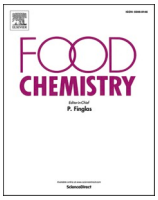
Food Chemistry 463 (2025) 141464



Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem





Determination of pesticide residues in beans using QuEChERS technique coupled to gas chromatography-mass spectrometry: Multivariate optimization of CEN and AOAC methods

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ARTICLE INFO

Keywords:
GC/MS
Multivariate
Pesticide residues
QuEChERS technique
Sample preparation

ABSTRACT

The use of pesticides has led to environmental pollution and posed a global health risk, since they remain as residues on foods. Beans one of the most widely cultivated crop in Africa, and susceptible to attack by insects both on field and during storage, leading to the application of pesticides to control pests' infestation. However, misuse of these chemicals by farmers on beans has resulted in the rejection of beans exported to European countries, due to the presence of pesticide residues at concentrations higher than the maximum residues levels (MRLs). In this study, the effectiveness of the Association Official Analytical Chemists (AOAC) Official Method and the European Committee of Standardization (CEN) Standard Method, were determined using multivariate approach for the analysis of organochlorine pesticide residues in 6 varieties of beans samples. The significance of factors (mass of sample, volume of acetonitrile, mass of magnesium sulphate, sample pH, centrifugation time and speed) affecting the efficiency of extraction was estimated using Plackett-Burman design, while central composite design was used to optimize the significant factors. The following optimum factors were subsequently used for method validation, recovery tests, and real sample analysis: 4 g of sample sludge (1:1 *v*/v), 10 mL of acetonitrile, 4.45 g of $MgSO_4$, and 5 min of centrifugation at 5000 rpm. The figure of merit of analytical methodology estimated using matrix-matched internal standard calibration method gave linearity ranging from 0.25 to 500 μg/kg, with correlation coefficient (R^2) greater than 0.99, the recovery ranged from 75.55 to 110.41 (RSD = 0.70–16.65), with LOD and LOQ of 0.23–1.77 μg/kg and 0.76–5.88 μg/kg, respectively.

1. Introduction

The capacity of food production, availability and stability is faced with an ever-growing number of challenges (Calicioglu, Flammini, Bracco, Bellu, & Sims, 2019), including a world population growing at geometrical rate to nearly 10 billion by 2050 (UN., 2019) and a falling ratio of arable land to population explosion (Akpan & Ebong, 2021). The imbalance in population growth and arable land utilization have led to high demand for food and increased in the use of pesticides to increase production and meet the ever-increasing demand for food supply (Abdulra'uf, Hammed, & Tan, 2012). The presence of pesticide residues on foods have become worrisome due to their health risks (including disruption of the endocrine system, alteration of hematological and hepatic systems, allergies and asthma (Pathak et al., 2022)). This has led to the establishment of maximum residue levels through the Codex Alimentarius Commission (Abdulra'uf, Chai, & Tan, 2012). Sample preparation stage is vital since the residues are present at trace level and embedded in the complex food matrices with diverse chemical and physical properties (Costa, Caldas, & Primel, 2014).

QuEChERS method was introduced in 2003 as "Quick, Easy, Cheap,

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https://doi.org/10.1016/j.foodchem.2024.141464
Received 2 June 2024; Received in revised form 24 September 2024; Accepted 26 September 2024
Available online 27 September 2024
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Effective, Rugged and Safe" with the aim of overcoming the limitation inherent in the existing multiresidue pesticide analysis methods and has since been modified and optimized to support a wide range of analytes (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Collimore & Bent, 2020; Kardani, Jelyani, Dahanzadeh, & Rashedinia, 2022; Su et al., 2024). It has a wide analytical scope with wide range of sensitivity and selectivity, due to its easy coupling to chromatographic instruments (gas and liquid chromatography (Lehotay et al., 2010). The QuEChERS method is widely used and is formalized in two documented methods, the Association of Official Analytical Chemists (AOAC) Official Standard Method (AOAC, 2007.01) (AOAC, 2007), involving the addition of acetate buffer and the European Committee for Standardization (CEN) Standard Method (EN 156223) (EN., 2008), where citrate buffer is added.

The basic step for both modified methods are the same: a liquid-liquid extraction between an organic phase (acetonitrile) and water with the use of salts for partitioning, followed by shaking and centrifugation, after which an aliquot of the organic phase is removed and subjected to a dispersive solid phase extraction (dSPE) using magnesium sulphate and a sorbent, that will bind matrix interference (Anastassiades, Scherbaum, Taşdelen, & Stajnbaher, 2007), the resulting supernatant can then be analyzed using gas chromatography (GC) or liquid chromatography (LC). GC is better for the analysis of volatile and semi-volatile analytes, while LC is used for the analysis of thermally labile analytes (Vázquez-Loureiro et al., 2021). The application of tandem mass analyzer has been observed to provide high selectivity with less background noise and reduced matrix effect (Schreiber, 2010), but single quadrupole mass spectrometry has also been shown to provide a cost effective analysis with comparable quantitative performance (Li et al., 2021; Xue et al., 2021), compared to tandem mass spectrometry. The QuEChERS technique is flexible and can be modified based on the properties of analytes, composition of the sample matrix and the available analytical instruments (Lehotay et al., 2010). It was observed that the differences in some analytical methods could be due to personal preferences, but different modifications of QuEChERS technique served as ways to improve efficiency (Lehotay et al., 2010).

Beans, the most widely cultivated and consumed grain legumes in Africa (Lawal, Olayinka, Abdulra'uf, & Etejere, 2020), and the third largest family of flowering plants, are easily grown but highly susceptible to pest problems, such as insects, slugs and snails, viral, fungal and bacterial diseases (Graham & Vance, 2003). They are considered to contain the second highest source of human dietary protein and calories (Maphosa & Jideani, 2017). The limiting factor for increase yield in beans production is the damage caused by insects (Abate & Ampofo, 1996) and the reason for introduction of integrated pest management through the application of chemical pesticides and other pest control methods.

