Journal of Oil Palm Research Vol. 36 (1) March 2024 p. 146-157
DOI: https://doi.org/10.21894/jopr.2023.0018

DEVELOPMENT AND IN-HOUSE VALIDATION OF MULTI-RESIDUE METHOD FOR TRIAZOLE FUNGICIDES IN PALM KERNEL, MESOCARP AND RAW AGRICULTURE COMMODITY (RAC) USING GAS CHROMATOGRAPHY-MICRO ELECTRON CAPTURE DETECTOR (GC-µECD)

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ABSTRACT

*A simple, fast and rapid multi-residue method for the analysis of triazole fungicide residues in palm kernel, mesocarp and raw agricultural commodity (RAC) was developed and validated using gas chromatography-micro electron capture detector (GC-µECD). The method was developed from a modified QuEChERS extraction method and followed by dispersive solid-phase extraction (*d*-SPE) clean-up procedure. Five triazole compounds were studied, namely tetraconazole, penconazole, hexaconazole, propiconazole and epoxiconazole. Optimisation of different solvent extractions was conducted in order to find a suitable solvents for triazole residue extraction. The developed method was validated in-house and satisfied the important validation parameters such as recovery, repeatability and reproducibility based on SANTE guideline. The recoveries for all fungicides at fortification levels of 0.1, 0.5 and 1.0 mg kg^{-1} in palm kernel, mesocarp and RAC were in the range of 80% to 108% with relative standard deviations (%RSD) of less than 10%. The limit of detection (LOD) and limit of quantification (LOQ) for the developed method were estimated to range between 0.12 and 0.23 mg kg^{-1} and 0.31 and 0.7 mg kg^{-1}, respectively.*

Keywords: mesocarp, modified QuEChERS, palm kernel, raw agricultural commodity (RAC), triazole.

Received: 23 November 2022; **Accepted:** 28 February 2023;

INTRODUCTION

Palm oil is one of the most versatile and efficient vegetable oils traded worldwide. Like any other major vegetable oil, palm oil trading is subjected to many international trading specifications and regulations. CODEX Standard 210-1999 has defined palm oil and palm kernel oil as products derived from fleshy mesocarp and kernel of the fruit of the oil palm (*Elaeis guineesis*), respectively (CODEX, 1999). CODEX classified oil palm fruit as Processed Food of Plant Origin under Nuts and Seeds (SO) and Group 023, Oilseed (FAO, 2021). Oilseed consists of seeds from a variety of plants used in the production of vegetable oils, seed meals and cakes for animal feed.

CODEX report on pesticide residues in food stated that any processed foods obtained from primary food commodities, which it is derived, needs to be analysed separately (CODEX, 1993). If the residue higher in the processed food than the RAC, separate maximum residue limit (MRL) should be considered for the processed food. Crude palm oil (CPO) and crude palm kernel oil (CPKO) are categorised under Class D: Processed Food of Plant Origin and divided into Type 067: Vegetable oil, crude which is derived products of plant

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origin. Based on this classification, oil palm fruits are considered as RAC and therefore the whole commodity components need to be analysed. According to the CODEX food category system, RAC means any food in its natural state intended for processing into food for sale to the consumer or as a food without further processing. It includes irradiated primary food commodities and products after removal of certain parts of the plant or parts of animal tissue (FAO, 2009). However, there is no published method available for the determination of pesticide residues in oil palm fruits and RAC.

On top of that, Codex Alimentarius Commission (CAC) also emphasised on other parts of commodities which are used as animal feeds. Palm kernel cake (PKC) is mainly used as a common ingredient for animal feed formulation. These commodities are listed under Class C: Primary Animal Feed Commodities. Previous studies had shown that PKC has high nutritional values (protein, fibre or lipids), provides balanced diet in amino acids and metabolisable energy (Alimon, 2004; Sharmila *et al.*, 2014; Sundu *et al.*, 2006). Many studies have reported that fermented PKC has some probiotic activity capable to enhance the performance of broilers and improve product quality (Aladi *et al.*, 2017; Halim *et al.*, 2021; Hanafiah *et al.*, 2017; Kalidas *et al.*, 2017). Despite the widespread uses of palm kernel in animal feed formulations, analytical method for the determination of pesticide residues in palm kernel matrices has never been established. There are many published methods for the determination of pesticide residues in CPO and CPKO (Halim *et al.*, 2020; Khairuddin *et al.*, 2021; Muhamad *et al.*, 2008; 2012a; Sobhanzadeh and Nemati, 2012; Sulaiman *et al.*, 2022; Yeoh *et al.*, 2006; Zainudin *et al.*, 2009). Under Malaysian Palm Oil Board Code of Practices (MPOB CoP), clause 4.8.2 suggests that CPO for edible purposes should be monitored frequently for pesticide residues (MPOB, 2021). However, to date, analytical method for the determination of pesticide in mesocarp is not available despite mesocarp is the source for CPO.

QuEChERS, is a common method used for multi-residue pesticides analysis in fruits and vegetables. QuEChERS stands for Quick, Easy, Cheap, Effective, Rugged, and Safe, is a simple sample preparation method that was introduced by Anastassiades *et al.* (2003). The principle of this method is based on a salt-out extraction using solvents; commonly acetonitrile followed by a d-SPE clean-up procedure using sorbents such as magnesium sulphate ($MgSO_4$) and primary secondary amine (PSA). QuEChERS method provides reliable results, simple analytical process for extraction and clean-up steps, less reagents consumption as well as minimum utilisation of laboratory instrument and apparatus. Nonetheless, further improvement to this method will broaden its applicability to other wide variety of commodities. This method gives a huge advantage to the food safety industry as it is cost-effective.

