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Derivative Spectrophotometric method for Simultaneous Determination the residue of Carboxin, Chlorothalonil and Pyriproxyfen in cabbage samples using Zero-crossing Technique

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Abstract

We developed and validated a spectrophotometric technique for determining Carboxin, Chlorothalonil and Pyriproxyfen residues in cabbage samples that is simple, accurate, sensitive and precise. The approach is based on recording the first derivative curves and utilising the zero-crossing method to compute the estimated values of each component. There are no earlier separation procedures in this procedure. This investigation led to the revelation that the correlation coefficient of the calibration curves was more than 0.9998. The calibration curves for this spectrophotometric approach for Carboxin, Chlorothalonil and Pyriproxyfen are linear in concentration ranges of (1-35) µg/mL, 0.5-7 µg/mL and (1-30) µg/mL, with a relative standard deviation of no more than 5.0%. The proposed approach has been utilised effectively to test Carboxin, Chlorothalonil and Pyriproxyfen residues in cabbage samples concurrently with a high rate of recovery, accuracy and precision.

Keywords: First derivative spectrophotometry, Zero-crossing Technique, Carboxin, Chlorothalonil, Pyriproxyfen

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طريقة القياس الطيفي المشتق للتحديد المتزامن لبقايا الكاربوكسين والكلوروثالونيل والبيربروكسيفين في عينات الملفوف باستخدام تقنية التقاطع الصفري

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الخلاصة

قمنا بالتطوير والتحقق من صحة تقنيه قياس الطيف الضوئي لتحديد بقايا الكاربوكسين والكلوروثالونيل والبيربروكسيفين في عينات الملفوف وهي بسيطة ودقيقة وحساسة و يعتمد النهج على تسجيل المنحنيات المشتقة الأولى واستخدام طريقة التقاطع الصفري لحساب القيم المقدرة لكل مكون ,لاتوجد إجراءات فصل سابقة في هذا الاجراء حيث أدى هذا التحقيق الى الكشف عن معامل الارتباط لمنحنيات المعايرة كان اكثر من 0.9998 منحنيات المعايرة لهذا النهج الطيفي الكاربوكسين والكلوروثالونيل والبيربروكسيفين من نطاقات التركيز من (1-35) و µg/mL, 0.5-7 µg/mL مع انحراف معياري نسبي لايزيد من 5%, حيث تم استخدام النهج المقترح بشكل فعال لاختباربقايا الكاربوكسين والكلوروثالونيل والبيربروكسيفين في عينات الملفوف بالتزامن مع معدل عال من الاستعادة والدقة.

الكلمات المفتاحية: منحنيات المشتقة الأولى ، طريقة التقاطع الصفري، الكاربوكسين، الكلوروثالونيل، البيربروكسيفين.

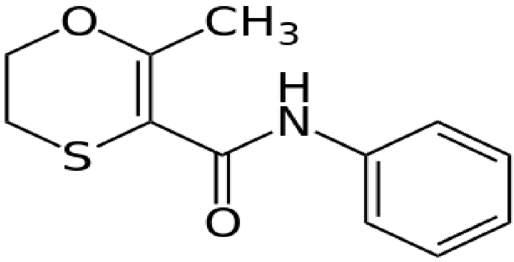
Introduction

Pesticides are chemicals used to control pests that damage agricultural goods during production, nutrient absorption and storage, resulting in product loss [1].