Organochlorine pesticides are continuously used illegally in developing countries, despite the ban by the United States and Europe countries (Oyinloye et al., 2021), and the continued export to developing countries has been questioned by the European Union (Sarkar, Gil, Keeley, Mohring, Jansen, 2021). The use of organochlorine pesticides was banned in Nigeria by the National Agency for Food Administration and Control (NAFDAC) in 2008 (NAFDAC, 2008). Thus, the study investigates the simultaeous optimization of AOAC2007.1 and EN156223 methods using design of experiment, for the analysis of organochlorine pesticide residues in 6 varieties of beans samples obtained in 4 different markets in Ilorin metropolis, Kwara State Nigeria. The pesticides under investigation were chosen based on the news of rejection and ban placed on Nigeria beans in 2015 by the European Union due to the presence of pesticide residues at concentration higher than the maximum residue levels (Anozie, 2015), the ban which is still in place (Nejo, 2022).

2. Materials and methods

2.1. Chemicals and reagents

The pesticide mixed standards (EPA 8081 pesticide standard mix containing a 200 μg/mL each of the pesticide standard) and the internal standard (triphenyl phosphate) used in this study were obtained from Sigma Aldrich, with more than 95 % purity. Analytical grade acetonitrile, acetic acid, formic acid and ethanol were obtained from Fisher Scientific. Anhydrous magnesium sulfate, sodium chloride, sodium acetate and sodium citrate were obtained from Merck. The dSPE kits (PK50 Supel Que PSA (EN), and PK50 Supel Que PSA/C18/ENVI-CARB (AOAC)) containing pre-weighed anhydrous magnesium sulfate, C18, and primary secondary amine (PSA) were purchased from Sigma Aldrich.

2.2. Sample collection and preparation

The method of systematic approach of sampling as recommended by the Codex Alimentarius Commission was adopted (CAC, 1999). One kilogram of each dried raw bean sample was collected from local sellers. Six varieties of beans were collected from each of the four wholesale markets as shown in Figs. 1, S1 and S2. The samples were collected in glass bottles, tightly sealed, labelled appropriately, transported to the laboratory and stored at 4 °C prior to analysis. The samples used for method validation, calibration and recovery studies were first analyzed to ensure the absence of the target pesticide residues (Souza-Silva, Lopez-Avila, & Pawliszyn, 2013).

2.3. QuEChERS sample preparation

The modified QuEChERS methods for the analysis of pesticide residues according to European Committee of Standardization, standard method and AOAC official method were adopted in this study. Briefly, 1 kg of dried beans samples was homogenized in a blender and was sieved through a 150-mesh. About 20 g of homogenized sample was transferred into a beaker followed by addition of 20 mL of double distilled water and the mixture was homogenized, then 10 g of the slurry formed was transferred into 50 mL polypropylene centrifuge tube and the slurry was subjected to EN15662 and AOAC modified QuEChERS as follows:

For European Committee of Standardization (EN15662:2008) standard method, 10 mL of acetonitrile was added to the mixture of the homogenized sample and was vortexed for 1 min, then 4 g of $MgSO_4$, 1 g of sodium chloride and 1 g of sodium citrate were added, followed by addition of 100 μL of internal standard (triphenyl phosphate at 20 μg/mL). The mixture was vigorously stirred using vortex mixer for 1 min, and was then centrifuged at 5000 rpm for 5 min. Exactly, 6 mL of the supernatant was transferred to the Supel QuE® dSPE kit, containing pre-weighed 900 mg of $MgSO_4$, 150 mg of PSA and 150 mg C18 and was centrifuged at 5000 rpm for 5 min. About 5 mL supernatant was acidified with 50 μL of 5 % formic acid in acetonitrile (10 μL/mL of extract) to stabilize the extract. The extract was then transferred into 5 mL amber glass vial for GC–MS analysis.

For AOAC official method, 10 mL of acetonitrile containing 1 % acetic acid was added to the aliquot of the homogenized sample mixture followed by addition of 6 g of $MgSO_4$ and 1.5 g of sodium acetate, then 100 μL of internal standard (triphenyl phosphate at 20 μg/mL), was added and the mixture was thoroughly mixed using vortex mixer for 1 min and then centrifuged at 5000 rpm for 5 min. About 6 mLof the supernatant was transferred to Supel QuE® dSPE kit containing pre-weighed dSPE sorbent of 900 mg of $MgSO_4$, 150 mg of PSA and 150 mg C18, shaken for 1 min and then was centrifuged at 5000 rpm for 5 min. About 5 mL supernatant was acidified with 50 μL of 5 % formic acid in acetonitrile (10 μL/mL of extract) to stabilize the extract. The extract was then transferred into 5 mLamber glass vial for GC–MS analysis.