The most frequent and widely used sorbents in QuEChERS method were PSA, graphite carbon black (GCB) and octadecyl (C18) sorbents combination and anhydrous $MgSO_4$ for clean-up step (Anagnostopoulos and Miliadis, 2013; Deme *et al.*, 2014). Numerous published reports on the analysis of pesticide residues in vegetable oil using d-SPE with different sorbent combinations and anhydrous $MgSO_4$ for clean-up step. PSA is a well-known sorbent for removing of fat content when necessary, the chemical structure of PSA provides high retention of free fatty acids and other polar matrix compounds (Deme *et al.*, 2014). GCB has a strong affinity for planar molecules, and thus effectively removes pigments such as chlorophyll and carotenoids, as well as sterols present in foods (Anastassiades *et al.*, 2003). Meanwhile, C18 can act as a clean-up sorbent resulted in a chromatogram with lower background and interfering peaks (Sobhanzadeh *et al.*, 2012). However, the use of PSA and GCB may raise some concerns especially on the recovery of pesticides with planar structures *e.g.*, chlorothalonil and thiabendazole (Lentza-Rizos *et al.*, 2001; Muhamad *et al.*, 2012).

Many studies reported the performance of modified QuEChERS method in analysing pesticide residues in various matrices such as edible oils (Chamkasem and Harmon, 2015; Halim *et al.*, 2020; Theurillat *et al.*, 2021), fruits and vegetables (Dong *et al.*, 2019; Li *et al.*, 2017) and soil (Asensio-Ramos *et al.*, 2010; Caldas *et al.*, 2011). Slight method modification involving the inclusion of liquid-liquid partition step and additional of water and iso-octane to the acetonitrile extracts facilitate the separation of the oil layers (Marchis *et al.*, 2012). New adsorbent materials such as cetyltrimethylammonium bromide (CTAB)-modified zeolite, synthetic magnesium silicate (florisil) and molecularly imprinted polymer (MIP) were also used as clean-up materials after the QuEChERS extraction (Batlokwa *et al.*, 2013; Salisaeng *et al.*, 2016; Wang *et al.*, 2017). Recently, metal-organic frameworks (MOFs), UiO-66 has shown its effectiveness as adsorbent in d-SPE clean-up for eliminating matrix interference compounds for determination of OP pesticides in edible vegetable oils (Mao *et al.*, 2019).

Fatty food commodities such as olives, oil seeds, oils, nuts, milk and other dairy products, fish and meat require a slight modification of the QuEChERS method. Specific modifications of QuEChERS method are crucial in order to obtain good recovery values and satisfactory extract purification (Rejczak and Tuzimski, 2015). Fatty foods pose great challenge due to some of the lipids are coextracted with acetonitrile and may cause difficulties in subsequent analysis or some fat-soluble nonpolar analytes could persist in fatty food samples and give poor extraction efficiency rates (Muhamad *et al*., 2012a). The aim of this study is to develop fast, simple and reliable multi-residue method for determination of triazole fungicide in palm kernel, mesocarp, and RAC. Quantification of triazole fungicide residues was performed using GC-μECD.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and acetonitrile of pesticide grade were purchased from Merck (Darmstadt, Germany). Formic acid and acetic acid for mass spectrometry with purity of 98.0% were obtained from Sigma-Aldrich (St. Louis, USA). Sodium chloride (NaCl) and magnesium sulphate ($MgSO_4$) were purchased from Sigma-Aldrich. For the d-SPE adsorbents, bulk PSA, DSC-18 (C18) and ENVI-Carb GCB were obtained from Supelco Inc. (Bellefonte, PA, USA). Pesticide reference standard of triazole group was purchased from Dr. Ehrenstorfer (Augsburn, Germany) with purity of 98.8%.

Instrumentation

Gas chromatography-micro-electron capture detector (GC-μECD) (Agilent Technologies 7693 Series) equipped with an automated injector was used throughout this study. The system was operated with an HP-5 (5% phenylmethylsiloxane) capillary column (30 m long, 250 μm internal diameter and 0.25 μm thickness). The column oven temperature was first set at an initial temperature of 55°C and maintained for 1 min. The temperature was then increased to 220°C at 5 min and kept at the final temperature of 300°C for 8 min. The injector in spitless mode and detector temperature were set at 250°C and 280°C, respectively. Nitrogen was used as carrier and make-up gas, with a flow rate of 0.8 and 60 mL min^{-1}, respectively. The injection volume was set at 1.0 μL.

Preparation of Reference Solution

Stock solution at concentration of 1000 mg L^{-1} of individual triazole fungicide was prepared by dissolving the appropriate amounts of individual triazole compound in acetonitrile into 10 mL volumetric flask. Then, the mixed reference stock solution (100 mg L^{-1}) containing all the triazole analytes was prepared by pipetting 1 mL of each individual stock solutions into a 10 mL volumetric flask and adding acetonitrile before mixing the solution for equilibrium. An intermediate working reference solution was prepared at the concentration of 10 mg L^{-1} through dilution using acetonitrile solvent in 10 mL volumetric flask. All solutions were stored in the dark at 4°C prior to analysis.

