SPECIAL GUEST EDITOR SECTION

Chemometric Study and Optimization of Headspace Solid-Phase Microextraction Parameters for the Determination of Multiclass Pesticide Residues in Processed Cocoa from Nigeria Using Gas Chromatography/Mass Spectrometry

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Solid-phase microextraction (SPME) is a solventless sample preparation method that combines sample preparation, isolation, concentration, and enrichment into one step. A simple and effective method coupling headspace-SPME to GC/MS was developed for the analysis of chlorpyrifos, fenitrothion, endosulfan I, and endosulfan II pesticide residues in cocoa powder. In this study, multivariate strategy was used to determine the significance of the factors affecting the SPME of the pesticides using a Plackett-Burman design, and the significant factors were optimized using central composite design. The analytes were extracted with 100 µm polydimethylsiloxane fibers according to the factorial design matrix and desorbed into a GC/MS instrument. The developed method was applied for the analysis of a cocoa powder sample, and it exhibited good figures of merit for the analytical methodology. Using the optimized conditions, the linearity ranged from 2.5 to 500 µg/kg (R^2 > 0.99) using an internal standard calibration method, and the average recoveries were between 75 and 95%, with RSD values between 3.8 and 9.7%.

Foods are usually analyzed for the presence of pesticide residues with a preliminary step of sample preparation due to the presence of the analytes at trace levels. The main objective of sample preparation is extraction and preconcentration of the target analyte in the presence of other interfering substances (1). Pesticides are unavoidable agricultural input and they play an important role in food production and sustainable agriculture, but their uses must be efficient and effectively sustainable in the environment (2). Organophosphorus and organochlorine pesticides are the most widely used pesticides on Nigeria cocoa farms due to their low environmental persistence. Although the use of pesticides in agriculture has led to an increase in production, their use has caused contamination of food and the environment. Pesticide use in Nigeria has drastically increased over the years, enabling cocoa farmers to attain an increase in the levels of cocoa production (3). The pesticides are biotransported along the food chain and bioacccumulate in animals and humans, thereby causing adverse effects (4) due to their toxicity (5). Therefore, due to the need to control the level of pesticide residues in food, maximum residues levels (MRLs) have been set by various regional, national, and international agencies for various pesticide residues (6).

To achieve isolation of the target analytes from complex sample matrixes, different microextraction techniques such as solid-phase microextraction (SPME; 7–10), liquid phase microextraction (11–17), stir bar sorptive extraction (18, 19), and microextraction in packed sorbent (20) have been developed to overcome the problems inherent in conventional extraction techniques (21), which are tedious, time-consuming, and use large amounts of toxic organic solvents (22). SPME is environmentally friendly because of its solventless nature, and therefore it has been used widely for extraction of pesticide residues in food, water (4, 23–25), environmental (26–29), and biological (30, 31) samples.

SPME was developed to facilitate rapid and sensitive extraction of analytes that saves time and cost of analysis (32, 33). It is easily automated and makes it easy to combine sampling, extraction, and preconcentration of analytes into one single step, which results in high throughput analysis (33). It involves the use of polymeric materials coated on fused silica or metal alloy fibers. The process involves partitioning of the analytes between the coated fibers and the sample matrix when the fiber is either immersed into the sample matrix (direct immersion mode) or exposed to the space above the sample matrix (headspace mode). Desorption of the extracted analytes into analytical instruments without any cleanup step has helped to reduce sample loss and contamination (34, 35). SPME is based on the establishment of equilibrium between the analyte in the sample matrix and the stationary polymeric materials. It has been widely used online with GC for semivolatile and volatile analytes and with LC for thermally labile and highly polar analytes (36, 37).

In the present study, the major factors affecting the extraction of residues of chlorpyrifos, fenitrothion, endosulfan I, and endosulfan II from cocoa powder were investigated using experimental factorial design. The multivariate experimental design is used to determine the major factors that significantly affect the SPME extraction efficiency, due to the large number of parameters to be optimized. This is achieved by use of a screening method such as Plackett-Burman (P-B) design (38, 39), where all interactions are ignored, resulting in the calculation of main effects using a reduced experimental run. The use of a screening technique helps to estimate the significant factors. The significant factors are then optimized using response surface methodology that is based on the

Guest edited as a special report on "New Trends in Pesticide Residue Analysis in Various Sample Matrixes" by Tomasz Tuzimski.