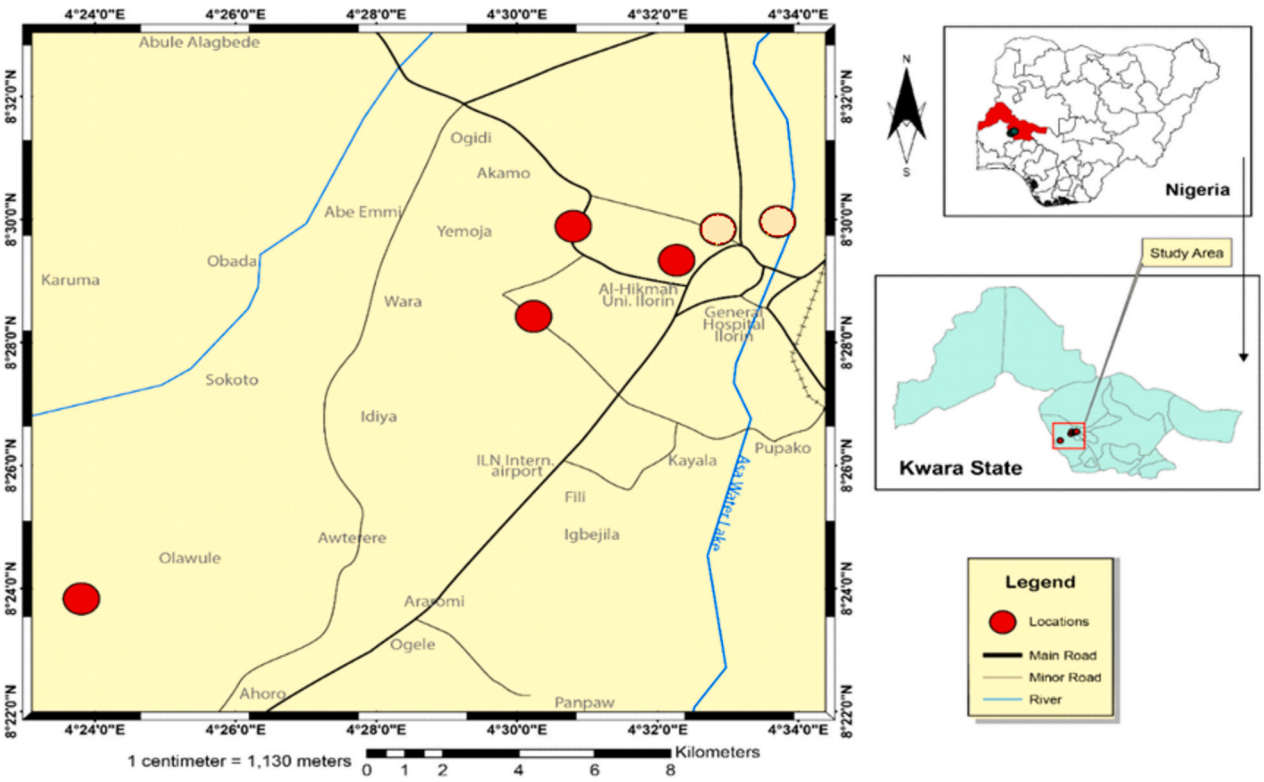


Fig. 1. Map showing the locations for sample collection.

2.4. GC–MS analysis

GC–MS (Shimadzu QP2010 Series) was operated in split/splitless mode at injection temperature of 270 °C. A DB-5MS fused capillary column equipped with 95 % dimethylpolysiloxane was used to separate the target analytes. A silanized narrow bore liner with high-pressure septumless injection were installed in the injection port. The linear velocity of the carrier gas (Helium) was set at 42 cm/s at a constant flow rate of 1.3 mL/min. The programmed temperature of the column oven used for the separation of the target analyte was specified as follows: The temperature was first set at 60 °C for 2 min, raised to 180 °C at the rate of 30 °C/min, and then at 3 °C/min to 210 °C and at 20 °C/min to 280 °C, which was maintained for 5 min, to give a runtime of 24.5 min. The mass spectrometry operation conditions were set to 70 eV (electron ionization), 200 °C (ion source) and 300 °C (transfer line). The scan mode was employed for the optimization of the methods, while selected ion monitoring was used for quantitation. The most abundant ion and two additional reference ions were used to monitor the target analytes. Identification of the target pesticides was done by matching the retention time of the standard and relative abundance and comparing the mass of the standard with NIST mass spectra library (Ogah, Coker, & Adepoju-Bello, 2011).

2.5. Validation of analytical methodology

Methods are validated as a form of quality assurance step to determine the effectiveness and desirability of multi-residue analysis. The guidance document of quality control and method validation requirements established by the European Commission for pesticide residues analysis in food and feed (SANTE, 2021) was employed to validated the developed method.

Beans samples to be used for method development, matrix-matched calibration and recovery studies was first analyzed to ensure that the target analytes are absent (Souza-Silva et al., 2013). The developed method was validated using the European Commission document for pesticide residues analysis in food and feed (SANTE, 2021). The parameters validated for the method include: precision, accuracy, linearity range, matrix effect, limit of detection (LOD) and limit of quantitation (LOQ).

The limits of detection (S/N = 3) and quantification (S/*N* = 10) were determined from the matrix match calibration curve using the standard deviation of intercept (Wenzl, Haedrich, Schaechtele, Robouch, & Stroka, 2016).

2.6. Statistical analysis

The multivariate experiment design matrices were generated using Minitab® statistical software package version 16, using the total chromatographic peak area (TCPA) as response for the Plackett-Burman (PB) and central composite (CC) designs.

2.7. Analysis of real samples

The QuEChERS method developed in this study was subsequently used for the quantitative determination of pesticide residues in the blank homogenized beans samples. The analysis was conducted in triplicate on six different varieties of beans samples obtained from four different markets in Ilorin metropolis, Kwara State, Nigeria. The residues obtained was then compared with the maximum residue limits (MRLs) as required by the EU Commission (EU., 2016).

3. Results and discussion

The development of extraction method requires the optimization of several factors which affect the efficiency of the method. In this study, the screening of parameters that significantly affect the extraction of the selected pesticides from beans samples was achieved using Plackett-Burman (PB) design. The factors were (Tables S1 and S2) initially optimized using one-factor-at-a-time (OFAT) method. The factors used for Plackett-Burman design are as shown in Table 1. Consequently, the

Table 1
Factors and level of variables used for P—B design.

S/N	Factors	Levels	
		Low (−)	High (+)
1	Mass of slurry (g) (A)	2	15
2	Mass of $MgSO_4$ (g) (B)	2	6
3	Volume of acetonitrile (mL) (C)	5	15
4	Percentage of acetic acid in acetonitrile (%) (D)	0	2
5	Mass of NaCl (g) (E)	0	2
6	Mass of buffering salt (g) (F)	0	2
7	Centrifugation speed (rpm) (G)	2000	6000
8	Centrifugation time (min) (H)	2	8

significant factors were further optimized using Central Composite Design (CCD).

Plackett-Burman design was used to determine the significant level of each factor. Table S2 showed the factors that were estimated for their significance using PB design matrix (Table S3). The model was further confirmed with ANOVA for the response (Total Chromatographic Peak Area (TCPA)) for the pesticide mix, and each sample was analyzed in triplicate (Stalikas, Fiamegos, Sakkas, & Albanis, 2009).

Other factors such as types of buffering salt and type of dSPE sorbent were not included in the PB design, because there was no significant difference between the application of sodium citrate and sodium acetate, although, use of sodium citrate produce extracts with high amounts of co-extractive. On the other hand, the use of PSA/C18/Envi-Carb/$MgSO_4$ was found to produce cleaner extracts. The significance of independence variables was estimated using the F-test, while the adequacy of the regression models in terms of lack of fit was checked using analysis of variance (ANOVA) (Bordagaray, Garcia-Arrona, & Millán, 2011). The experiments were randomly performed to reduce the effect of extraneous variables (Narenderan, Meyyanathan, Karri, Babu, & Chintamaneni, 2019).