Palm Kernel, Mesocarp and RAC Samples for Fortification

Fresh mesocarp and palm nuts were collected after manual pressing of oil palm fruit in laboratory. Palm nuts sample was washed with hexane to remove oil, air-dried and manually crush to separate shell and kernel. Then, palm kernel was ground to powder form using laboratory blender (Waring 8010S/G). Fresh mesocarp was air-dried, cut into size of 0.5-1.0 cm using scissors and ground to homogeneous size. Fresh RAC was cut layer by layer into small pieces (0.2-0.5 cm) and ground using laboratory blender. All samples (palm kernel, mesocarp and RAC) were used for blanks and fortified samples in validation and matrix-matched standards preparation for calibration. The samples were stored in the freezer (-4°C) prior to analysis. Processed palm kernel, mesocarp and RAC samples are shown in *Figure 1*.

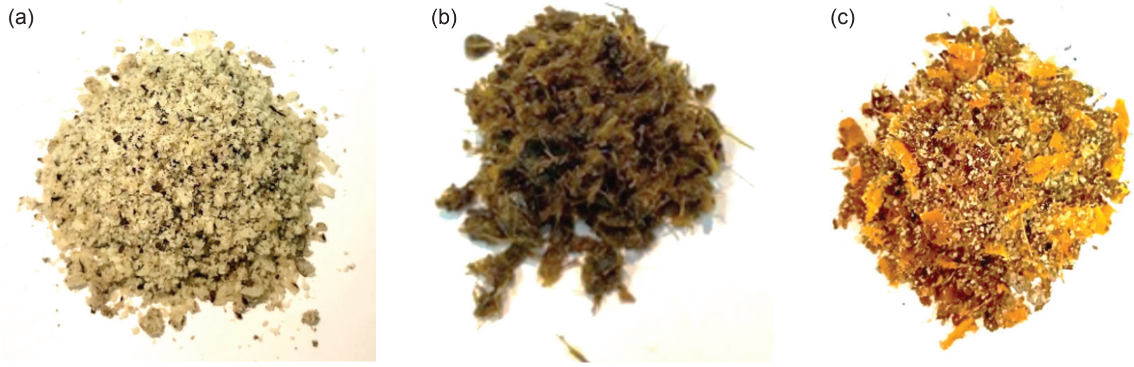

Figure 1. Processed (a) palm kernel, (b) mesocarp and (c) RAC samples.

Establishment of Matrix-Matched Calibration Curve

Matrix-matched calibration was established in a series of mixed standard at concentration of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mg L^{-1} in palm kernel, mesocarp and RAC blank extracts. Samples of 1.0 g palm kernel, mesocarp and RAC were weighed in 50 mL screw cap centrifuge tubes and fortified with working standard solution to give final concentrations of 0.5, 1.0 and 5.0 mg g^{-1}. The tubes were mixed well using a vortex mixer and allowed to stand for 10 min to reach equilibrium. Then, 10 mL of acidified acetonitrile (1% acetic acid) was added into the spiked samples followed by 4 g of $MgSO_4$ and 1 g of NaCl. The tubes were then shaken for 3 min using a vortex mixer and underwent centrifugation at 5000 rpm for 5 min. Thereafter, 1 mL of supernatant was transferred into a d-SPE tube containing various amounts of $MgSO_4$, PSA and GCB. The matrix effect was calculated and expressed as percentage difference of the slope of the calibration curve in solvent and in matrix using Equation (Ferrer *et al.*, 2011):

$$\begin{array}{c}\%\ \text{Matrix}\\ \text{effect (ME)}\end{array} = \left[\frac{\text{slope matrix}}{\text{slope solvent}} - 1\right] \times 100\% \qquad (1)$$

Modified QuEChERS Method

Preliminary tests were conducted to evaluate the modified QuEChERS with original QuEChERS (Anastassiades *et al.*, 2003). For the evaluation of the extraction step, blank matrix was spiked at 1 mg kg^{-1} in five replicates and matrix match calibration was used for recovery calculation.

One gram of sample was weighed in a 50 mL polypropylene (PP) tube. The extraction solvent was added and the samples were hand shaken for 1 min. The solvent added in each test tube was as follows: (a) 10 mL of acetonitrile (100%), (b) 10 mL of 1% (v/v) acetic acid in acetonitrile, (c) 10 mL of 3% (v/v) acetic acid in acetonitrile, (d) 10 mL of 1% (v/v) formic acid in acetonitrile and (e) 10 mL of 3% (v/v) formic acid in acetonitrile. Then, 4 g of $MgSO_4$ and 1 g of NaCl were added to initiate partitioning process. The centrifuge tube was vortexed for 3 min for palm kernel and RAC and 5 min for mesocarp, followed by centrifugation at 5000 rpm for 5 min. Afterwards, 1 mL of supernatant was subjected to clean-up step using d-SPE. The optimum extraction solvent was selected upon the highest value of mean recovery of the analytes. The flow chart of QuEChERS procedure for the pesticide residual analysis is summarised in *Figure 2*.

Dispersive Solid-Phase Extraction (*d*-SPE) Clean-up

Various sorbent combinations were investigated using *d*-SPE for clean-up procedure prior to GC-μECD analysis. An aliquot (1 mL) of the upper layer was transferred into a *d*-SPE tube containing one of the following for testing: (1) 0.45 g of anhydrous $MgSO_4$ and 0.20 PSA, (2) 0.45 g of anhydrous $MgSO_4$, 0.25 g of PSA and 20 g of GCB, (3) 0.45 g of anhydrous $MgSO_4$, 0.25 g of PSA and 25 g of GCB. The tubes were then tightly capped and shaken for 1 min before centrifugation at 3000 rpm for 1 min.

