Malays. J. Anal. Sci. Volume 29 Number 4 (2025): 1363

MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES

Journal homepage: https://mjas.analis.com.my/

Research Article

Development of QuEChERS method combined with ultraviolet-visible spectroscopy for residual nanopesticide analysis in crops and vegetables

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Received: 23 September 2025; Revised: 3 July 2025; Accepted: 21 July 2025; Published: 22 August 2025

Abstract

This study reports the development of a modified QuEChERS method combined with UV-Visible spectroscopy for the analysis of residual nanohexaconazole in rubber, chilli, and eggplant matrices. Conventional QuEChERS methods face major limitations, including incomplete removal of matrix interferences and insufficient sensitivity, especially when applied to nanomaterials. Detecting nanopesticides presents additional challenges due to their small particle size, enhanced penetration into plant tissues, and interactions with biological compounds that can alter detection signals. To address these issues, the method was improved using acidified acetonitrile extraction and matrix-matched calibration to enhance both precision and accuracy. The study specifically focuses on detecting the newly developed nanohexaconazole in various matrices, including fruit, leaf, and soil samples. The results revealed strong signal suppression in leaf matrices, particularly in chilli leaf (ME% = -412.2%), followed by eggplant and rubber leaves (ME% = -153.5% and -194.2%). In contrast, eggplant fruit and topsoil showed moderate signal enhancement (ME% = 39.2% and 27.9%). All matrix-specific calibration curves achieved R^2 values greater than 0.9, confirming excellent linearity. This modified method provides a reliable and accurate approach for the analysis of nanopesticide residue across complex agricultural matrices, thereby supporting food safety monitoring and regulatory compliance.

Keywords: QuEChERs method, nanopesticide, residue analysis, sustainable nanomaterials

Introduction

The increasing use of nanopesticides in modern agriculture has raised significant concerns about their residual presence in food products, particularly in crops and vegetables [1]. Nanopesticides, due to their small particle size, often in the range of 1-100 nm, can penetrate deep into plant tissues, unlike conventional pesticides that typically remain on the surface [2]. This deep penetration into crop tissues raises additional concerns regarding food safety. Due to their enhanced bioavailability, nanoparticles can be more readily absorbed by plant tissues and potentially transferred to humans upon consumption [3]. The ability of nanoparticles to cross biological barriers also increases the risk of accumulation in food products, thereby necessitating advanced analytical methods capable of detecting residues within the whole plant matrix, not just on the surface.

Traditional pesticide analysis methods are often inadequate for detecting nanomaterials due to their unique physicochemical properties. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, originally developed for pesticide residue analysis, has proven to be an effective extraction technique, particularly when combined with advanced analytical tools like UV-Visible Spectroscopy. QuEChERS offers a simple and cost-effective approach to sample preparation by integrating solvent extraction with dispersive solid-phase extraction, making it highly suitable for complex food matrices such as fruits and vegetables [4]. The combination of QuEChERS with UV-Visible Spectroscopy provides enhanced sensitivity and specificity for detecting residual nanopesticides. This combination allows for the identification of optical signatures unique to nanomaterials, offering a rapid, non-destructive, and reliable analytical method.

Moreover, one of the primary challenges in detecting pesticide residues in agricultural products is the matrix effect. The matrix effect refers to the alteration of analyte signals when co-extracted substances from the sample matrix interfere with the analytical process, resulting in either signal suppression or enhancement [5]. In the case of crops and vegetables, compounds such as pigments, fatty acids, sugars, and other naturally occurring substances may distort the accuracy of pesticide quantification by either masking the presence of residues or falsely elevating the detected levels [6]. This complexity makes it essential to rigorously evaluate and mitigate matrix effects during method validation to ensure reliable results.

For this reason, proper sample cleanup is crucial in pesticide residue analysis. Techniques like QuEChERS are designed to simplify and streamline this process by offering a multi-step purification procedure, which includes the use of dispersive solid-phase extraction to eliminate matrix interferences. However, even with such methods, it remains crucial to study and account for the matrix effect in each specific sample type, as chemical composition can vary significantly across different crops and vegetables [7]. Thus, the ability to accurately detect residual nanopesticides using UV-Visible Spectroscopy combined with QuEChERS depends heavily on addressing and compensating for the matrix effect, ensuring that the analytical method remains robust and accurate across diverse sample types.

This work aims to develop a modified QuEChERS method combined with UV-Visible spectroscopy analysis for detecting a newly developed nanohexaconazole formulation (comprising 85% w/w chitosan and 15% w/w hexaconazole) in crops (rubber) and vegetables (chilli and eggplant), encompassing samples from fruit, leaf, and soil matrices to ensure the method's reliability and accuracy. Chitosan, a biopolymer known for its biocompatibility and biodegradability, was chosen as a nanocarrier for hexaconazole, an antifungal agent widely used in agriculture. The development of these nanoparticles has demonstrated remarkable antifungal efficacy, showing a threefold increase in activity compared to their non-nano counterpart, highlighting the potential of nanotechnology to enhance the effectiveness of agrochemicals [8]. Rubber (*Hevea brasiliensis*), chilli (*Capsicum annuum*), and eggplant (*Solanum melongena*) were selected due to their agricultural importance in Malaysia and their distinct tissue characteristics. Rubber represents a key industrial crop, while chilli and eggplant are widely consumed vegetables with varying levels of pigments, phenolics, and structural components, making them suitable for evaluating matrix effects across diverse sample types.

Matrix-matched calibration standards are essential for accurately assessing pesticide residue levels in complex matrices by compensating for matrix-induced effects such as signal suppression or enhancement. These standards are prepared by spiking blank matrix extracts with known concentrations of the analyte, ensuring that the matrix's influence on detection is accurately reflected. By accounting for matrix-induced variations, they provide a more realistic measurement of pesticide levels under real-world conditions. This approach ensures that the modified QuEChERS extraction method, combined with UV-Visible spectroscopy, delivers consistent and reliable results for pesticide residue monitoring in crops and vegetables, thereby enhancing food safety and ensuring compliance with regulatory standards.