The Pareto chart of standardized effect as shown in Fig. 2a, graphically represents the main effect of each factor and it implied that the length of the bar is proportional to the absolute value of the main effect, and the vertical line indicates significance level at $p \leq 0.05$. If the reference line is exceeded by the standardized effect, it shows the significant effect of the factor at 95 % confidence interval (Wei, Wu, Xiao, & Wang, 2020). The normal plot (Fig. 2b) showed the significance of each factor, while the half-normal plot (Fig. 3a) showed the level of their significance. The significance factors as shown in Fig. 2a and b are as follows: mass of magnesium sulphate, volume of acetonitrile, mass of sodium chloride, mass of buffering salts (sodium acetate) and centrifugation time. The variation of the mass of slurry, percentage of acetic acid in acetonitrile and mass of NaCl were found not to significantly affect extraction, but are needed for an effective and efficient extraction process and were all fixed at values optimized using univariate design. The mass of sodium chloride and the centrifugation time showed negative effect, while the mass of magnesium sulphate, volume of acetonitrile and mass of buffering salt showed significantly positive effects (Fig. 3a).

The significance of the mass of magnesium sulphate agreed with the finding of Wei and co-workers in the determination of pesticide multiresidues in tomatoes using QuEChERS and GC–MS/MS (Wei et al., 2020). Souza and co-workers in their study on the determination of pyrethroid and organophosphate pesticides in honey, reported that, the mass of magnesium sulphate showed significantly negative effect, while significantly positive effect was reported for the mass of sodium chloride at $p \leq 0.1$ (Souza, Petrarca, de Campos Braga, Rodrigues, & Reyes, 2021). Similar observation was reported by Narenderan and co-workers, in the determination of organophosphorus insecticides in carrot and potato samples (Narenderan, Meyyanathan, Karri, Babu, & Chintamaneni, 2020). The observations implied that, the significant of each factor depends on the physicochemical properties of pesticide residues and the nature of sample matrix (Bulaić Nevistić & Kovač Tomas, 2023). The ANOVA results of the Plackett-Burman design (Table S4), showed that the *P*-value of the model is significant ($0.029 < 0.05$).

The volume of acetonitrile showed significantly positive effect, which agreed well with the fact that the efficiency of QuEChERS technique is associated with the extraction solvent employed, because of the chemical properties of pesticide residues (Manav, Dinç-Zor, & Alpdoğan, 2019). The effect of centrifugation time was significantly negative and agreed with the report of Narenderan and co-workers (Narenderan et al., 2019). The regression equation in uncoded units is given in eq. 1. The significant factors were further optimized using central composite design.

$$TCPA = 4 - 457A + 1817B + 1584C + 4303D - 5521E + 11251F - 0.177G - 2331 - 14524\ Ct\ Pt \quad (1)$$

3.1. Central composite design (CCD)

The second order response surface methodology such as central

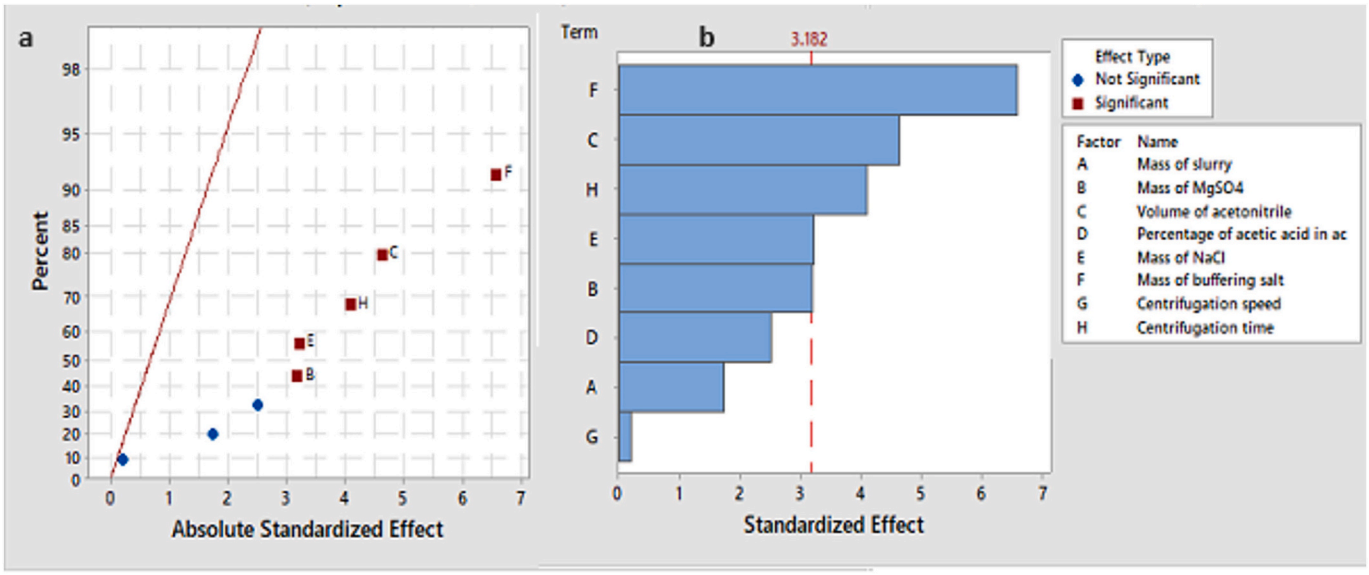


Fig. 2. (a) Pareto chart of standardized main effect (b) Normal plot of standard effect (response is TCPA α = 0.05).