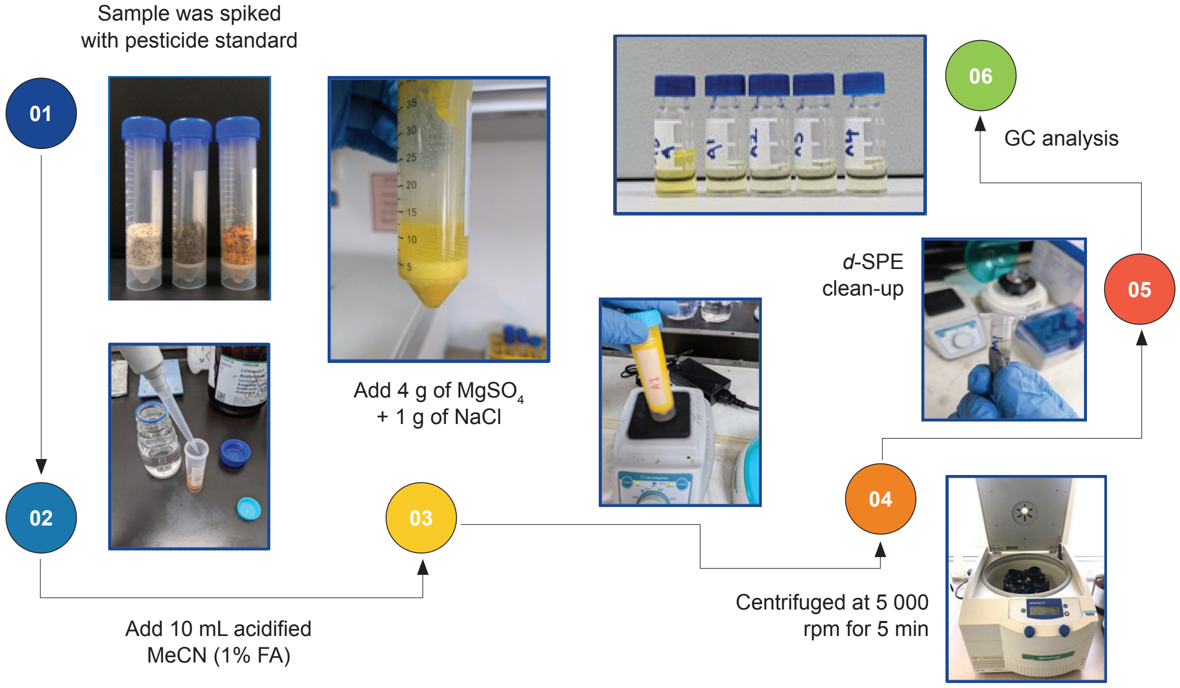

Figure 2. Flow chart of modified QuEChERS procedure.

Finally, 1 mL aliquot was filtered through a 0.22 µm nylon filter into an autosampler vial prior to GC-µECD analysis.

Analytical Method Validation for Pesticide Residual Analysis

In-house validation study was conducted in order to evaluate accuracy, precision, linearity, LOD, LOQ, matrix effects and repeatability as described in Document No. SANTE/2017/11813 (SANTE, 2020).

Statistical Analysis

All analyses were performed in six replications and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's test (α=0.05) were used for comparison of the treatments using Minitab 21 Statistical Software (USA).

RESULTS AND DISCUSSION

Linearity, Matrix-Matched Calibration Curve and Matrix Effect

Calibration curves for triazole fungicides were constructed by plotting the individual peak area against the concentration of corresponding calibration standards at 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mg L^{-1} in acetonitrile. A good linearity with the regression coefficients (R2) of > 0.99 was obtained for all triazole residual. *Figure 3* shows chromatogram of the triazole mixture standard analysed using GC-µECD. Matrix effect was calculated by comparing the slope of calibration curves of acetonitrile and matrix. The matrix effects for triazole fungicides in palm kernel, mesocarp and RAC are listed in *Table 1*. It can be seen that triazole signal suffered from ion enhancement effect caused by the matrix (>30% of matrix effect). The highest matrix suppression was observed in RAC when compared to palm kernel and mesocarp. Epoxiconazole showed high matrix suppression in all matrices studied. From this observation, it was decided that further work on method development and validation of triazole fungicides would be based on the matrix-matched calibration curve. *Figure 4* shows the calibration curve of tetraconazole in acetonitrile, palm kernel, RAC and mesocarp matrix.

LOD and LOQ

LOD and LOQ of an individual analytical procedure is the lowest amount of analyte presence in a sample which can be detected and quantitatively determined, respectively (European Medicines Agency, 1995). The equations to calculate LOD and LOQ for each triazole analytes are as follows:

$$LOD = (3.3\ \sigma)/S \qquad (2)$$

$$LOQ = (10\ \sigma)/S \qquad (3)$$

where σ is denoted as the standard deviation of the regression line and S is denoted as the slope of the calibration curve. The LOD and LOQ obtained were in the range between 0.12 and 0.23 mg kg^{-1} and 0.31 and 0.70 mg kg^{-1}, respectively. Chromatogram of triazole analytes in the blank and spiked matrix of palm kernel, mesocarp and RAC are shown in *Figure 5*. Method validation study was evaluated in terms of apparent recovery, repeatability, linearity, limit of quantification, matrix effects, reproducibility as well as intra-day and inter-day precision as described in Document No. SANTE/11945/2017 (SANTE, 2020).