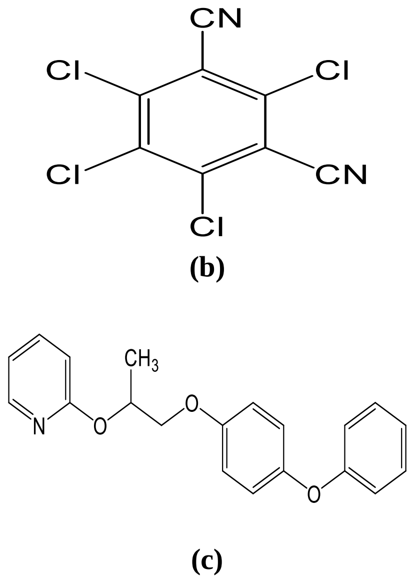
Carboxylate (CAR) commonly known as Vitavax (2,3-dihydro-6-methyl-oxathine-5-carboxanilide) is one of the most popular of several systemic fungicides used in agriculture to eliminate harmful fungi. The chemical composition of carboxin is shown in Figure (1a) [2]. Fungicide residue levels in foods are typically regulated to reduce consumer exposure to harmful or unnecessary pesticide ingestion, despite the fact that it has minimal mammalian toxicity [3]. For the carboxin determination, many analytical techniques were applied, either alone or in conjunction with other pesticides including gas liquid chromatography [4], high-performance liquid chromatography [5,6] and derivative spectrophotometry [7].

Chlorothalonil (CHL) is a chemical compound (2,4,5,6-tetrachloroisophthalonitrile). Fungicide chlorothalonil chemical structure is shown in Figure (1b). It is not poisonous [8]. Chlorothalonil is an extensively used pesticide for combating fungi that destroy vegetables, trees, little fruits and other agricultural crops [9]. The most widely used analytical methods for locating chlorothalonil residues in various matrices include gas chromatography-mass spectrometry, gas chromatography with electron capture detection, high-performance liquid chromatography with UV detection and high-performance liquid chromatography-diode array detection [10-16].

Pyriproxyfen (PYR) is poisonous to insects throughout their embryonic, final larval or reproductive stages. Whiteflies, mealworms, scales and thrips are among the pests that Pyriproxyfen is especially efficient against [17]. Pyriproxyfen [4-phenoxyphenyl (RS)-2-(2-pyridyloxy)-propyl ether] its chemical name. It is a crucial pesticide used to protect plants from whiteflies [18]. Pyriproxyfen chemical structure is given in Figure (1c) is a pyridine-based pesticide which is effective on a variety of arachnoid [19]. Traditional techniques for detecting pyriproxyfen and other pesticides in agricultural goods such as fruits and vegetables include gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry [20, 21]. Pyriproxyfen and other pesticides were quantified using high-performance liquid chromatography and HPLC with UV detection [22,23], as well as derivative spectrophotometry [24]. The aim of this study is to establish a method to resolve a ternary mixture of pesticides in cabbage samples.



(a)



(b)

(c)

Figure 1: The chemical structure of (a) Carboxin (b) Chlorothalonil (c) Pyriproxyfen

Experimental

1. Apparatus

The spectroscopic observations were performed using a Shimadzu (UV-1800, Japan) UV-visible double beam spectrometer with a fixed bandwidth of 1 nm and a 1 cm quartz cell. A double beam spectrophotometer linked to a computer was used to record carboxin, chlorothalonil and pyriproxyfen mixed solution zero order spectra as well as data on absorption spectra. Data for Carboxin, Chlorothalonil and Pyriproxyfen with their combination solutions were transformed from zero order spectra to first order derivative spectra using the UV Probe software.

2. Chemicals and Materials

Santa Cruz Biotechnology in the United States provided 99% pure versions of carboxin, chlorothalonil, and pyriproxyfen. The HPLC solvents acetonitrile and methanol were provided by Merck (Darmstadt, Germany). Sigma Aldrich provided the Primary Secondary Amin (PSA). BDH supplied anhydrous sodium acetate and magnesium sulphate (VWR Chemicals BDH, England). MgSO4 was heated for activation at 400 °C for 4 hours to remove phthalates. It was then kept cold until it was placed in a desiccator.

3. Standard Stock Solution preparation

Each 10 mg of insecticide were dissolved in 100 ml of methanol to create stock solutions (100 µg /mL), which were then placed in bottles with stained glass stoppers and kept at -18 °C. In less than two months, all stock solutions were kept in storage. Stock solutions are diluted with methanol to form regularly used standard solutions.