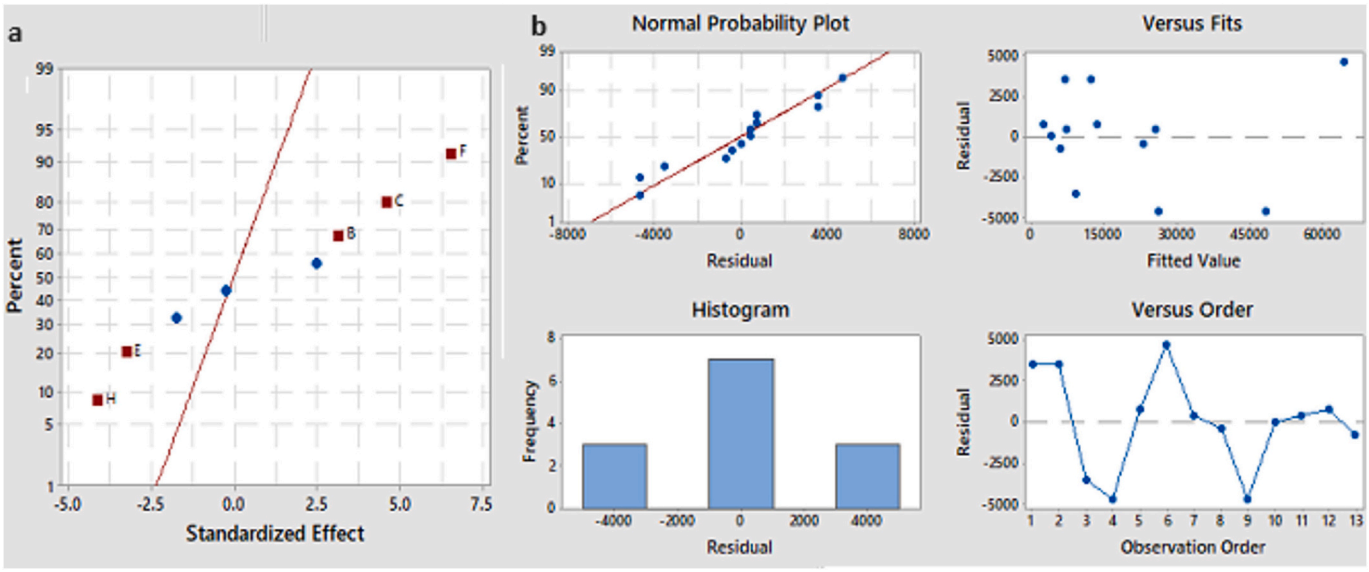


Fig. 3. (a) Half normal plot of standardized effect; (b) Residual plot of TCPA in Plackett-Burman Design.

composite design was used to optimize the significant factors estimated using the screening Plackett-Burman design (Table S5). The CCD design matrix (Table S6), was generated with Minitab® statistical software version 17 using the following factors (mass of magnesium sulphate (A), volume of acetonitrile (B), mass of sodium chloride (C), mass of sodium acetate (D) and centrifugation time (E). The design matrix contained 16 runs for cube points, 6 runs for center points in cube, 10 runs for axial points and 0 run for center points in axial, making a total of 32 runs. The regression equation in uncoded units for the CCD is given in eq. 2. Other factors which were found not to significantly affect the extraction

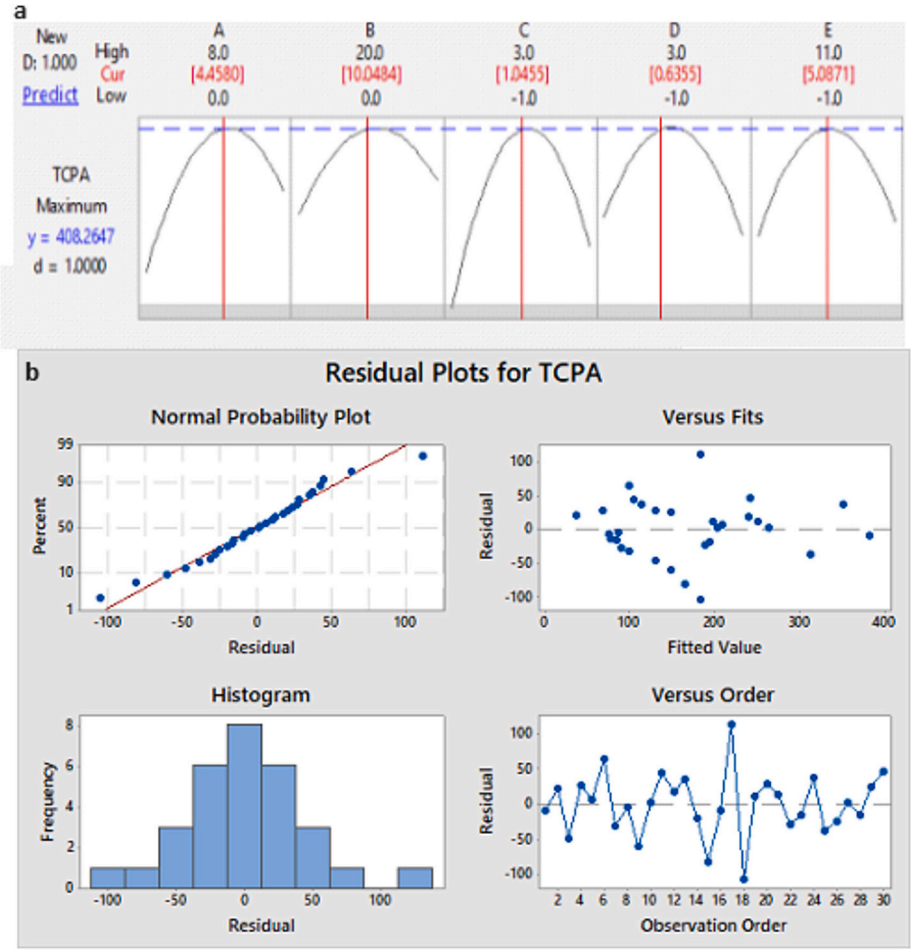


Fig. 4. (a) Response optimizer for the optimum parameters; (b) Residual plot for TCPA of the CCD design.

efficiency but are needed for efficient extraction were fixed at the optimized values estimated using univariate analysis.