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DOI: 10.5740/jaoacint.SGEAbdulrauf

Table 1. Design matrix for 2^{7-4} P-B design[a]

Run order	Extraction temp., °C	Extraction time, min	Salt added, %	pH	Stirring rate, rpm	Desorption time, min	Desorption temp., °C
1	30	60	10	4	600	5	250
2	60	30	10	4	300	5	270
3	30	60	10	4	600	5	250
4	60	60	5	8	300	5	250
5	60	60	5	8	600	5	270
6	60	60	5	8	600	5	270
7	30	60	10	8	300	10	270
8	60	30	10	4	300	5	270
9	60	30	5	4	600	10	270
10	60	30	10	8	300	10	250
11	30	60	10	8	300	10	270
12	60	60	10	4	600	10	250
13	30	30	5	4	300	5	250
14	30	30	5	4	300	5	250
15	30	60	5	4	300	10	270
16	60	30	5	4	600	10	270
17	30	30	10	8	600	5	270
18	30	30	10	8	600	5	270
19	30	60	5	4	300	10	270
20	60	30	10	8	300	10	250
21	30	30	5	8	600	10	250
22	60	60	5	8	300	5	250
23	60	60	10	4	600	10	250
24	30	30	5	8	600	10	250

[a] Generated using Minitab statistical software.

central composite design (CCD; 31, 38) in order to find the best experimental conditions.

Experimental

Materials and Reagents

All reagents used were pesticide grade with purity greater than 99%. Pesticide standards (100 µg/mL) were purchased from Accustandard, Inc. (New Haven, CT) with purity greater than 97% and stored at 18°C, while the solvents were purchased from Fisher Scientific (Loughborough, UK). Working standard mixtures containing 10 µg/mL of each pesticide were prepared daily by diluting the stock standard solution in methanol. Double distilled deionized water using a Milli-Q system (EMD Millipore, Billerica, MA) was used in experiments.

SPME Device

The SPME device consists of an autosampler and a replaceable fiber coated with polydimethylsiloxane (PDMS, 100 µm) purchased from Supelco (Bellefonte, PA). The fiber was used without modification and was conditioned in the GC/MS injector at 250°C for 30 min prior to first use as recommended by the manufacturer. All analyses were conducted in 20 mL amber glass vials (Supelco) that were rinsed with acetone and then dried in an oven at 120°C for 2 h before use.

Sample Preparation and SPME Procedure

The cocoa powder used was purchased in Nigeria (Cocoa Oluji). An aliquot of 2 g of cocoa powder was accurately weighed into a 20 mL amber glass vial, and optimum dilution was made with 4 mL double distilled deionized water, filtered with a Milli-Q system containing 10% NaCl. The mixture was spiked with 50 µL working standard solution to prepare a 100 µg/kg solution. The mixture was shaken ultrasonically for 1 min, and the sample was subjected to SPME using a 100 µm PDMS-coated fiber. The mixture were agitated and incubated for 5 min at 60°C in the CTC combiPAL autosampler (CTC Analytics, Industriestrasse, Zwingen, Switzerland) before the SPME fiber was exposed to the headspace of the sample, and the analysis was conducted according to the P-B design matrix in Table 1, which was prepared according to the variables as shown in Table 2 generated using a Minitab (State College, PA) statistical software version 15. After each extraction, the extracted analytes were desorbed into the GC/MS system for 7 min at 270°C

GC/MS Analysis

The analysis was performed using a GC/MS [Shimadzu (Tokyo, Japan) QP2010 Series] equipped with a CTC combiPAL autosampler and operated in the split/splitless mode. The DB-5MS capillary column containing 5% diphenyl and 95% dimethylpolysiloxane was 30 m long, 0.25 mm id, and 0.25 µm film thickness. The GC oven temperature was programmed as follows: initial temperature 60°C, held for 2 min and increased to 180°C at 30°C/min, then to 210°C at 3°C/min, ramped to 280°C at 20°C/min, and held for 5 min. The MS operation conditions included transfer line 300°C, ion source 200°C, and ionization 70 eV. The carrier gas (helium, 99.999%) was used at a constant flow rate of 1.3 mL/min. The method development was carried out in the scan mode, while quantitation was done in the selective ion monitoring mode. In case of co-elution, easy

Table 2. Factors and levels of the variables

Factor	Level	
	Low (–)	High (+)
Extraction temperature, °C	30	60
Extraction time, min	30	60
Salt addition, %	5	10
Stirring rate, rpm	300	600
pH	4	8
Desorption temperature, °C	250	270
Desorption time, min	5	10

Table 3. Design matrix for 2^3 full factorial CCD

Run order	Blocks	Extraction temp., °C	Extraction time, min	pH
1	2	45.00	45.00	6.00
2	2	69.50	45.00	6.00
3	2	45.00	69.50	6.00
4	2	45.00	45.00	6.00
5	2	20.51	45.00	6.00
6	2	45.00	45.00	2.73
7	2	45.00	20.51	6.00
8	2	45.00	45.00	9.27
9	1	60.00	60.00	8.00
10	1	45.00	45.00	6.00
11	1	45.00	45.00	6.00
12	1	60.00	60.00	4.00
13	1	60.00	30.00	8.00
14	1	30.00	60.00	4.00
15	1	30.00	30.00	4.00
16	1	45.00	45.00	6.00
17	1	30.00	60.00	8.00
18	1	45.00	45.00	6.00
19	1	30.00	30.00	8.00
20	1	60.00	30.00	4.00

[a] Generated using Minitab statistical software.

spectral identification and integration were achieved by using the deconvolution feature of the GC/MS system.

