



CHEMOMETRIC STUDY AND OPTIMIZATION OF HS-SPME PARAMETERS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN PROCESSED COCOA POWDER

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ABSTRACT

Solid-phase microextraction (SPME) is a solvent-less sample preparation method which combines sample preparation, isolation, concentration and enrichment into one step. A simple and effective headspace-solid phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME/GC-MS) method was developed for the analysis of 4 (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 solid phase microextraction of the pesticides using a Plackett-Burman design and the significant factors were optimized using central composite design (CCD). The analytes were extracted with 100 μ m PDMS fibers according to the factorial design matrix and desorbed into a gas chromatography-mass spectrometry detector. The developed method was applied for the analysis of cocoa powder sample and it exhibited good figure of merit of analytical methodology with low limits of detection, which were lower than the MRLs for cocoa and a good precision in terms of relative standard deviations (RSD) (less than 10%) with average recovery of 75 to 95%. The linearity ranges from 5 – 500 μ g.kg⁻¹ with correlation coefficient greater than 0.99.

Keyword: GC-MS, SPME, pesticide residues, chemometrics study

INTRODUCTION

Pesticides are unavoidable agricultural input and they play an important role in food production and sustainable agriculture, but their uses must be efficient and effective sustainable in the environment [1]. Organophosphorus and organochlorine pesticides are the most widely used pesticides in Nigeria cocoa farm, this is due to their less environmental persistence. Although, the use of pesticides in agriculture have led to an increase in agriculture production but their use has led to contamination of food and environment. Pesticide use in Nigeria has drastically increased over the year, this has enable cocoa farmers to attain an increase in the levels of cocoa production. [2]. Therefore, due to the need to control the level of pesticide residues in food, the maximum residues levels (MRLs) have been set up by various regional,

national and international agencies by setting up different maximum residue levels (MRLs) for various pesticide residues [3].

Solid phase microextraction was developed in order to facilitate a rapid and sensitive extraction of analytes, to save time and cost of analysis [4, 5]. It is easily automated and makes it easy to combine sampling, extraction and pre-concentration of analytes into one single step, which results in high throughput analysis [5]. It involves the use of polymeric materials coated on fused silica or metal alloys fiber. The process involves the partitioning of the analytes between the coated fibers and the sampling 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) and the desorption of the extracted analytes into analytical instruments, without any [6, 7] clean-up step and has helped to reduce sample loss and contamination. It has been widely used on-line with gas chromatography for semi-volatile and volatile analytes and with liquid chromatography for thermally mobile and high polar analytes [8, 9].

In the present study, the major factors affecting the extraction of four pesticide residues (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 which significantly affect the SPME extraction efficiency, due to the large number of parameters to be optimized. This is achieved by the use of screening method such as Plackett-Burman Design [10, 11], where all interactions are ignored, resulting in the calculation of main effect using a reduced experimental run. The use of screening technique helps to estimate the significant factors. The significant factors were then optimized using response surface methodology which is based on the central composite design [10, 12], in order to get the best experimental conditions

EXPERIMENTAL

Materials and Reagents

All reagents used were pesticide grade with purity greater than 99 %. Pesticide standards ($100 \mu\text{g.mL}^{-1}$) were purchased from Accustandard Inc, New Haven CT, USA, with percent purity greater than 97% and stored at 18°C , while the solvent were purchased from Fisher Scientific, Loughborough, UK. Working standard mixture containing $10 \mu\text{g.mL}^{-1}$ of each pesticide were prepared daily by diluting the stock standard solution in methanol. Experimental method was developed using double distilled deionized water filtered using Milli-Q system.

SPME Device

The SPME device consist of autosampler and a replaceable fiber coated with polydimethylsiloxane (PDMS, $100 \mu\text{m}$), purchased from Supelco (Bellefonte, PA, USA). The fiber were 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 analysis was conducted in 20 mL amber glass vial (Supelco), which was rinsed with

acetone and then dried in oven at 120°C for 2 hr before use

Sample Preparation and SPME Procedure

The cocoa powder used was purchased in Nigeria (Cocoa Oluji). An aliquot of 2g of cocoa powder was accurately weighed into the 20 mL amber glass vial and optimum dilution was made with 4 mL of Milli-Q filtered double distilled water containing 10 % NaCl. The mixture was spiked with $50 \mu\text{l}$ of the working standard solution to prepare $100 \mu\text{g.kg}^{-1}$. The mixture was shaken ultrasonically and sample was subjected to SPME procedure using $100 \mu\text{m}$ PDMS coated fiber. The fiber was exposed to the headspace of the sample and the analysis was conducted according to P-B design matrix in Table 2 which was prepared according to the variables to screened (Table 1) using a Minitab® statistical software. After each extraction, the extracted analytes were desorbed into the GC-MS system for 7 min at 270°C