The response surface methodology (RSM) values were 4.45 g of magnesium sulphate, 10 mL of acetonitrile, 1 g of sodium chloride, 0.64 g of sodium acetate and 5 min of centrifugation time as shown in the response optimizer (Fig. 4a). The residual plot (Fig. 4b) illustrated the response surface of the interaction of various variables that yielded the highest total chromatographic peak areas. As shown in Fig. 4a, all the significant factors were observed to increase extraction efficiency up to the optimum values, and beyond the optimum values, the extraction efficiency was observed to decrease. Consequently, the optimized QuEChERS extraction parameters were represented in Table S8, while taking into consideration the results obtained from univariate analysis. The *P*-value observed for the central composite design was found to be statistically insignificant ($0.194 > 0.05$) (Table S7).

$$\begin{aligned}\text{TCPA} = & -150 + 36.5\,\text{A} + 12.1\,\text{B} + 64.9\,\text{C} - 30.4\,\text{D} + 35.2\,\text{E} \\ & + 1.59\,\text{A*A} + 0.894\,\text{B*B} + 8.5\,\text{C*C} + 36.0\,\text{D*D} - 0.86\,\text{E*E} \\ & - 3.49\,\text{A*B} - 22.68\,\text{A*C} - 0.01\,\text{A*D} + 4.41\,\text{A*E} - 0.82\,\text{B*C} \\ & - 0.87\,\text{B*D} - 3.63\,\text{B*E} + 22.6\,\text{C*D} - 1.15\,\text{C*E} - 5.61\,\text{D*E}\end{aligned} \quad (2)$$

3.2. Method validation

The desirability and effectiveness of the developed method is determined by validation of its figures of merit (Lawal, Wong, Tan, Abdulra'uf, & Alsharif, 2018). This helps to determine if the developed method is suitable for its intended purpose (Abdulra'uf, 2014). The method validation also serves as quality assurance step for the determination of contaminants in food samples (Thompson, Ellison, & Wood, 2002). The method validation is achieved by determining the linear range, accuracy in terms of average recoveries, inter-day and intra-day precision in term of relative standard deviation, limit of quantitation and limit of detection, according to EU Commission decision (SANTE, 2021). The validated parameters were all determined using the optimum QuEChERS extraction parameters.

3.3. Calibration curve and linearity range

The calibration curve for each pesticide was constructed by analyzing the working pesticide standard at seven (7) different concentration levels. The concentration was ranged from 0.5 to 500 μg/kg. The calibration curves of all the investigated pesticides were linear over the tested concentration range. The linearity, correlation coefficient (r^2) which was found to be greater than 0.99 for all pesticide residues and the linear equation are given in Table 2.

3.4. Accuracy and precision

The relative recovery was determined to estimate the accuracy of the method (Table 3). Blank beans spiked with the mixed pesticide standard at two concentration levels (50 and 100 μg/kg) were subjected to extraction using the optimized parameters. The relative recoveries were determined along with relative standard deviation (intra- and inter-day precisions) by analysis three separately spiked samples a day, to determine the intra-day precision and analysis three separately spiked samples a day for three days to determine the inter-day precision. Table 3 show the relative recoveries and precision. The average recoveries ranged from 75.55 to 110.41 % with intra-day and inter-day precision ranging from 0.70 to 16.65 % and 0.62–17.93 % respectively. The observed average recoveries in this study were with the acceptable range of 70–120 % and relative standard deviation of less than 20 %, according to the SANTE guidelines (SANTE, 2021). The result obtained in this study agreed well with the results (89–104 %) of Odion and co-researchers, who determined organochlorine pesticide residues in beans samples using Soxhlet apparatus followed by gas chromatography–mass spectrometry (Odion, Usifoh, Abolagba, Igene, & Usifoh, 2020).

Table 2
Linearity, linearity equation and correlation coefficient (r^2) of the developed method in the beans sample spiked with 50 μg/kg of the working standard solution.

Pesticides	Range (μg/kg)	Linear Equation	r^2
Aldrin	2.5–250	$y = 0.003\times + 0.0006$	0.9998
α-BHC	2.5–250	$y = 0.0062\times + 0.0685$	0.9973
δ-BHC	0.5–250	$y = 0.0191\times + 0.0712$	0.9983
Lindane	1–500	$y = 0.0009\times + 0.0385$	0.9999
β-BHC	10–500	$y = 0.0061\times + 0.0664$	0.9996
α-Chlordane	1–500	$y = 0.001\times + 0.0051$	0.9998
γ-Chlordane	1–500	$y = 0.0038\times + 0.0389$	0.9997
4,4′-DDD	1–500	$y = 0.0255\times + 0.0565$	0.9998
4,4′-DDE	1–500	$y = 0.0046\times + 0.0143$	0.9999
4,4′-DDT	1–500	$y = 0.0068\times$ - 0.0217	0.9997
Decachlorobiphenyl	1–500	$y = 0.002\times + 0.0174$	0.9999
Dieldrin	0.5–500	$y = 0.0055\times + 0.015$	0.9993
α-Endosulfan	5–500	$y = 0.001\times$ - 0.0007	0.9995
β-Endosulfan	5–500	$y = 0.002\times + 0.0203$	0.9993
Endosulfan sulfate	2.5–500	$y = 0.0136\times + 0.0661$	0.9997
Endrin	2.5–500	$y = 0.0024\times + 0.0732$	0.9994
Endrin aldehyde	0.5–500	$y = 0.0165\times + 0.0624$	0.9989
Endrin ketone	5–500	$y = 0.0009\times + 0.0038$	0.9978
Heptachlor	5–500	$y = 0.0036\times$ - 0.0447	0.9983
Heptachlor *exo*-epoxide	5–500	$y = 0.1492\times$ - 0.2242	0.9995
Methoxychlor	2.5–250	$y = 0.0076\times + 0.0152$	0.9984
2,4,5,6-Tetrachloro-m-xylen	2.5–250	$y = 0.0359\times + 0.0825$	0.9993

3.5. Limits of detection (LOD) and quantitation (LOQ)

The LOD ranged from 0.23 to 1.77 μg/kg, while the LOQ were found between 0.76 and 5.88 (Table S9). These were the range found by Odion and co-workers in the analysis of pesticide residues for the analysis of organochlorine pesticides in cowpea using Soxhlet extraction (Odion et al., 2020). The observed LOQs were found to be lower than those found by Abdallah and co-workers, in the analysis of clothianidin residues in green bean (Abdallah, Abd El-Hamid, & Abdel Raheem, 2019).