Statistical Design of the Experiment.

The experiment was carried out with seven independent variables by generating a P-B design for the screening of major variables (Table 2) affecting extraction efficiency. As shown in Table 1, a 2^{7-4} saturated P-B design generated by Minitab statistical software version 15 was used to evaluate the significance factors. After the screening, the significant factors were evaluated using a general full factorial CCD as shown in Table 3, which was also used to build a quadratic model between the dependent and independent variables.

Results and Discussion

P-B Design

The efficiency of SPME for the determination of pesticide residues in complex food samples depends on different parameters that must be optimized. The selection of the fiber is important since the method sensitivity depends on the distribution constant between the coated fiber and the sample matrix. The significance of seven factors (Table 2) was evaluated based on the literature and research experience (40), while the significant factors were optimized. The response variable was the total chromatographic peak areas of the selected pesticides.

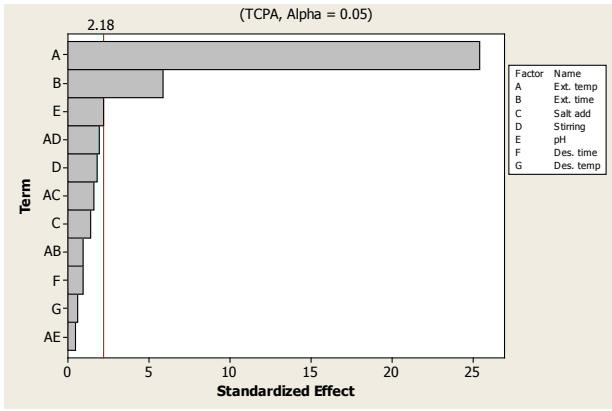


Figure 1. Pareto chart of the standardized effects.

The design was executed in two blocks, one daily to allow the elimination of sources of daily variability.

The data obtained were evaluated using the analysis of variance test, and the significance of the main effects was evaluated by the *F*-test and graphically represented by a Pareto chart (Figure 1). The significance of each factor was evaluated from the significance value ($P = 0.05$) with a confidence level of 95%; high significance of the regression model is indicated by low probability of the *P*-value (not shown; 41). The length of each bar is proportional to the standardized effect of each factor, while the vertical line indicates the significance level at the 95% confidence level.

From the Pareto chart and the normal plot, three factors were shown to be significant; extraction temperature and extraction time showed a positive effect, while the pH value showed a negative effect, as shown in Figure 2. Other factors that showed no significant effect were fixed: desorption temperature at 270°C, desorption time at 7 min, stirring rate at 300 rpm, and salt addition at 10% NaCl.

CCD

The next step involved the optimization of the significant factors. This was achieved by generating a 2^3 full factorial CCD (Table 3), comprising eight cube points, four center points in cube, six axial points, and two center points in axial with a total of 20 randomized chromatographic runs. The axial

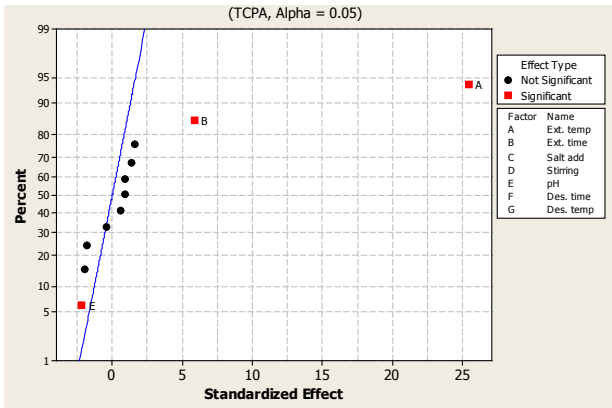


Figure 2. Normal plot of the standardized effects.