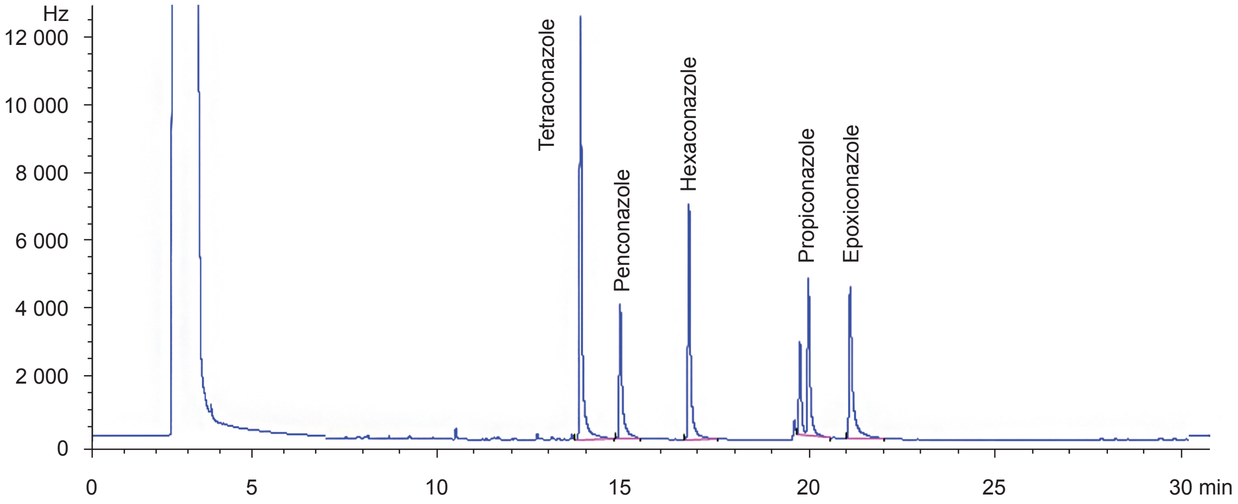

Figure 3. GC-µECD chromatogram of triazole residuals at concentration of 1.0 mg L^{-1}.

TABLE 1. MATRIX EFFECT IN PALM KERNEL, MESOCARP AND RAC

Analytes	Retention time	Palm kernel (%)	Mesocarp (%)	RAC (%)
Tetraconazole	14.14	31.28	26.79	33.52
Penconazole	15.25	39.30	29.63	43.72
Hexaconazole	17.17	54.64	48.69	63.73
Propiconazole	20.08	197.55	197.70	225.04
Epoxiconazole	21.46	288.26	264.71	317.33

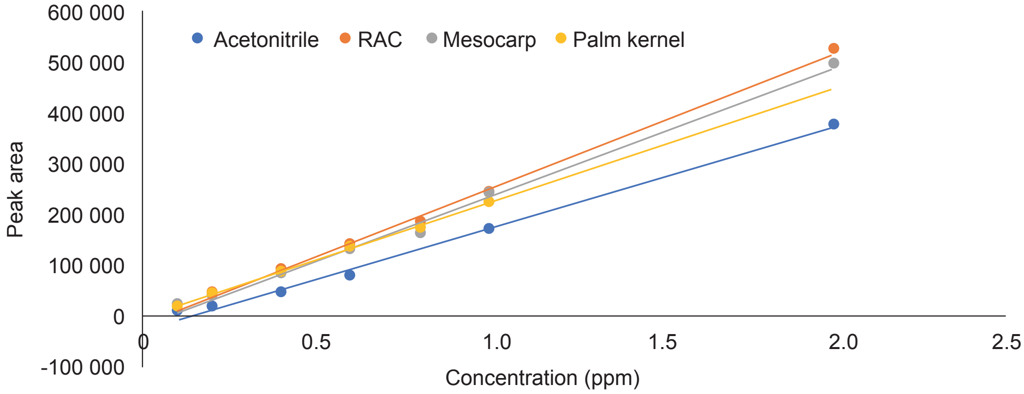

Figure 4. Calibration curve of tetraconzole in acetonitrile, palm kernel, mesocarp and RAC extract.