3.6. Matrix effect

The enhancement or suppression of analyte recoveries from the prepared sample is determined by measurement of the matrix effect (ME), which also determines the effectiveness of matrix interference. It has been proposed that, when matrix effect is less than or equal to – 20 %, the analyte recovery is suppressed, while it is enhanced if matrix effect is greater than or equal 20 % (Lawal et al., 2018). It was also proposed that ME value ranging from 10 to 50 % indicates moderate increase in the signal (moderate enhancement), and values greater than 50 % indicates high increase in the signal (high enhancement) (Steiner, Krska, Malachová, Taschl, & Sulyok, 2020; Stremel et al., 2018). As shown in Table S10, the matrix effect ranged from – 81.45 to 6.65 %, which were found to be minimal. The matrix effect observed in this study could be as a result of the effective clean-up step, which helped to remove matrix interference during the QuEChERS extraction clean-up stage (Lawal, Wong, Tan, & Abdulra'uf, L. B., 2019). The observed matrix effect was lower than the reported matrix effect (33.3–103.5) in the analysis of organochlorine pesticides from food samples (Stremel et al., 2018).

3.7. Analysis of real samples

The developed QuEChERS method was consequently employed for the analysis of pesticide residues in 6 varieties of beans samples

Table 3
Average recoveries, intra-day and inter-day precision of the spiked pesticides.

Pesticides	Added (μg/kg)	Recovery (%)	Intra-day RSD (n = 3) (%)	Inter-day RSD (*n* = 9) (%)
Aldrin	50	97.13	3.23	14.20
	100	102.69	1.98	1.79
α-BHC	50	99.71	3.02	14.22
	100	104.35	3.57	10.06
δ-BHC	50	94.63	4.19	4.77
	100	103.63	4.79	4.11
Lindane	50	104.24	1.87	4.05
	100	99.06	2.33	1.82
β-BHC	50	90.75	2.37	2.65
	100	99.45	4.93	5.45
α-Chlordane	50	106.25	5.20	5.89
	100	98.54	2.49	2.19
γ-Chlordane	50	98.92	4.14	1.24
	100	103.04	2.38	2.10
4,4′-DDD	50	88.40	9.92	11.07
	100	98.19	2.49	2.20
4,4′-DDE	50	93.70	13.88	11.41
	100	102.07	4.80	4.23
4,4′-DDT	50	99.33	6.91	8.31
	100	109.14	2.24	1.98
Decachlorobiphenyl	50	83.77	14.48	12.99
	100	106.52	7.92	8.97
Dieldrin	50	110.41	16.65	14.36
	100	108.55	4.34	2.44
α-Endosulfan	50	103.55	15.80	17.93
	100	109.39	0.70	0.62
β-Endosulfan	50	78.25	4.64	5.02
	100	75.55	6.48	7.17
Endosulfan sulfate	50	80.56	11.51	15.49
	100	79.86	8.67	11.75
Endrin	50	106.54	7.97	8.12
	100	85.15	3.67	4.00
Endrin aldehyde	50	99.46	10.75	9.33
	100	100.89	8.37	9.18
Endrin ketone	50	77.61	6.21	6.43
	100	98.13	7.46	7.26
Heptachlor	50	77.61	6.21	6.43
	100	102.57	12.85	10.86
Heptachlor exo-epoxide	50	87.28	9.70	10.04
	100	102.63	11.78	13.19
Methoxychlor	50	82.46	8.90	10.24
	100	94.95	5.77	8.20
2,4,5,6-Tetrachloro-m-xylen	50	82.78	10.59	11.66
	100	79.04	4.12	4.98
Range		75.55–110.41	0.70–16.65	0.62–17.93

collected from 4 different major markets in Ilorin metropolis. This was done to determine the reliability and effectiveness of the developed method in the extraction of target analytes in real samples. As shown in Table S11 – S14 (Supplementary materials), it was observed that, 4,4′-DDE (74.96 ± 6.53 μg/kg) and 4,4′-DDT (75.76 ± 4.61 μg/kg) had the highest residue level in Enyenkorin (EM) (Table S11), Mandate (MM) (Table S12) and Ita-nmo (IM) (Table S13) markets, respectively, while α-endosulfan (89.19 ± 1.37 μg/kg) has the highest residues in Baboko market (BM) (Table S14). The lowest concentration of pesticide was found in Brown milk beans (B1) obtained from Baboko market (BM) (Table S14) with 1.73 ± 0.44 μg/kg of dieldrin. Brown oloyin beans was found to have lower incidence of the organochlorine pesticides in all of the markets, as 11 out of the 21 pesticide residues were not detected in the sample.

Organochlorines have relatively high chemical stability and it is persistent in the environment (Kaushik & Kaushik, 2007), with field half-life ranging from 9 days to 15 years (Ansari et al., 2024). Researches have shown that, the persistent of organochorines in the environment in due to the fact that, 99.9 % of the pesticides applied on the field are released into the environment as runoff, infiltration and residues on crop, while only 0.1 % reach the target pests (Pimentel, 1995; Zhang, Jiang, & Ou, 2011). Thus, 4,4′-DDT and 4,4′-DDE were detected in all samples obtained in Eyenkorin market, with concentration ranging from 41.92 to 69.04 μg/kg, only DDT was detected in all samples obtained from mandate market with concentration ranging from 47.10 to 72.34 μg/kg. Baboko market had the highest incidence of pesticide residues, all the beans collected contained 4,4′-DDT (47.10–77.57 μg/kg), 4,4′-DDE (55.14–84.22 μg/kg), 4,4′-DDD (8.72–61.43 μg/kg), dieldrin (1.73–26.58 μg/kg), and eldrin (4.33–14.67 μg/kg), while Ita-mo market has the lowest incidence of pesticide residues with only DDD detected at concentration ranging from 52.48 to 75.76 μg/kg in all the beans samples collected.