Materials and Methods

Chemicals and Materials

Hexaconazole (95% purity) was used as a standard in this study and was sourced from Changzhou Aiteng (Jiangsu, China). The nanohexaconazole, with a mean particle diameter size of 18 nm as determined by High-Resolution Transmission Electron Microscopy (HRTEM), was formulated following previously published methods [8]. The synthesised nano hexaconazole appeared as a yellowish-white powder, consistent with earlier reports on similar formulations. The nanoparticles were created by encapsulating hexaconazole within chitosan nanocapsules using an ionic gelation method. This method involved the use of sodium tripolyphosphate as a crosslinking agent and Tween-80 as a stabilising agent, ensuring the structural integrity and dispersibility of the nanoparticles. The final formulation comprised 85% w/w of chitosan, serving as the nanocarrier, and 15% w/w of hexaconazole, the active antifungal agent.

QuEChERS extraction tubes packed with 150 mg $MgSO_4$, 50 mg of a Primary-Secondary Amine (PSA), 50 mg of Graphitised Carbon Black (GCB), and 50 mg of octadecyl (C_{18}) were purchased from United Chemical (Bristol, Pennsylvania). Acetonitrile (MeCN), magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl) were purchased from Supelco (Bellefonte, PA, USA). Hydrochloric acid (37%) was purchased from Fisher Scientific (Selangor, Malaysia). Rubber (*Hevea brasiliensis*) seedlings were purchased from TSH Greenview Nursery (Kedah). Chilli (*Capsicum annuum*) seedlings were purchased from QST Garden Nursery (Sungai Nibong, Pulau Pinang). Eggplant (*Solanum melongena*) seedlings were purchased from a plant nursery located in Kepala Batas, Pulau Pinang.

Sample processing

Using six seedlings each, the leaves of eggplant, chilli, and rubber were collected and thoroughly cleaned to eliminate any external contaminants. The leaves were then left to air-dry at room temperature until they were completely dry. Once dried, the leaves were ground into a fine powder using a mortar and pestle. From the resulting powder, a 5 g portion was measured and divided into multiple centrifuge tubes for further extraction, ensuring consistency and accuracy in the subsequent analysis of nanopesticide residues.

Fruits from the eggplant and chilli plants were collected from six different seedlings and thoroughly washed with distilled water to remove any external impurities. After cleaning, the fruits were left to air-dry at room temperature for a brief period. Once dried, the fruits were pulverised using a blender to obtain a uniform consistency. A 5 g portion of the pulverised fruit was then measured and divided into multiple centrifuge tubes for further extraction, ensuring proper sample preparation for the subsequent nanopesticide residue analysis.

Soil samples (topsoil) were collected and air-dried at room temperature to remove any moisture content. Once fully dried, the soil was sieved to eliminate larger particles and debris, ensuring a fine and consistent texture. A 5 g portion of the sieved soil was then measured and transferred into multiple centrifuge tubes for further extraction, ensuring uniformity in the sample preparation for nanopesticide residue analysis.

Control samples were prepared using untreated plant tissues, fruits, and topsoil that had not been exposed to nanohexaconazole. These samples were processed using the same extraction and cleanup procedures as the test samples. The resulting blank extracts were used to confirm the absence of background interference and to prepare matrix-matched calibration standards by spiking with known concentrations of nanohexaconazole. Although no recovery tests were conducted, method reliability was assessed through the performance of calibration curves generated for each matrix. All matrices yielded R^2 values greater than 0.9, indicating consistent analytical response and supporting the overall reliability of the method.

QuEChERS extraction method

A significant modification was made to the QuEChERS analytical procedure [3]. Initially, a 5 g portion of the sample was placed into a 50 mL polypropylene centrifuge tube, followed by the addition of 30 mL of acetonitrile containing 1% (v/v) hydrochloric acid (HCl). The mixture was then agitated using a vortex mixer for two 30-second cycles. To facilitate the partitioning process, 4 g of magnesium sulphate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were added, and the tube was vigorously shaken for another 30 seconds. The sample was then centrifuged at 5000 rpm for 20 minutes. After centrifugation, a 5 mL aliquot of the supernatant was transferred to a QuEChERS tube for dispersive solid-phase extraction (d-SPE). The solution underwent a second round of centrifugation at 5000 rpm for 10 minutes. The resulting extract was filtered using a PTFE syringe filter with a 0.22 µm pore size and 13 mm diameter. Finally, the extracted matrix solutions were stored at room temperature.

The matrix-matched calibration curve, the limit of detection (LOD), and the limit of quantification (LOQ)

A stock solution of nanohexaconazole was prepared using a solvent of N,N-dimethylformamide and 1% v/v acetic acid and was subsequently serially diluted to obtain working standard solutions at concentrations of 1, 2, 4, 6, 8, 10, 12, 16, 18, and 20 mg/mL. The stock solution was also used for the matrix-matched solution by serial dilution at the same concentrations in the extracted matrix solutions. To assess the Matrix Effect (ME) in the calibration, ME % was then calculated using Equation 1 [3].

$$\text{ME}\ (\%) = \left(1 - \frac{\text{solvent slope}}{\text{Matrix−matched slope}}\right) \times 100 \tag{1}$$

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using Equations 2 and 3, respectively [3].

$$\text{LOD} = 3.3 \times \frac{\text{standard deviation of the regression line}}{\text{slope}} \tag{2}$$

$$\text{LOQ} = 10 \times \frac{\text{standard deviation of the regression line}}{\text{slope}} \tag{3}$$

In this study, a Shimadzu UV-Vis Spectrophotometer UV-2600 (Tokyo, Japan) was used to plot the calibration curves and analyse the extracted samples. The analysis was conducted at a wavelength range of 200-800 nm.