GC-MS Analysis

The extraction and analysis were performed using a GC-MS (Shimadzu QP2010 Series) equipped with CTC combiPAL autosampler and operated in the split/splitless mode. The capillary column is made up of DB-5MS containing 5 % diphenyl and 95 % dimethylpolysiloxane with 30 m long, 0.25 mm internal diameter and 0.25 μm film thickness. The GC over temperature was programmed as follows: initial temperature of 60°C , held for 2 min and increased to 180°C at $30^\circ\text{C.min}^{-1}$, then to 210°C at 3°C.min^{-1} and finally ramped to 280°C at $20^\circ\text{C.min}^{-1}$, held for 5 min. The MS operation conditions include transfer line of 300°C , ion source of 200°C , electron ionization (EI) of 70 eV.

Statistical Design of Experiment

The experiment was carried out with 7 independent variables by generating a P-B design for the screening of major variables (Table 1) affecting extraction efficiency. A 2^{7-4} saturated Plackett-Burman design generated with 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 central composite design (CCD), which was also used to build a quadratic model between the dependent and independent variables.



Table 1: Factors and levels of the variables

Factors	Code	Level	
		Low (-)	High (+)
Extraction temperature ($^{\circ}\text{C}$)	A	30	60
Extraction time (min)	B	30	60
Salt addition (% NaCl)	C	5	10
Stirring rate (rpm)	D	300	600
pH	E	4	8
Desorption temperature ($^{\circ}\text{C}$)	F	250	270
Desorption time	G	5	10

RESULTS AND DISCUSSION

The data obtained was evaluated using ANOVA test, and the significant of the main effects was evaluated by F-test and graphically represented by a Pareto chart (Fig 1a). The significant of each factor was evaluated from the significant value ($p=0.05$), with confidence level of 95% and high significance of the regression model is indicated by low probability of p-value (not shown) [13]. The length of each bar is proportional to the standardized effect of each factor, while the vertical line indicates the significance level at 95 % confidence level. From the Pareto chart and the normal plot, three (3) factors are shown to be significant; Extraction temperature and extraction time show positive effect, while the pH value show negative effect. Other factors that show no significance effect are fixed: desorption temperature at 270°C , desorption time at 7 min, stirring rate at 300 rpm and salt addition at 10 % NaCl.

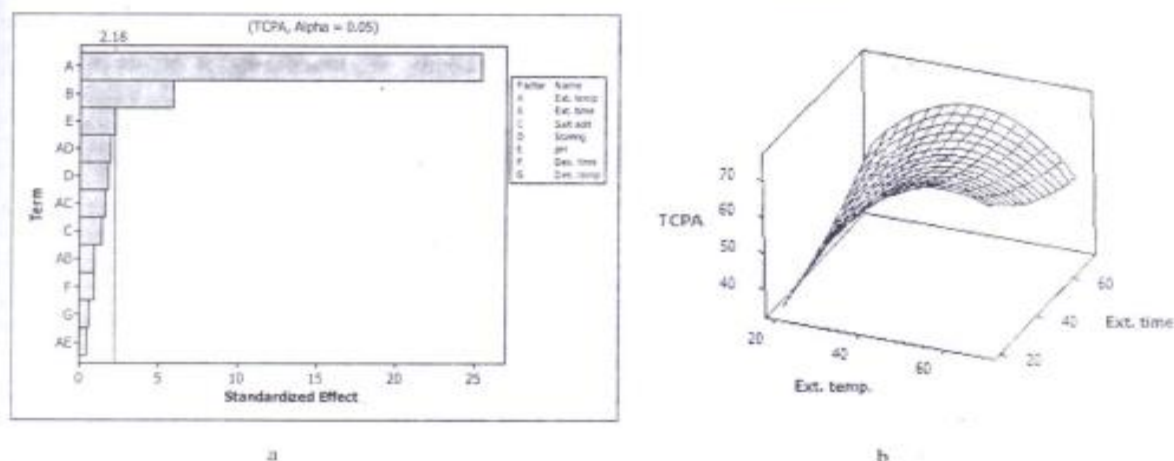


Fig 1: (a) Pareto chart of the standardized effects: (b) Response surface response plot of the TCPA



As can be observed in response surface and response optimizer plots (Fig 1b), the experimental domain which results in the best global response is given at extraction temperature of 60 °C, extraction time of 30 min and pH value of 5. The response surface plot (Fig 1b) was also used to investigate the interaction effect of extraction temperature and extraction time. Therefore the working SPME conditions are 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).