The pesticide residues detected in beans in this study aligned with the study of Otitoju and Lewis, who assessed the health risk of pesticide residues in bean samples obtained from Wukari, Taraba State (Otitoju & Lewis, 2021) and with the study of Ogah and co-workers, who analyzed organochlorine pesticides in beans from markets in Lagos State (Ogah et al., 2011), and also with the findings of Olutona and Aderemi, who analyzed the presence of organochlorine pesticides in leguminous food crops from selected markets in Ibadan, Nigeria (Olutona & Aderemi, 2019). Aldrin, 4,4′-DDE, 4,4′-DDT and β-Endosulfan were found at concentration higher than the maximum residue levels in all the beans samples obtained from the four markets. α-BHC, δ-BHC and endrin were found at concentrations higher than the MRLs in white big beans and white small beans in all the four markets, while they were either not detected in some beans varieties or were detected at concentration lower than the MRLs (Table S11 – S14).

In Eyenkorin markets (Table S11), 12 different residues were detected in brown milk beans (B1) (8 residues found lower than the MRLs), 6 in brown oloyin beans (B2) (3 residues found in concentrations lower than the MRLs), 7 in brown drum beans (B3) (4 residues lower than the MRLs), 10 in brown big beans (B4) (8 residues lower than the MRLs), 5 in white big beans (B5) (all residues found were above the MRLs) and 9 in white small beans (B6) (2 residues lower than the MRLs). In Mandate market (Table S12), 12 residues were detected in brown milk beans (B1) (9 residues lower than MRLs), 8 in brown oloyin beans (B2) (3 residues lower than the MRLs), 7 in brown drum beans (B3) (3 residues lower than the MRLs), 12 in brown big beans (B4) (9 residues lower than the MRLs), 5 in both white big (B5) (only 1 residue lower than the MRL) and white small beans (B6) (all residues found were above the MRLs). In Ita-mo market (Table S13), the occurrence of the residues detected showed that brown milk beans (B1) contained 14 (10 residues were found below the MRLs), 6 in brown oloyin beans (B2) (3 residues were found below the MRLs), 6 in brown drum beans (B3) (3 residues were found below the MRLs), 10 in brown big beans (B4) (4 residues were below the MRLs), 5 in white big beans (B5) (all were above the MRLs) and 7 in white small beans (B6) (all were found above the MRLs), while 15 residues were detected in brown milk beans (B1) (11 residues were below the MRLs), 7 in brown oloyin beans (B2) ((3 residues found below the MRLs), 10 in brown drum beans (B3) (6 residues found below the MRLs), 7 in brown big beans (B4) (with 2 residues found below the MRLs), 12 in white big beans (B5) (only 1 residues below the MRLs) and 10 in white small beans (B6) (all residues were above the MRLs) in Baboko market (Table S14).

On an average, higher number of pesticides were applied to the brown milk beans varieties, followed brown big beans, then brown drum beans and white small beans had the same incidence of pesticide residues, while brown oloyin beans and white big beans had the lowest incidence of pesticide residues. There was statistically significant difference ($p \leq 0.05$) between the incidence of pesticide residues in all the beans samples. Commercial pesticide products usually contain at least one active ingredient. A farmer or vendor may use more than one pesticide to control pest either on the field or in the store. It was gathered from the interaction with wholesalers, that most of the beans sold in Ilorin metropolis were bought from Niger State, while few are from Kano and Sokoto States and others from Cameroon and Niger Republic.

Pesticides are valuable components, and are useful for food production and also for control and eradication of vectors of human disease.

However, some pesticides are poisonous and carcinogenic. There is urgent need to increase the surveillance by National Agency for Food Drug Administration and Control (NAFDAC), Standard Organization Nigeria (SON) and the Federal Ministry of Agriculture on the manufacturing, importation and use of banned pesticides in the country. Routine and periodic monitoring of pesticide residues in food samples in warehouses and in the markets is recommended to protect consumers from unscrupulous food supply agents, and good agricultural practices (GAP) should also be encouraged.

4. Conclusion

The misuse of pesticides in Nigeria is of increasing concern. It has economic and health implications, therefore, there is a need for frequent monitoring of pesticide residues in food samples, especially at the point of sale. To this end, there is urgent need for fast and simple extraction method for the efficient determination of pesticide residues in food samples. QuEChERS satisfied these requirements and also at low cost and environmentally friendly.

Plackett-Burman (PB) design was applied as a screening method to determine the factors that are significant to the extraction of pesticide residues from beans samples. The PB design, which is a first order design with reduced factorial is time-saving. Central composite (CC) design was employed for the optimization of significant factors in the extraction and clean-up of the extract, before introduction into GC–MS, for separation, identification and quantification of the pesticide residues with little or no interference.

The EU commission decision was used for the determination of figures of merit of analytical methodology in a pesticide free bean samples spiked with known amount of the pesticide mix standard. The result of method validation was found to be satisfactory and therefore was used for the determination of 21 pesticide residues in 6 varieties of beans obtained from 4 different wholesale markets in Ilorin metropolis.

The study has shown that the application of design of experiment to QuEChERS extraction technique followed by gas chromatography–mass spectrometry analysis is time-saving and low cost with high sample throughput in pesticide analysis. The study also revealed that, beans farmers and beans wholesalers used high percentage of organochlorine pesticides, which have been banned in Nigeria.

It can be concluded from the study that most of the beans obtained in Ilorin wholesale markets, contained organochlorine pesticides which may pose health risks to consumers. The results of this study showed that beans samples sold in Ilorin metropolis contain pesticide residues some of which were found to be higher than the maximum concentration legally permitted to be on the food.

CRediT authorship contribution statement

Lukman Bola Abdulra'uf: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Abibat Monisola Junaid:** Funding acquisition, Formal analysis. **Amudalat Ranti Lawal:** Investigation, Formal analysis. **Hassan Bisiriyu Ibrahim:** Investigation, Formal analysis. **Guan Huat Tan:** Project administration, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

This study was supported by the Tertiary Education Trust Fund (TETFUND) Institutional-Based Research (IBR) grant, through the Centre for Research Development, Innovation, Incubation and In-House Training (CREDIIIT), Kwara State College of Education, Ilorin, Kwara State, Nigeria.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.141464.

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