Results and Discussion

UV-Visible spectrum and calibration curves: Solvent

The UV-Vis spectra of nanohexaconazole dissolved in solvent at concentrations ranging from 1 to 20 mg/mL (**Figure 1A**) show a prominent absorbance peak at approximately 275 nm, which corresponds to the maximum absorbance wavelength (λ_{max}) for hexaconazole. As the concentration increases, the absorbance at λ_{max} also increases, indicating a direct correlation between concentration and absorbance.

As shown in **Figure 1B**, the calibration curve of absorbance at the peak wavelength (275 nm) against the concentration of the analyte was plotted. The calibration curve exhibits a linear relationship and showed excellent linearity with $R^2 = 0.9461$. The slope (0.1465) represents the sensitivity of the method, while the y-intercept (0.7917) represents the baseline absorbance, possibly due to the solvent or background noise. The R^2 value indicates a strong positive correlation, confirming the reliability and accuracy of the UV-Vis spectroscopic method for quantifying the analyte within the studied range.

Leaf matrices

The UV-Visible absorption spectra of nano hexaconazole at varying concentrations within a rubber leaf matrix are shown in **Figure 2A**. The spectra display a distinct absorption peak at 279 nm, corresponding to the characteristic absorbance of hexaconazole within the rubber leaf matrix. This peak is slightly right-shifted compared to the absorption peak of hexaconazole in solvent, indicating potential interactions between the hexaconazole molecules and the components of the rubber leaf matrix. Consequently, the calibration curve for hexaconazole in the rubber leaf matrix was plotted, as shown in **Figure 2B**. The curve demonstrates a clear linear relationship between absorbance and concentration, with a high coefficient of determination ($R^2 = 0.9818$), indicating excellent linearity.

The UV-Visible absorption spectra of nanohexaconazole at varying concentrations within a chilli leaf matrix are shown in **Figure 3A**. The spectra exhibit a distinct absorption peak around 300 nm, which demonstrates a significant right shift from the absorption peak of hexaconazole in solvent, recorded at 275 nm. This shift indicates potential interactions between hexaconazole and the specific components of the chilli leaf matrix, which could alter the optical properties of the pesticide. Consequently, the calibration curve for hexaconazole in the chilli leaf matrix was plotted, as shown in **Figure 3B**. The calibration curve demonstrates a strong linear relationship between absorbance and concentration, with a high coefficient of determination ($R^2 = 0.9316$), indicating excellent linearity.

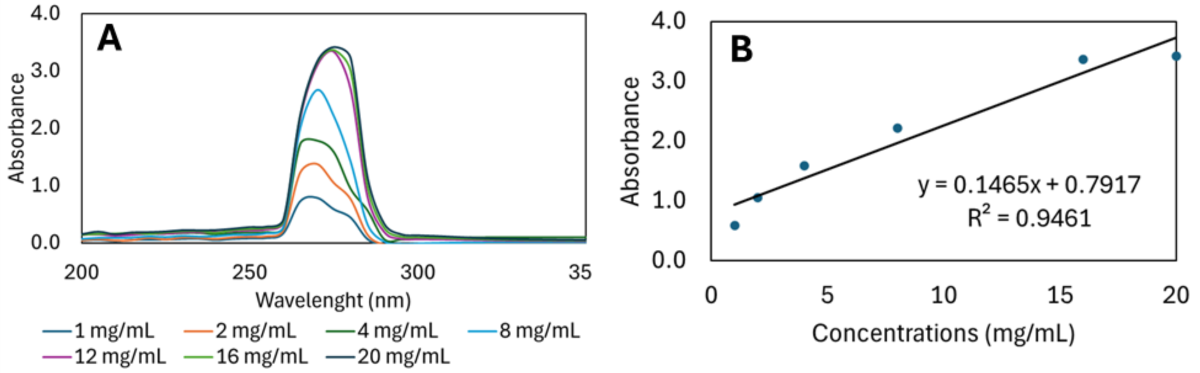

Figure 1. (A) UV-Visible spectrum of different concentrations of hexaconazole in solvent and (B) calibration curve of hexaconazole in solvent

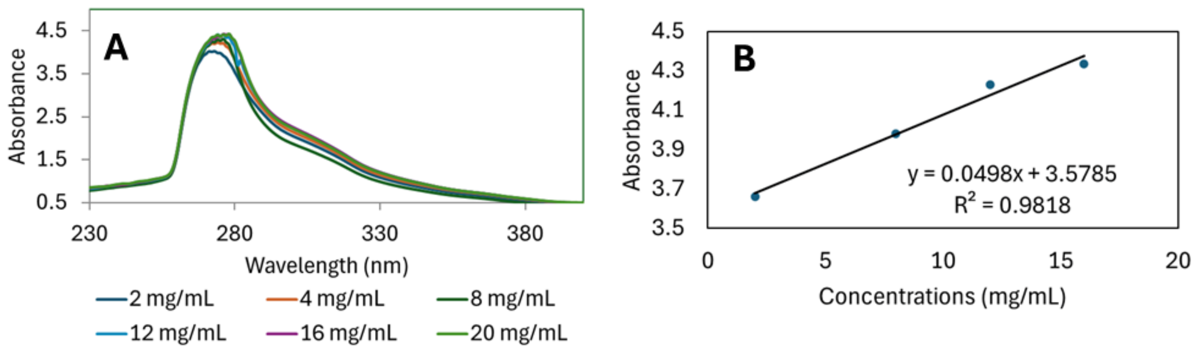

Figure 2. (A) UV-Visible spectrum of different concentrations of hexaconazole in rubber leaf matrix and (B) calibration curve of hexaconazole in rubber leaf matrix