Following the establishment of the optimal SPME condition, the developed method was used to validate the analytical figure of merit for each pesticide under investigation. The accuracy and precision of the method was evaluated in terms of linearity, limit of detection and quantification, recovery.

Table 2: Figure of merit of the developed method

Analytes (MRLs)	Linearity ($\mu\text{g.kg}^{-1}$)	R ²	LOQ ($\mu\text{g.kg}^{-1}$)	MRLs ($\mu\text{g.kg}^{-1}$)	Recovery (%)
Fenitrothion	5 – 200	0.9989	15.58	50	95
Chlorpyrifos	10 – 500	0.9993	28.90	100	75
Endosulfan I	10 – 500	0.9991	31.95	100	88
Endosulfan II	10 – 500	0.9995	23.74	100	79

The method developed was extended for the extraction of the target analytes in cocoa powder obtained from Nigerian market and chlorpyrifos, which is one of the currently approved pesticide for use in cocoa farm in Nigeria [2], was detected but not quantified, as it was present at a level lower than the limit of quantitation. This implies that the pesticide present in the investigated cocoa powder were lower than MRLs set by the European Commission [14] and the joint WHO/FAO food standard established through the Codex Alimentarius Commission [3].

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REFERENCES

1. J.L. Tadeo, R.A. Pérez, B. Albero, A.I. García-Valcárcel, and C. Sánchez-Brunete, *Review of sample preparation techniques for the analysis of pesticide residues in soil*, J. AOAC Int., 2012. **95**(5): p. 1258-1271.
2. E.U. Asogwa and L.N. Dongo, *Problems associated with pesticide usage and application in Nigerian cocoa production: A review*, African J. Agric. Res, 2009. **4**(8): p. 675-683.
3. WHO/FAO, *The Codex Alimentarius Commission International Food Standard*
4. C.L. Arthur and J. Pawliszyn, *Solid phase microextraction with thermal desorption using fused silica optical fibers*, Anal. Chem., 1990. **62**(19): p. 2145-2148.
5. S. Risticvic, V.H. Niri, D. Vuckovic, and J. Pawliszyn, *Recent developments in solid-phase microextraction*, Anal. Bioanal. Chem., 2009. **393**(3): p. 781-795.
6. J. Pawliszyn, *Theory of Solid-Phase Microextraction*, in *Handbook of Solid Phase Microextraction*, J. Pawliszyn, Editor, 2012, Elsevier: Waltham, USA. p. 13-59.
7. J. Pawliszyn, *Development of SPME Devices and Coatings*, in *Handbook of Solid Phase Microextraction*, J. Pawliszyn, Editor, 2012, Elsevier: Waltham, USA. p. 60-97.
8. H. Lord, *Strategies for Interfacing Solid-Phase Microextraction with Liquid Chromatography*, J. Chromatogr. A, 2007. **1152**(1-2): p. 2-13.



9. C. Nerín, J. Salafranca, M. Aznar, and R. Battle, *Critical review on recent developments in solventless techniques for extraction of analytes*. Anal. Bioanal. Chem., 2009. **393**(3): p. 809-833.
10. C. Stalikas, Y. Fiamegos, V. Sakkas, and T. Albanis, *Developments on chemometric approaches to optimize and evaluate microextraction*. J. Chromatogr. A, 2009. **1216**(2): p. 175-189.
11. S.L.C. Ferreira, W.N.L. Dos Santos, C.M. Quintella, B.B. Neto, and J.M. Bosque-Sendra, *Doehlert matrix: A chemometric tool for analytical chemistry - Review*. Talanta, 2004. **63**(4): p. 1061-1067.
12. R. López, F. Goñi, A. Etxandia, and E. Millán, *Determination of organochlorine pesticides and polychlorinated biphenyls in human serum using headspace solid-phase microextraction and gas chromatography-electron capture detection*. J. Chromatogr. B, 2007. **846**(1-2): p. 298-305.
13. J.N. Miller and J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*. 6th ed. 2010, Essex, England: Pearson.
14. EU, *Regulation (EC) No 396/2005*, in *EU Pesticide Database*. 2005.