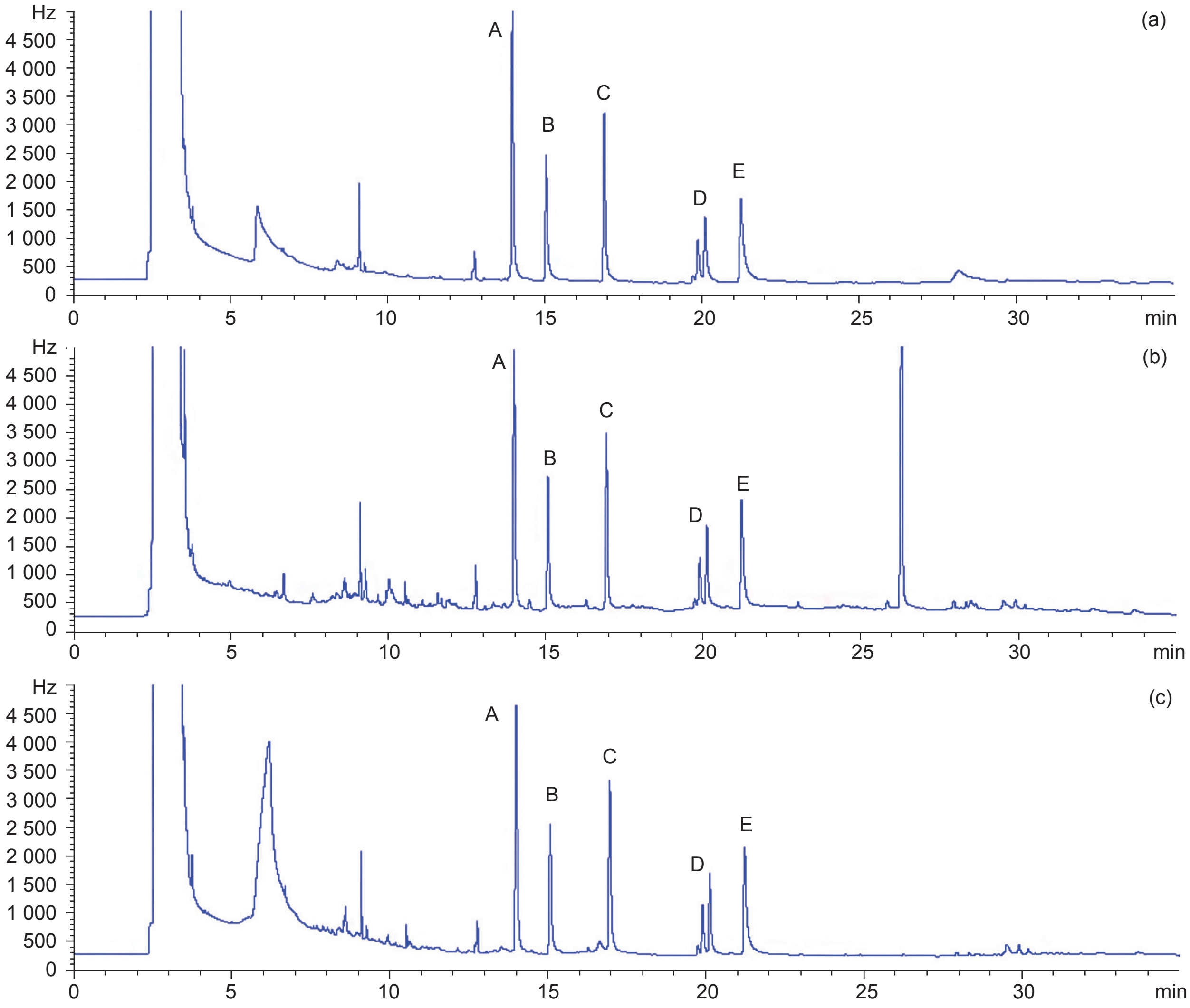

Figure 5. Chromatograms of (A) tetraconazole, (B) penconazole, (C) hexaconazole, (D) propiconazole and (E) epoxiconazole in (a) spiked palm kernel (b) mesocarp and (c) RAC at 0.5 mg kg^{-1}.

Optimisation of Modified QuEChERS

Optimisation of different extraction solvents namely 100.00% acetonitrile, acidified acetonitrile containing 1.00% acetic acid, 3.00% acetic acid, 1.00% formic acid and 3.00% formic acid were investigated to find suitable solvent for pesticide residuals extraction from palm kernel, mesocarp and RAC. For mesocarp matrix, one additional solvent extraction containing 10.00% distilled water was tested. It was indicated from all figures that the acidified acetonitrile with 1.00% acetic acid was the most effective solvent for pesticide residue extraction. Results showed that acidified acetonitrile with 1.00% formic acid gave good recoveries in palm kernel and RAC matrices, with mean recoveries obtained ranged from 81.53% to 96.93%. For mesocarp sample, acidified acetonitrile with 1% acetic acid and 10.00% distilled water gave the highest recoveries in the ranged of 104.00% to 111.87% with %RSD of less than 6.00%. Addition of water in dry sample is necessary to loosen up the interaction between the analytes and the matrix hence, enhancing separation of the analytes (Santana-Mayor *et al.*, 2019). Numerous studies have combined acetonitrile with other solvents such formic acid, acetic acid, citric acid, ethyl acetate, methanol, dichloromethane, and hexane to create acidified acetonitrile in order to improve the extraction efficiency (Perestrelo *et al.*, 2019).

Acetonitrile was shown to be the most suitable extraction solvent (Lentza-Rizos *et al.*, 2001). It was reported that acetonitrile is able to extract a wide range of pesticide residues and has higher capacity and selectivity as compared to the other solvents (González-Curbelo *et al.*, 2015; Maštovská and Lehotay, 2004). In addition, acetonitrile offers the possibility of subsequent liquid-liquid clean-up step as it forms distinct partitioning phases with non-polar solvents. Acidified solution is more effective to extract triazole pesticides due to the presence of 1,3-imidazole or 1,2,4-triazole ring which is capable of protonating the molecules of fungicides, and high activity of hydrogen ions in anhydrous solution (Zayats *et al.*, 2015). Wang *et al.* (2017) reported that acidification of acetonitrile with 1% acetic acid showed extraction recovery improvement for all Alternaria toxins in Jujube samples. Reichert *et al.* (2018) found that acidified acetonitrile with 1% acetic acid as extraction solvent provides the best extraction efficiency of 117 pesticides and 30 myotoxins analytes in raw coffee when compared to acidified acetonitrile with 4% of formic acid.