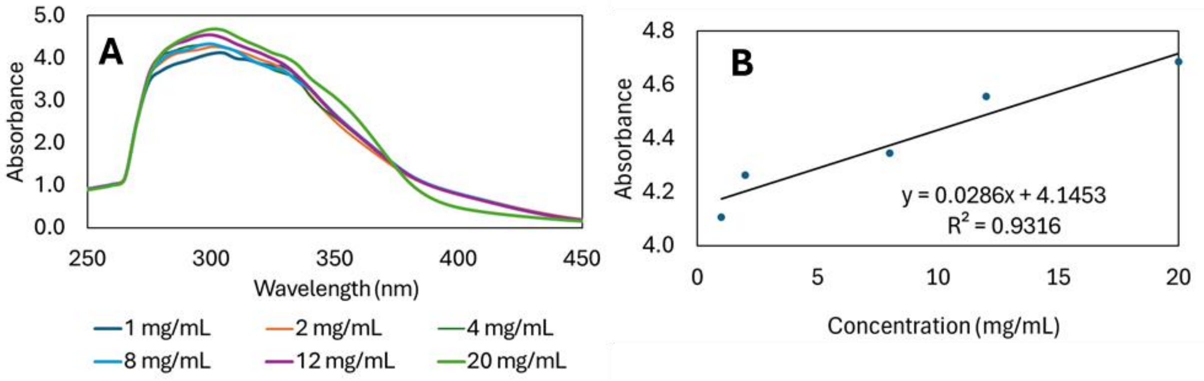

Figure 3. (A) UV-Visible spectrum of different concentrations of hexaconazole in chilli leaf matrix and (B) calibration curve of hexaconazole in chilli leaf matrix

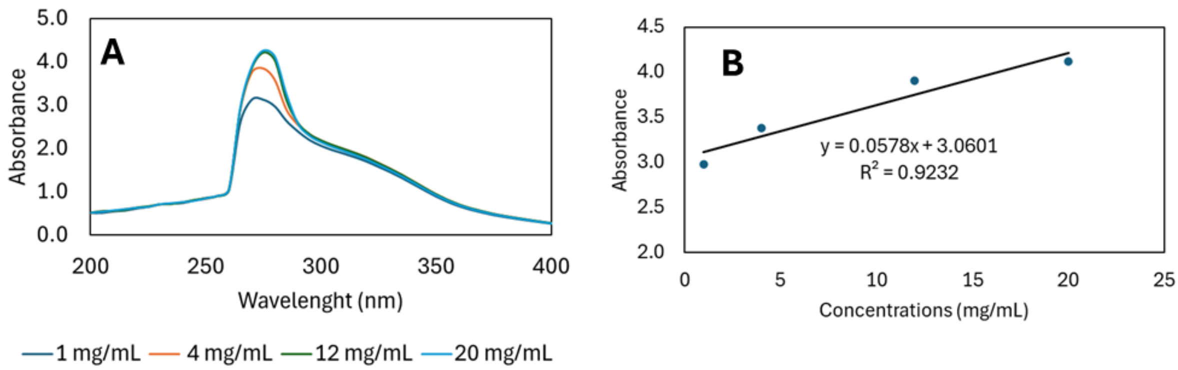

Figure 4. (A) UV-Visible spectrum of different concentrations of hexaconazole in eggplant leaf matrix and (B) calibration curve of hexaconazole in eggplant leaf matrix

The UV-Visible absorption spectra of nano hexaconazole at varying concentrations within an eggplant leaf matrix are shown in **Figure 4A**. The spectra exhibit a distinct absorption peak around 280 nm, which is slightly shifted compared to the absorption peak of hexaconazole in solvent, recorded at 275 nm. This right shift indicates potential interactions between hexaconazole and the components of the eggplant leaf matrix. Consequently, the calibration curve for hexaconazole in the eggplant leaf matrix was plotted, as shown in Figure 4B. The calibration curve demonstrates a strong linear relationship between absorbance and concentration, with a high coefficient of determination ($R^2 = 0.9232$), indicating excellent linearity.

These results suggest that nanohexaconazole undergoes notable spectral shifts in leaf matrices, with the absorption peak shifting from 275 nm in solvent to 280–300 nm in plant tissues. This behaviour indicates strong matrix effects, likely driven by pigments, proteins, phenolics, and other bioactive compounds, which complicate quantification using UV–Vis detection.

Leaf matrices are inherently complex, containing a range of plant-derived substances that can significantly influence analytical processes and interfere with pesticide detection. One key factor is the presence of pigments, including chlorophylls, carotenoids, and anthocyanins, which are abundant in leaves and absorb light at different wavelengths. Chlorophyll a absorbs strongly around 430 nm and 662 nm [9], while carotenoids absorb between 400 and 500 nm [10], and anthocyanins typically absorb between 500 and 550 nm [11]. Although these wavelengths are above hexaconazole's absorption peak (below 300 nm), their presence can still interfere with UV-Visible spectroscopy by affecting baseline absorbance, scattering light, or introducing noise, potentially complicating accurate detection and quantification. Additionally, fatty acids, found in the leaf cuticle, can impact the extraction and quantification of pesticides by interacting with the analytical reagents, potentially complicating accurate detection [12].

Another major group of interfering compounds is sugars, such as glucose, fructose, and sucrose, which are prevalent in leaves [13]. These sugars can create challenges in analysis by introducing background noise or complicating the quantification of target analytes. Proteins, particularly enzymes and structural proteins, can further interact with analytical reagents and influence the outcome of the analysis [14].

Furthermore, phenolic compounds (**Table 1**) present additional complications by binding to other molecules, which can mask or alter the detection of pesticides. Organic acids, such as citric and malic acid, can change the pH of the matrix and impact analyte stability, making it more difficult to achieve accurate measurements [15].