4. Sample preparation

CAR, CHL and PYR residues were extracted from cabbage samples using the modified QuEChERS approach described by Hou et al. [13]. After weighing and homogenising 500 g of chopped material, three levels of the standard stock solution were introduced to a 50 mL teflon centrifuge tube containing 10 g of newly weighted sample. 10 mL of an extraction solvent composed of toluene (1:1, v/v) and acetonitrile with 5% acetic acid were added using a transfer pipette. The mixture was vortexed for 1 minute after being agitated for 1 minute in an air bath at 22 °C. The liquid was immediately vortexed for 1 minute after 4 g of anhydrous MgSO4 and 1 g of anhydrous sodium acetate were added. The extracts were then centrifuged for 5 minutes at 2500 rpm. After that, the top layer was separated into 10 mL sections and added to a 15 mL Teflon centrifuge tube together with 1.5 g of MgSO4 and 300 mg of PSA. After one minute of vortexing, the samples were centrifuged at 8800 rpm for five minutes. The solution was filtered using a 0.45-m filter and 10 mL of the filtrate was transferred to a 15-mL tube and gradually concentrated with nitrogen until nearly dry. In order to evaluate the pesticide residue, the extract was diluted in methanol according to the recommended protocol using the standard addition method.

5. Calibration Graph and Zero-crossing method

Three sets of calibration flasks, each having a capacity of 10 mL and containing a mixture of CAR, CHL and PYR diluted to volume with methanol, were used to create a sufficient stock solution for the samples. In the first sequences, CHL and PYR concentrations are fixed at (1 and 5) µg/mL, respectively, whereas CAR concentrations range from (1 to 35) µg/mL. The second sequences had fixed CAR and PYR concentrations of 5 µg/mL each and variable CHL concentrations of (0.5-7) µg/mL. The third sequences had constant CAR and CHL concentrations of 5 and 1 µg/mL, respectively, together with variable PYR concentrations of (1 to 30) µg/mL. On a white background, absorption spectra between (200 and 350) nm were visible. The computer application SHIMADZU UV Probe Data System converts zero-order spectra of CAR, CHL and PYR in ternary combinations into first-derived spectra in the (200-350) nm range (Version 2.43). Figures (2,3) depict the overlap spectra (zero, first order) for the three insecticides in the ternary combination (3).

Results

1. Calibration curve and the statistical data

Table 1 displays the analytical characteristics and statistical information of the calibration curves of the suggested method for each pesticide with an estimated CAR, CHL and PYR, including the linear range of the calibration graph, correlation coefficients, LOD, LOQ and relative standard deviation. According to the table, the calibration curves for the suggested technique have strong correlation coefficient values that are greater than 0.999, which indicates that the calibration curves are linearly consistent. Lower LOD and LOQ values show how sensitive the suggested techniques are. The precision and accuracy of the proposed first derivative spectrophotometric technique based on the zero-cross methodology for the simultaneous measurement of CAR, CHL and PYR were also investigated. The relative standard deviation percentage (RSD%) for each pesticide was less than 5% based on the results of five replicate measurements of three different CAR, CHL and PYR concentrations and the recovery percentage (Recovery%) was greater than 95.04%, demonstrating the suggested approach's acceptable precision and accuracy. The results are shown in the table (2).

In the (200-350 nm) region, the usual UV absorption spectra of CAR, CHL and PYR completely overlap (Figure 2). As a result, it is impossible to use normal spectrophotometry to separate a mixture and detect three pesticides at once in a ternary mixture. Therefore, interference has been reduced and overlapping spectra have been cleaned up using the zero-crossing method of derivative spectrophotometry.