Optimisation of *d*-SPE

The selection of adsorbents was conducted to assess the effectiveness of the clean-up method. The clean-up step is vital to remove co-extractives in the samples (Tuzimski and Rejczak, 2016). Prior to injection, aliquot sample was added into *d*-SPE tubes containing several sorbents and subjected to centrifugation to provide immediate contact between the sample and sorbent and subsequently improving the effectiveness of the clean-up technique (Senes *et al.*, 2020). The final clear aliquot obtained is then injected into GC or LC for quantification.

Table 2 shows the effect of three different ratios of $MgSO_4$ and PSA on the mean recoveries obtained from spiked PKC. The mean recoveries of d-SPE clean-up in the absence of GCB (45:20:0) was the highest in all analytes when compared to d-SPE clean-up with GCB. For PKC matrix, GCB was not required in d-SPE clean-up due to absence of colouring supernatant. Result showed that increment of PSA ratio had no significant effect on the recovery ($p<0.05$). Statistical analysis also showed that there was no significant ($p<0.05$) effect of different ratios of $MgSO_4$ and PSA on the mean recovery. Thus, it was concluded that the ratio of 45:20 for both $MgSO_4$ and PSA was found to be the optimum clean-up sorbents ratio proven by the highest recovery for all analytes.

Effect of different ratios of absorbents; $MgSO_4$, PSA and GCB at ratios of 45:20:0, 45:20:20 and 45:25:25 from fortified mesocarp is tabulated in *Table 3*. After extraction, the supernatant was observed to have high amount of yellowish coloured pigment. The presence of pigment in the aliquot did not contribute to any peaks but it could reduce the half-life of GC column. Hence, GCB was introduced to the treatment to enable removal of colour pigment. Results showed satisfactory mean recovery for all pesticide residual was obtained in the range of 80.52% to 93.15% with %RSD less than 10.00% when the absorbent ratio was 45:20:20. Similar results was also found for RAC samples where the ratio of 45:20:20 gave acceptable range of recoveries of 81.54% to 93.15% with %RSD less than 5.00% (*Table 4*). It was also observed that the increment of GCB ratio demonstrated a negative effect on recovery. The mean percentage of recovery was slightly reduced when the portion of GCB increased from 20.00% to 25.00%. The use of PSA and GCB could raise some concerns such as loss of analytes and low in recovery especially for certain pesticides (Deme *et al.*, 2014; Muhamad *et al.*, 2012b). Guan *et al.* (2013) agreed that extensive use of adsorbent might affect the recovery of target analytes, since the target analytes could be adsorbed by the sorbent.

Method Accuracy

Recovery studies. Three fortification levels of 0.5, 1.0 and 5.0 mg kg^{-1} were evaluated for recovery determinations. All recovery experiments were performed in six replicates at each level as suggested in SANTE document. *Tables 5, 6* and *7* show the mean recoveries and %RSD at different concentration levels spiked in palm kernel, mesocarp and RAC, respectively. The mean recoveries ranged from 85.29% to 104.91% with RSD less than 6.00% were observed in palm kernel samples. For mesocarp samples, most of analytes were recovered in the range of 89.95% and 106.28% with RSD of less than 5.00%. For RAC samples, the mean of recoveries was recorded within 80.58% and 108.47% with %RSD of less than 8.00%. Relatively low %RSD obtained from the recovery assessment indicates that the developed method has good repeatability (Khairuddin *et al.*, 2021).

Method Precision

Repeatability. Repeatability is estimated by determining the inter- and intra-day relative standard deviation (RSD, %) by repeated analysis (n=6) of a spiked palm kernel, mesocarp and RAC samples. Two fortification levels of 1.0 mg kg^{-1} and 5.0 mg kg^{-1} were tested from run-to-run over 1 and 5 days. The mean recovery for palm kernel replicates on day 1 and 5 were within 80.32% and 103.99% with %RSD below 6.00%. For mesocarp and RAC matrices, the recoveries for repeatability study performed at day 1 and 5 were ranged within 86.05% and 111.80%, and 97.89% and 107.99%, respectively. The %RSD calculated from recovery assessment are less than 10.00%.

Reproducibility. Reproducibility is assessed by comparing the relative standard deviation of the recovery of spiked samples within the same day (intra-day) by two analysts. Method reproducibility is expressed as mean recovery (%), and relative standard deviation (%RSD) spiked at 1.0 mg kg^{-1} in palm kernel, mesocarp and RAC. Analyses performed by analyst-2 was found comparable with that of analyst-1. Mean recoveries observed for analyst-1 were within the range of 78.26% and 101.06%. For analyst-2, mean recoveries were found between 80.37% and 92.89%. The %RSD found were less than 10.00% for both analysts.

TABLE 2. EFFECT OF $MgSO_4$: PSA: GCB RATIO ON MEAN RECOVERY OF PESTICIDES RESIDUAL FROM FORTIFIED PKC AT 1.0 mg kg^{-1}

Pesticide residual	% Mean recovery ± SD		
	45:20:0	45:25:0	45:30:0
Tetraconazole	93.19 ± 1.80^{a}	82.30 ± 1.92^{a}	90.83 ± 2.50^{a}
Penconazole	92.96 ± 4.01^{a}	81.48 ± 1.28^{a}	86.91 ± 3.02^{a}
Hexaconazole	93.77 ± 1.27^{a}	78.87 ± 1.38^{a}	85.98 ± 3.09^{a}
Propiconazole	93.20 ± 5.62^{a}	82.70 ± 0.37^{a}	88.92 ± 2.35^{a}
Epoxiconazole	89.85 ± 6.38^{a}	88.20 ± 3.94^{a}	88.16 ± 2.96^{a}

Note: Mean values with different letter superscripts indicate significant differences between ratio ($p<0.05$).