Finally, structural components like fibres, including cellulose and lignin, can complicate the sample preparation process, making it harder to extract the analyte efficiently [19]. These biological components collectively contribute to the strong matrix effects observed in leaf matrices, highlighting the need for matrix-matched calibration and robust extraction methods to ensure accuracy.

Fruit matrices

Further evaluation of the matrix effect was conducted on fruit matrices. As shown in **Figure 5A**, the UV-Visible absorption spectra of nanohexaconazole in the chilli fruit matrix displayed a distinct peak at 280 nm, slightly right-shifted compared to its behaviour in solvent. In contrast, hexaconazole in the chilli leaf matrix exhibited a peak at 300 nm, indicating stronger interactions with leaf components. It has been reported that the total flavonoid content in chilli leaves is 1.8 times higher than that in chilli fruits, highlighting the leaf's richer and more complex chemical composition [20]. These compounds significantly interfere with pesticide absorption, altering its behaviour and causing a more pronounced shift in the absorption peak. On the other hand, the chilli fruit matrix, which contains more water, sugars, and organic acids, facilitates fewer interactions with hexaconazole, leading to a smaller shift in the absorption peak [15]. The simpler chemical composition of fruit tissues reduces interference, as fruit typically contains fewer interfering compounds compared to leaves, allowing for more reliable detection. The calibration curve (Figure 5B) demonstrates a strong linear relationship between absorbance and concentration, with a high coefficient of determination (R^2 = 0.9275), indicating good linearity.

Table 1. Phenolic contents in leaves of chilli, eggplant and rubber

Type of leaves	Phenolic contents
Chilli	Ferulic acid (0.40-5.20 ppm), caffeic acid (0.10-1.20 ppm), epigallocatechin gallate (0.02-0.45 ppm), sinapic acid (0.09-1.90 ppm), gallic acid (0.18-1.10 ppm), and quercetin (0.07-1.30 ppm) [16].
Eggplant	Anthocyanins (9.3-52.8 mg/100 g), total phenols (966.5-2072.6 mg/100 g), *o*-diphenols (349.7-741.2 mg/100 g), flavonoids (154.0-375.6 mg/100 g), flavonols (462.9-733.7 mg/100 g), and tannins (2809.0-4840.2 mg/100 g) [17].
Rubber	Total phenolic content (0.003-0.020 mg GAE/mL) and total flavonoid content (0.086 to 0.200 mg CAE/mL) [18].

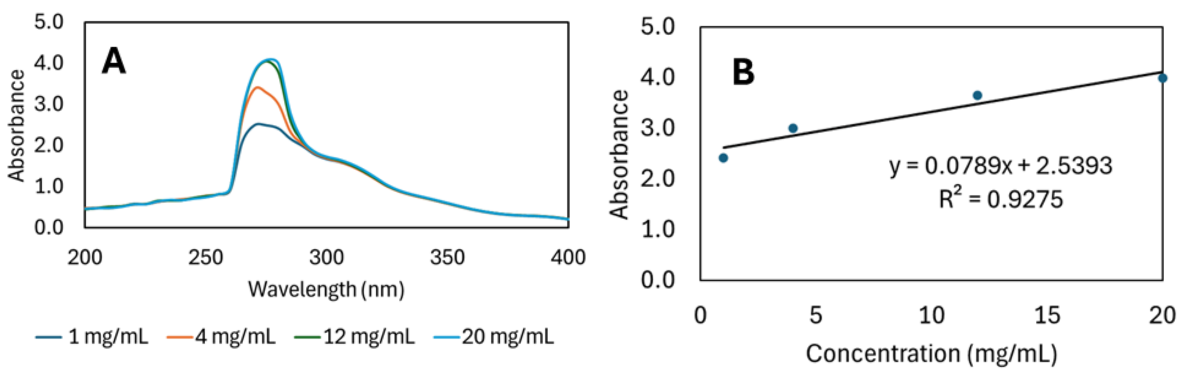

Figure 5. (A) UV-Visible spectrum of different concentrations of hexaconazole in chilli fruit matrix and (B) Calibration curve of hexaconazole in chilli fruit matrix

As shown in **Figure 6A**, the UV-Visible absorption spectra of nanohexaconazole in the eggplant fruit matrix display a distinct peak at 275 nm, consistent with its absorption in solvent, indicating minimal matrix interaction. In contrast, the eggplant leaf matrix shows a slight right shift to 280 nm, following a similar trend observed in chilli matrices, where the absorption peak shifts due to stronger interactions between hexaconazole and leaf tissue components. These shifts prove that the higher presence of pigments and organic compounds in leaf matrices leads to more pronounced matrix interference compared to fruit matrices. The calibration curve, shown in **Figure 6B**, reveals a strong linear relationship between absorbance and concentration, with a high coefficient of determination ($R^2 = 0.9979$), indicating excellent linearity. These findings suggest that although matrix effects are present, the method is highly reliable for accurately quantifying hexaconazole residues in the eggplant fruit matrix.

In contrast, fruit matrices exhibited less pronounced spectral shifts compared to leaf matrices, indicating weaker matrix–analyte interactions. For example, in the eggplant fruit matrix, the absorbance peak remained at 275 nm, consistent with the peak observed in the solvent, and suggests minimal optical interference. However, despite this spectral similarity, differences in the slope of matrix-matched calibration curves compared to solvent-based standards (as discussed in Equation 1) may still indicate underlying matrix effects that could influence quantification accuracy. This emphasises the need to assess matrix influence not only through peak shifts but also through changes in calibration response. These considerations will be further discussed in the next section in the context of method reliability.

Soil matrix

Moreover, the matrix assessment was further tested on topsoil. This analysis was crucial because nanopesticides, due to their small particle size and enhanced bioavailability, can persist in the soil for extended periods [21]. This prolonged presence in the soil environment could disrupt the soil's pH balance and alter its microbial composition, potentially affecting plant growth and soil health. The ability of nanopesticides to bind with soil particles or penetrate deeper into the soil layers also raises concerns about long-term environmental contamination [22].