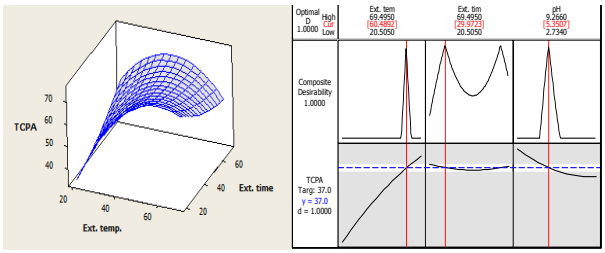


Figure 3. Response surface (a) plot and response optimizer plot (b) of the TCPA.

distance of α = 1.633 was selected to establish the rotatability condition. The CCD was used to establish the main effects, their interaction, and their quadratic components.

As can be observed in the response surface and response optimizer plots (Figure 3a), the experimental domain that results in the best global response is given at an extraction temperature of 60°C, extraction time of 30 min, and pH value of 5. The response surface plot (Figure 3b) was also used to investigate the interaction effect of extraction temperature and extraction time. Therefore, the working SPME conditions were as follows: extraction temperature, 60°C; extraction time, 30 min; stirring rate, 300 rpm; salt addition, 10% NaCl; desorption temperature, 270°C; and desorption time, 7 min. The optimized parameters were subsequently used to extract the target analytes from the sample matrix spiked at 100 µg/kg, and the GC/MS chromatogram obtained is as shown in Figure 4.

Method Validation and Sample Analysis

Following the establishment of the optimal SPME conditions, the developed method was used to validate the analytical figures of merit for each pesticide under investigation. The accuracy, precision, linearity, LOD, and LOQ were evaluated. For the method validation study, cocoa powder free of the investigated pesticides (obtained from Malaysian hypermarket) was used.

The method analytical performance parameters are listed in Tables 4 and 5. The linearity, estimated using the residual SD of the intercept and slope of the calibration curve (41), ranged from 5 to 500 µg/kg, LOD and LOQ were low, and recovery was 75–95%. The LOD estimated at S/N 3 was between 4.67 and 9.59 µg/kg, while the LOQ estimated at S/N 10 ranged from 15.58 to 23.74 µg/kg, which indicates remarkably sensitive detection. For the precision studies, intraday repeatability

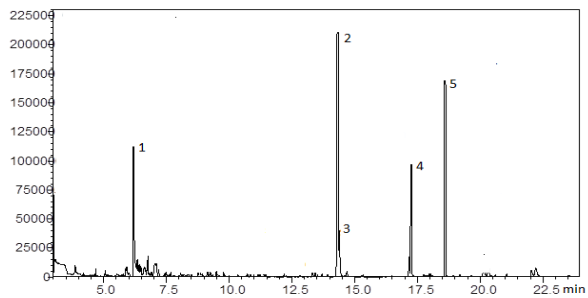


Figure 4. GC/MS chromatogram of cocoa powder spiked with 100 µg/kg of pesticide mixture: 1, Internal standard; 2, fenitrothion; 3, chlorpyrifos; 4, endosulfan I; and 5, endosulfan II.

Table 4. Figures of merit of the developed method

Analytes	Linearity, µg/kg	R^2	LOD, µg/kg	LOQ, µg/kg	MRL, µg/kg
Fenitrothion	5–200	0.9989	4.67	15.58	50
Chlorpyrifos	10–500	0.9993	8.67	28.90	100
Endosulfan I	10–500	0.9991	9.59	31.95	100
Endosulfan II	10–500	0.9995	7.12	23.74	100

Table 5. Accuracy and precision of the developed method

Analyte	Added, µg/kg	Found, µg/kg	Intraday RSD, % (*n* = 3)	Interday RSD, % (*n* = 9)
Fenitrothion	50	43	10.5	13.8
	100	95	9.4	10.2
Chlorpyrifos	50	35	10.9	9.7
	100	75	7.2	8.4
Endosulfan I	50	39	10.1	12.0
	100	88	7.8	8.3
Endosulfan II	50	36	9.8	10.3
	100	79	6.0	8.2

(*n* = 3) and interday reproducibility (*n* = 9; three extractions daily for 3 days) were evaluated by spiking the sample at 50 and 100 µg/kg levels, and the RSD values were less than 15% (Table 5). The method developed was applied for the extraction of the target analytes in cocoa powder obtained from a Nigerian market, and chlorpyrifos, which is one of the currently approved pesticides for use on cocoa farms in Nigeria (3), was detected but not quantified, as it was present at a level lower than the LOQ. This implies that the pesticides present in the investigated cocoa powder were lower than MRLs set by the European Commission (42) and the joint World Health Organization/Food and Agricultural Organization food standard established through the Codex Alimentarius Commission (6).

Acknowledgments

The University of Malaya Research Management Centre support through the Institut Pengurusan dan Pemantauan Penyelidikan (IPPP) grant PV009/2011B and UMRG grant RG227/12AFR is gratefully acknowledged.

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