TABLE 3. EFFECT OF $MgSO_4$: PSA: GCB RATIO ON MEAN RECOVERY OF PESTICIDES RESIDUAL FROM FORTIFIED MESOCARP AT 1.0 mg kg^{-1}

Pesticide residual	% Mean recovery ± SD		
	45:20:0	45:20:20	45:25:25
Tetraconazole	68.81 ± 5.32^{a}	80.52 ± 1.85^{a}	69.01 ± 4.06^{a}
Penconazole	75.81 ± 5.70^{b}	93.15 ± 2.01^{a}	70.22 ± 4.94^{b}
Hexaconazole	78.83 ± 6.05^{b}	85.87 ± 1.84^{a}	67.70 ± 2.19^{c}
Propiconazole	70.50 ± 4.51^{a}	81.54 ± 5.06^{a}	72.48 ± 5.22^{a}
Epoxiconazole	75.58 ± 5.77^{a,b}	81.25 ± 4.22^{a}	70.56 ± 3.68^{b}

Note: Mean values with different letter superscripts indicate significant differences between ratio ($p<0.05$).

TABLE 4. EFFECT OF $MgSO_4$: PSA: GCB RATIOS ON MEAN RECOVERY OF PESTICIDES RESIDUAL FROM FORTIFIED RAC AT 1.0 mg kg^{-1}

Pesticide residual	% Mean recovery ± SD		
	45:20:0	45:20:20	45:25:25
Tetraconazole	69.20 ± 1.75^{b}	81.94 ± 2.27^{a}	68.71 ± 4.06^{b}
Penconazole	73.16 ± 2.23^{b}	78.77± 2.48^{a}	71.22 ± 1.69^{b}
Hexaconazole	80.19 ± 2.80^{a}	78.14 ± 3.01^{a}	77.27 ± 1.97^{a}
Propiconazole	71.61 ± 1.88^{b}	81.04 ± 3.69^{a}	70.71 ± 1.45^{b}
Epoxiconazole	68.08 ± 3.72^{b}	89.58 ± 3.77^{a}	68.09 ± 1.97^{b}

Note: Mean values with different letter superscripts indicate significant differences between ratio ($p<0.05$).

TABLE 5. MEAN RECOVERY (%) AND RSD (%) FOR TRIAZOLE RESIDUES FROM SPIKED PALM KERNEL AT THREE LEVELS OF CONCENTRATIONS

Pesticide residual	Palm kernel					
	0.5 mg kg^{-1}		1.0 mg kg^{-1}		5.0 mg kg^{-1}	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Tetraconazole	87.78	4.22	94.01	4.52	81.10	2.50
Penconazole	85.29	3.03	94.64	4.44	80.82	3.02
Hexaconazole	87.33	1.36	86.82	4.78	76.31	3.09
Propiconazole	89.22	5.32	88.98	4.63	82.87	2.35
Epoxiconazole	94.32	4.87	94.68	3.10	90.95	2.96

TABLE 6. MEAN RECOVERY (%) AND RSD (%) FOR TRIAZOLE RESIDUES FROM SPIKED MESOCARP AT THREE LEVELS OF CONCENTRATIONS

Pesticide residual	Mesocarp					
	0.5 mg kg^{-1}		1.0 mg kg^{-1}		5.0 mg kg^{-1}	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Tetraconazole	106.28	0.98	100.55	1.54	86.04	4.36
Penconazole	106.11	0.53	101.48	2.70	83.46	3.11
Hexaconazole	105.49	0.25	101.27	3.24	82.48	4.13
Propiconazole	106.13	0.70	101.70	1.89	84.39	3.21
Epoxiconazole	100.85	4.48	99.06	4.48	87.57	2.87

TABLE 7. MEAN RECOVERY (%) AND RSD (%) FOR TRIAZOLE RESIDUES FROM SPIKED RAC AT THREE LEVELS OF CONCENTRATIONS

Pesticide residual	RAC					
	0.5 mg kg^{-1}		1.0 mg kg^{-1}		5.0 mg kg^{-1}	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Tetraconazole	101.20	1.20	86.30	2.58	95.21	3.92
Penconazole	88.28	4.11	81.53	2.70	80.93	4.27
Hexaconazole	108.47	1.71	82.54	3.17	101.41	2.04
Propiconazole	80.58	1.72	90.13	4.73	89.61	4.72
Epoxiconazole	81.47	4.01	96.93	3.42	92.99	4.84

CONCLUSION

A simple, fast and reliable method from modified QuEChERS approach was successfully developed and optimised for the determination of triazole residues in palm kernel, mesocarp and RAC matrices. The method is successfully validated in-house, and fulfil the requirements of important validation parameters such as linearity, recovery study and method precision (repeatability and reproducibility). The LOD and LOQ fell between 0.12-0.23 mg kg^{-1} and 0.31-0.70 mg kg^{-1}, respectively. From this work, the developed method can be used to determine the level of triazole fungicides residues in palm kernel, mesocarp and RAC matrices in meeting the standard legislative limits of importing countries.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish the article.

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