As shown in **Figure 7A**, the UV-Visible absorption spectra of nanohexaconazole in the topsoil matrix reveal a distinct absorption peak at 275 nm, aligning with its absorption in solvent. This suggests minimal matrix interaction in the soil compared to more complex biological matrices like leaves or fruits. The calibration curve in **Figure 7B** demonstrates a high coefficient of determination ($R^2 = 0.9723$), indicating excellent linearity. This suggests that, despite the complex nature of the soil matrix, the method remains reliable for quantifying hexaconazole in soil samples.

However, as previously mentioned, further analysis of the slope of matrix-matched calibrations might reveal a different outcome. Although the absorption peak remains stable, suggesting fewer matrix interactions, the matrix effect may still influence the accuracy of the quantification. These studies collectively emphasise that soil, due to its complex composition of organic matter, minerals, and microbial life, can significantly influence pesticide detection, making matrix-specific adjustments essential for accurate analysis across different environmental contexts [23].

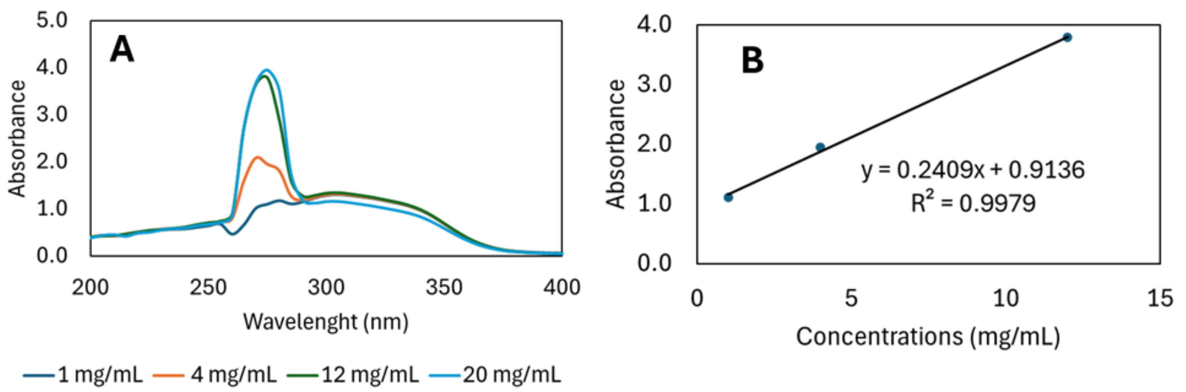

Figure 6. (A) UV-Visible spectrum of different concentrations of hexaconazole in eggplant fruit matrix and (B) calibration curve of hexaconazole in eggplant fruit matrix

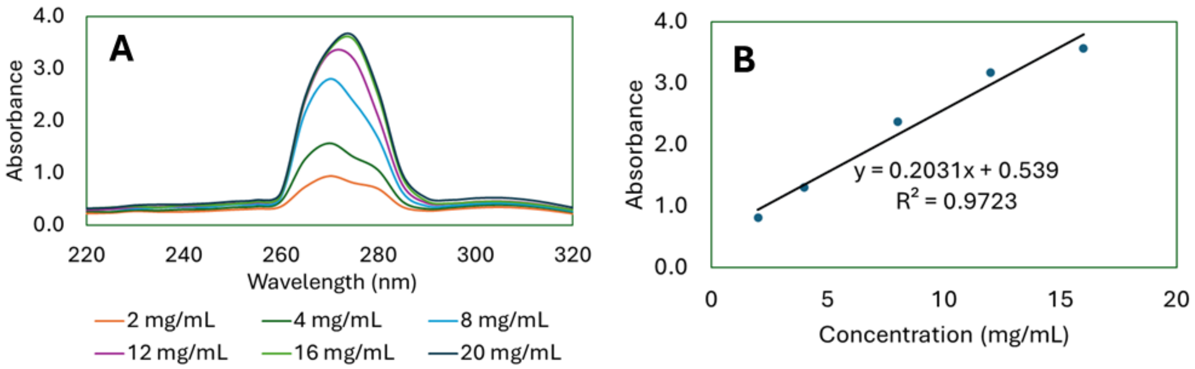

Figure 7. (A) UV-Visible spectrum of different concentrations of hexaconazole in topsoil matrix and (B) Calibration curve of hexaconazole in topsoil matrix

Matrix effect, the limit of detection (LOD), and limit of quantification (LOQ)

The findings presented in **Table 2** reveal that the Matrix Effects (ME%), Limits of Detection (LOD), and Limits of Quantification (LOQ) for nanohexaconazole vary significantly across different matrices. The chilli leaf matrix exhibited the most severe signal suppression, with an ME% of -412.2% and a large shift in the absorbance peak to 300 nm, indicating strong interactions between hexaconazole and the leaf's chemical components. Rubber leaf and eggplant leaf also showed considerable suppression, with ME% values of -194.2% and -153.5%, respectively, coupled with shifts in λ_{max}, reflecting substantial matrix interference. The consistent signal suppression observed across all leaf matrices aligns with the higher shift in the absorbance peak, as discussed earlier, due to the rich presence of chlorophyll, pigments, phenolics, and other organic compounds in leaf structures. These compounds can interfere with pesticide detection by scattering light, causing baseline noise, or chemically interacting with the analyte. Such interferences significantly reduce the sensitivity of the method and increase signal suppression, making leaf matrices more complex and challenging for accurate detection and quantification compared to fruit or soil matrices.