Table 1: The statistical parameter for determination of carbon, chlorothalonil, and pyriproxyfen by proposed methods

Compounds	Λmax (nm)	Linearity µg/mL	Regression equation	r2	LOD µg/mL	LOQ µg/mL
Carboxin	313.20 318.20	1-35	Y=1.7932x-0.1711	0.9998	0.154	0.466 0.355
Chlorothalonil	225.20	0.5-7	Y=1.5409x-0.0954 Y=4.1494x-0.2814	0.999 0.998	0.117 0.066	0.355 0.201
Pyriproxyfen	279.40 283.60	1-30	Y=1.041x+0.0954 Y=1.041x-0.2429	0.9998 0.9999	0.173 0.150	0.526 0.454

Table 2: The accuracy of the propose methods for simultanous determination of carbon, chlorothalonil, and pyriproxyfen in a ternary mixture

Compound	Methods of Analysis	Concentration µg/mL	Recovery %	RSD%
Carboxin	Zero-crossing technique at 313.20nm	1	103.22	4.98
		20	98.62	0.778
		35	100.01	0.668
	Zero-crossing technique at 318.20nm	1	95.04	3.51
		20	908.98	1.36
		35	99.02	0.661
Chlorothalonil	Zero-crossing technique at 318.20nm	0.5	96.02	4.84
		4.0	100.02	1.096
		7.0	99.08	0.951
Pyriproxyfen	Zero-crossing technique at 279.40nm	1	96.37	3.30
		15	96.33	1.744
		30	97.80	1.433
	Zero-crossing technique at 283.60nm	1	105.48	4.92
		15	97.16	0.840
		30	100.50	0.756

Discussion

1. Normal absorption spectra of Carboxin, Chlorothalonil and Pyriproxyfen solution

CAR, CHL and PYR UV absorption spectra, as well as their combination, were measured against methanol as a blank reagent. Figure (2) depicts the absorption spectra of Carboxin and Pyriproxyfen solutions at 10 µg/mL and Chlorothalonil solution at 4.0 µg/mL, respectively. The CAR, CHL and PYR spectrums clearly overlapped one another, as demonstrated. As a result of substantial overlapping and spectral interference, identifying each of these chemicals under investigation based on their usual absorption spectra when present in the same solution is extremely challenging.

