were between 0.5 and 50 µg.L⁻¹. The recovery calculated by comparing the peak area of spiked sample with that of standard solution at the same concentration and extraction conditions ranged from 80 – 105% with an RSD less than 20% for all pesticides investigated.

3.3 Analysis of Commercial Apple Samples

The HS-SPME procedure was routinely applied to apple sample purchased in Malaysia markets. In order to ascertain the applicability of the method, analyses were made in triplicate. A fiber blank was also carried out in order to check the effect of carry-over. The pesticide chlorpyrifos was detected at concentration of 2 µg.L⁻¹, which is below the MRL (50 µg.L⁻¹) for apples [13].

4. Conclusions

The experimental factorial design was successfully used for the extraction of fenobucarb, diazinon, chlorothalonil and chlorpyrifos in water, and the developed method was applied to the extraction of apple sample obtained in the Malaysian market. As observed, the optimum conditions are dependent on temperature, time, and salt addition to the sample matrix. The recovery and relative standard deviation obtained were comparable with official method for pesticide residue analysis.

An experimental factorial design method developed has shown that it can be applied for the study of conditions affecting the extraction of pesticide residues from fruits and vegetables. It has been

demonstrated in this study that the application of factorial design to HS-SPME technique, allows in a simple and fast ways, the simultaneous determination of factors affecting extraction conditions and their interactions. The sample preparation time is drastically reduced compared to univariate determination which involves many experiments due to the optimization of each factor at a time and also does not allow the determination of interaction of various factors.

Acknowledgments

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