In contrast, the chilli fruit matrix showed moderate suppression at -85.7% and a smaller shift in λ_{max} to 280 nm, while eggplant fruit demonstrated signal enhancement (ME% = 39.2%) at the same wavelength, suggesting less interference and even some amplification of the signal. The topsoil matrix, with minimal interference (ME% = 27.9%) and no shift in λ_{max}, suggests that this matrix has a relatively simple composition compared to plant tissues, leading to fewer interactions.

It is important to clarify that the strong signal suppression observed in the chilli leaf matrix (ME% = –412.2%) does not reflect superior method performance but rather highlights the extent of matrix interference caused by complex biological components such as pigments, phenolics, and proteins. Similar findings have been reported in previous studies. Nevistić and Tomas observed that matrix suppression exceeding –80% was common in food matrices such as spelt and sunflower seeds, yet satisfactory recoveries ranging from 70% to 120% were achieved using matrix-matched calibration [24]. Zhang et al. reported matrix effects between –32% and –72% in herbal matrices including rosemary and ginger, where accurate quantification was only possible after applying matrix-matched calibration [25]. These studies reinforce that strong matrix effects are characteristic of complex samples and must be corrected for using appropriate calibration techniques, rather than being interpreted as a limitation of the analytical method. In this study, despite significant suppression in several matrices, the consistent linearity observed across all calibration curves ($R^2 > 0.9$) confirms that the modified QuEChERS–UV-Visible method provides reliable and accurate results when matrix-specific calibration is applied.

In addition, the solvent showed the lowest LOD (0.91 mg/mL) and LOQ (2.75 mg/mL), reflecting minimal matrix interference and ideal conditions for detection. In contrast, eggplant leaf had the highest LOD (2.44 mg/mL) and LOQ (7.39 mg/mL), indicating substantial suppression and matrix complexity, making it harder to detect hexaconazole accurately. Similarly, chilli leaf showed a relatively high LOD (2.27 mg/mL) and LOQ (6.88 mg/mL), consistent with the severe suppression observed.

Fruit matrices such as eggplant fruit and chilli fruit showed moderate LOD and LOQ values, with eggplant fruit exhibiting a signal enhancement (ME% = 39.2%) and an LOD of 2.57 mg/mL, suggesting less matrix interference. On the other hand, topsoil demonstrated the lowest LOD (0.80 mg/mL) and LOQ (2.82 mg/mL) among the matrices tested, reflecting minimal interference and highlighting that soil, compared to plant tissues, allows for more sensitive detection.

Thus, these findings show that all matrices have a significant impact on hexaconazole detection, despite the absorption peak remaining unchanged in some cases, such as in topsoil and eggplant fruit. The positive ME% values in some matrices enhance detection sensitivity, while negative ME% values, such as those in leaf matrices, suppress the signal and require matrix-specific calibration for accurate quantification. This highlights the necessity of considering matrix effects in nanopesticide residue analysis to ensure reliability across different agricultural and environmental samples.

As reported in various studies, matrix-induced signal suppression is more frequently encountered than enhancement in pesticide residue analysis (**Table 3**). This suppression often results from complex co-extracted substances such as pigments, proteins, phenolics, sugars, and organic acids. These compounds can interfere with the analyte signal by absorbing or scattering light, altering baselines, competing for ionisation, or reducing analyte solubility. In UV-Visible spectroscopy, such interference can lead to inaccurate absorbance readings, reduced sensitivity, spectral distortion, and compromised quantification [26].

For instance, a study using GC-MS/MS found that strong suppression was prevalent in matrices with high starch/protein and low water content, such as spelt kernels and sunflower seeds, with suppression reaching up to –82.6% [24]. Likewise, complex herbal matrices caused notable suppression for most organophosphorus and carbamate pesticides, especially in *Perillae folium*, where isocarbophos experienced –86.9% suppression, while a mild enhancement (+14.5%) was reported in *Astragali radix* for sulfonylureas [27]. In aromatic spices and herbs, such as rosemary, Amomum tsao-ko, Sichuan pepper, and ginger, strong suppression (up to –72%) was observed, attributed to dense phytochemical loads interfering with ionisation [25].

In addition, sugarcane honey presented a unique case where matrix effects varied by compound and detection method. Using LC-ESI-MS/MS, 2,4-D showed suppression of –53.4%, while diuron was enhanced by +35.1%. However, when analysed by HPLC-DAD, no matrix effects were observed, highlighting the significant influence of the ionisation technique on matrix interference [28]. Apple–blueberry sauce, peas, and limes offered further nuance in matrix behaviour. In this study, minor matrix effects were recorded for apple–blueberry (<16%) and peas (<12%), whereas suppression in limes reached up to –80% (notably for imazalil). Slight enhancement was also observed in apple–blueberry for azoxystrobin and pymetrozine when citrate-buffered QuEChERS was used [29]. This supports the view that matrix behaviour can shift depending on extraction buffer composition and target analyte characteristics.

Further evidence of enhancement was observed in matrices high in water and organic acid content. For example, in grapes and apples, signal enhancement reached up to +77.7%, while in tomatoes, linuron exhibited an exceptionally high enhancement of +314% [24],[30]. A comprehensive study across 12 vegetable matrices revealed that leafy vegetables such as lettuce, parsley, and spinach produced matrix effects ranging from –2% suppression to +282% enhancement, depending on the herbicide tested [30].