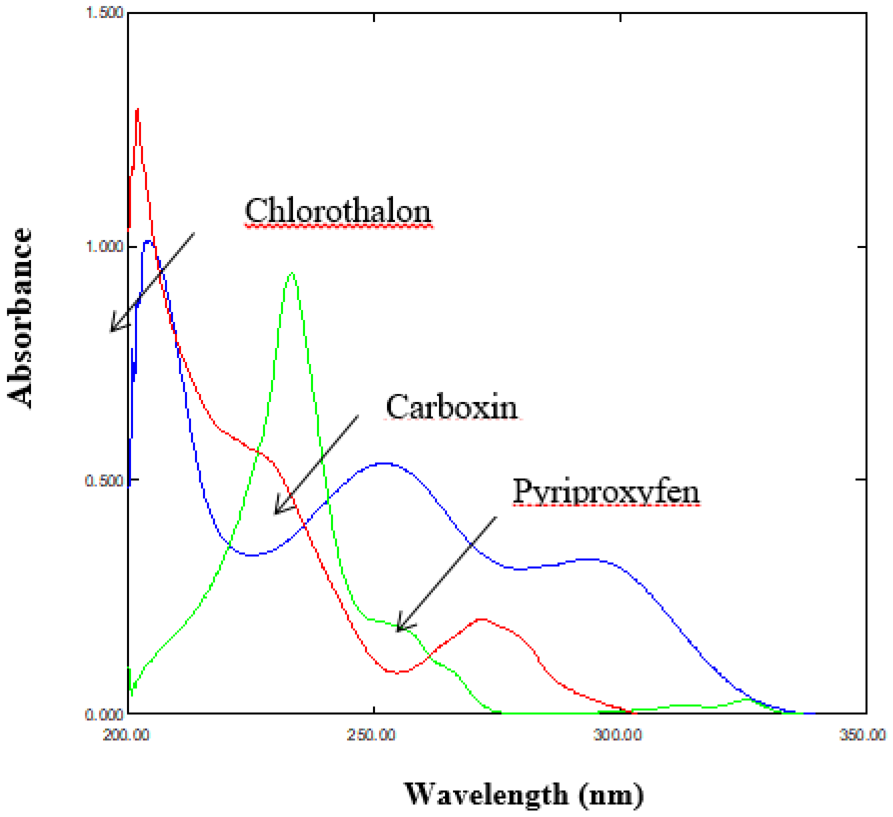


Figure 2: Zero-order spectra of 10 µg/mL each of Carboxin and Pyriproxyfen and 4 µg/mL of Chlorothalonil

2. Derivative spectra of the mixture of Carboxin, Chlorothalonil and Pyriproxyfen solution

Derivatizing the zero-order spectrum can aid in the separation of overlapping signals as well as the reduction of noise produced by the presence of other substances in a sample. The aforementioned features can enable for the measurement of one or a few analytes without the need for prior separation or purification [25]. From this perspective, first and second derivative spectra for CAR, CHL and PYR were obtained. The best results for simultaneous determination were obtained with CAR, CHL and PYR first derivative spectra. Because each CAR, CHL and PYR first derivative spectrum in Figure (3) includes several zero-crossing points, CAR, CHL and PYR may all be computed concurrently with good precision and accuracy using this approach.

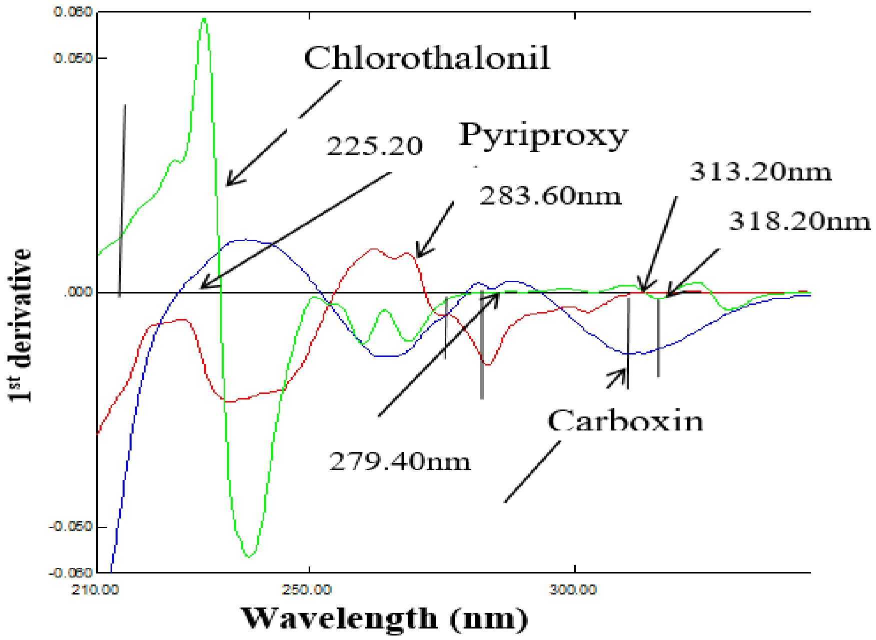


Figure 3: First derivative spectra of 10 μg/mL each of Carboxin and Pyriproxyfen and 4 μg/mL of Chlorothalonil