Table 2. The limit of detection (LOD) and limit of quantification (LOQ), maximum absorbance (λ_{max}), and matrix effect percentage (ME%) of nanohexaconazole in different matrices of crops and vegetables

Matrix-Matched Calibration	LOD (mg/mL)	LOQ (mg/mL)	λ_{max} (nm)	ME (%)
Solvent	0.91	2.75	275	NA
Rubber leaf	1.29	3.91	279	-194.2
Chilli leaf	2.27	6.88	300	-412.2
Eggplant leaf	2.44	7.39	280	-153.5
Chilli fruit	3.03	9.18	280	-85.7
Eggplant Fruit	2.57	7.78	275	39.2
Topsoil	0.80	2.82	275	27.9

Table 3. Comparative matrix effect in pesticide residue analysis

Matrix Sample	Pesticide(s)	Suppression/ Enhancement Effect	Method (Extraction + Quantification)	Ref
Apples, grapes, spelt kernels and sunflower seeds	>200 multi-residue pesticides including organophosphates, cypermethrin, pirimiphos-methyl	Strong matrix effects were observed across all matrices Enhancement: +72.5% to +77.7% (apples, grapes) Suppression: –65.2% to –82.6% (spelt kernels, sunflower seeds) Spelt kernels showed the highest suppression, while grapes showed the strongest enhancement.	QuEChERS + GC-MS/MS	[24]
Medicinal herbs (*Pinelliae rhizome*, *Astragali radix*, *Dendrobii officinalis caulis*, *Lonicerae japonicae flos*, *Perillae folium*)	28 pesticides and metabolites, including phorate, methamidophos, aldicarb-sulfoxide, chlorsulfuron, and metsulfuron-methyl	Predominantly suppression effects. Average ME: –17.6% (*Astragali radix*) to –41.8% (*Perillae folium*) Strongest: –86.9% (isocarbophos in *Perillae folium*) Enhancement noted in *Astragali radix* for sulfonylureas (+14.5% for metsulfuron-methyl).	QuEChERS + UHPLC-MS/MS	[27]
Sugarcane honey	2,4-D, diuron, fipronil	Mixed matrix effects observed via LC-ESI-MS/MS: Suppression: –53.4% (2,4-D) Enhancement: +35.1% (diuron) No matrix effect was observed by HPLC-DAD.	QuEChERS + HPLC-DAD and LC-ESI-MS/MS;	[28]
Apple–blueberry sauce, peas, limes	32 pesticides, including azoxystrobin, pymetrozine, thiabendazole, imazalil, and dichlorvos	Minor matrix effects in apple–blueberry and peas (<16% and <12%). Suppression in limes: up to –80% (imazalil). Slight enhancement in apple–blueberry: azoxystrobin, pymetrozine (citrate-buffered).	QuEChERS + LC–MS/MS and GC–MS;	[29]
Bay leaf, ginger, rosemary, *Amomum tsao-ko*, Sichuan pepper, cilantro, garlic sprout	73 pesticides, including chlorsulfuron, phosmet, chlorpyrifos, tau-fluvalinate, and methidathion	Predominantly suppression effects across spices: Suppression: up to –72% (e.g., fludioxonil in Sichuan pepper) Enhancement: up to +146% (e.g., fludioxonil in soybeans).	QuEChERS + UPLC-MS/MS and UPLC-QTOF-MS	[25]
Cucumber, squash, pumpkin, melon, tomato, eggplant, pepper, potato, lettuce, parsley, dill, spinach	57 herbicides, including butachlor, linuron, flufenacet, chlorpropham, pyriproxyfen, chlorsulfuron, and cinidon ethyl	Strong matrix effects across families Suppression: –2% to –79% (e.g., parsley, pepper, cucumber) Enhancement: +122% to +379% (e.g., tomato, lettuce, melon) Parsley showed the highest suppression, while tomato showed the strongest enhancement (linuron: +314%).	QuEChERS + LC-MS/MS	[30]

Therefore, accurate quantification in complex matrices requires appropriate correction strategies. In this study, matrix-specific calibration was applied to each matrix, including rubber leaf, chilli leaf, eggplant leaf, fruit tissues, and topsoil. Despite notable variation in matrix effects, all calibration curves demonstrated strong linearity ($R^2 > 0.90$), indicating acceptable analytical performance across the tested range. For example, eggplant fruit yielded an R^2 of 0.9979, while rubber leaf produced an R^2 of 0.9818. Correlation coefficients above 0.90 are generally considered acceptable for preliminary or screening-level methods, particularly in spectrophotometric analysis involving complex biological matrices at low analyte concentrations. This threshold is supported by international validation guidance, which recommends that linearity be assessed based on method purpose, sample complexity, and signal consistency [31]. The observed linear trends without irregular deviation confirm that the method is sufficiently robust for early-stage quantification of nanopesticide residues and may be further developed for confirmatory applications.

Additionally, the method follows key green chemistry principles by minimising solvent consumption, avoiding hazardous reagents, and employing simple UV–Visible instrumentation. These practices are consistent with criteria established in green analytical chemistry frameworks, which emphasise low environmental impact through reduced waste, safer solvents, and energy efficiency [32]. Although a formal greenness assessment was not performed, the characteristics of this method indicate a strong alignment with sustainable analytical practices. A comprehensive greenness evaluation will be considered in future studies to support this potential.

Conclusion

This study established a modified QuEChERS extraction method coupled with UV-Visible spectroscopy for the detection of nanohexaconazole residues in complex agricultural matrices. Despite substantial matrix interferences, particularly in leaf samples where signal suppression exceeded 400%, matrix-specific calibration curves yielded high linearity ($R^2 > 0.9$), confirming the method's reliability across diverse sample types. The results confirm that matrix effects remain a significant challenge in nanopesticide residue analysis. Without proper correction, they can compromise both sensitivity and accuracy. This work demonstrates that careful calibration within each matrix is essential to achieving valid quantitative results, particularly when using optical detection techniques that are more prone to baseline shifts and interference. The simplicity and accessibility of UV-Vis spectroscopy, combined with targeted matrix-matched calibration, provide a promising platform for preliminary screening of nanopesticide residues. However, future research should focus on improving sensitivity at lower concentration levels, integrating this approach with confirmatory methods, and validating its applicability across a wider range of nanopesticide formulations and environmental conditions.

Acknowledgement

This work was supported by a Universiti Sains Malaysia, Short-Term Grant with Project No: 304/PKIMIA/6315732.

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