CAR, CHL and PYR mixed solutions with fixed concentrations of CHL and PYR (1 µg/ml) and (5 µg/ml), respectively and varied concentrations of CAR for CAR determination in the presence of CHL and PYR in the same solution were created. The produced mixture's first derivative spectra were obtained in the (210-350) nm region, as shown in Figure (4). The results reveal that across the concentration range (1-35) µg/ml, the CAR concentration is linearly proportional to the amplitude from peak to baseline at 313.20 nm and 318.20 nm CAR, CHL and PYR mixed solutions with fixed concentrations of CHL and PYR (1 µg/ml) and (5 µg/ml), respectively and varied concentrations of CAR for CAR determination in the presence of CHL and PYR in the same solution were created. The produced mixture's first derivative spectra were obtained in the (210-350) nm region, as shown in Figure (4). The results reveal that across the concentration range (1-35) µg/ml, the CAR concentration is linearly proportional to the amplitude from peak to baseline at 313.20 nm and 318.20 nm. with detection limits of 0.154 µg/ml and 0.117 µg/ml, r2 = 0.9998, and 0.9999 respectively. Figures (7) and (8) show the calibration graph.

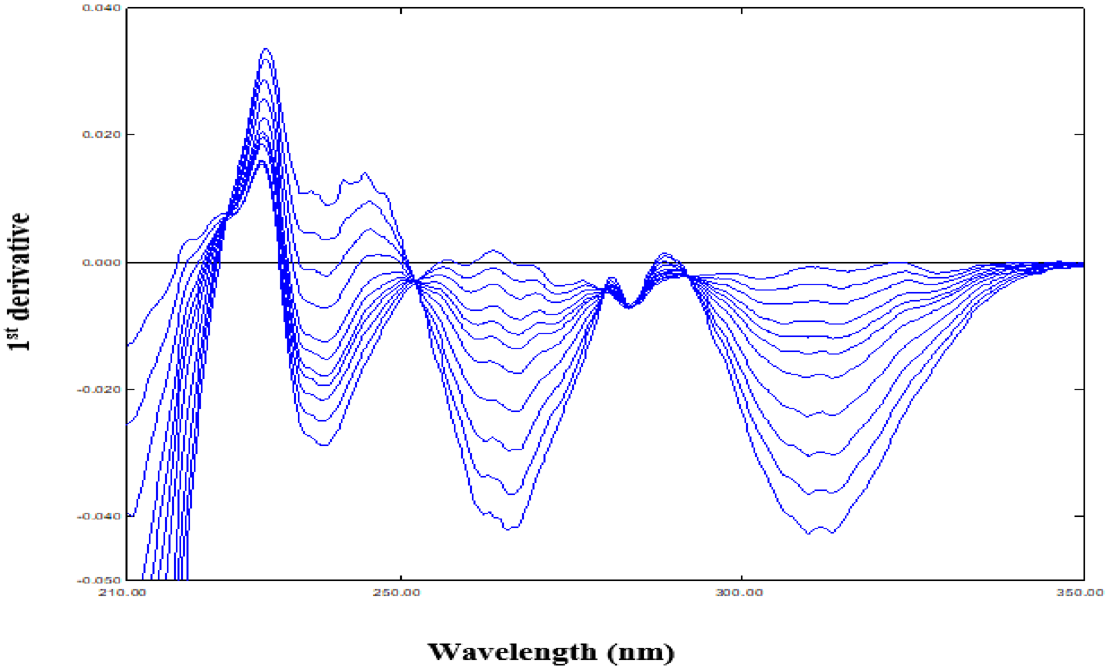


Figure 4: First derivative spectra of ternary mixture (1, 3, 5, 8, 10, 12, 15, 20, 25, 30, and 35 µg/mL) of CAR in the presence of 5 µg/mL of PYR and 1 µg/mL CHL

To measure CHL in the presence of CAR and PYR in the same solution, several mixed solutions of CAR, CHL and PYR were produced in such a manner that the concentrations of CAR and PYR remained constant (5 µg/mL) of each with variable concentrations of CHL. The first derivative spectra of the combination solutions were obtained in the (210 - 350) nm region, as shown in Figure 1. (5). It was established that the concentration of CHL is directly proportional to the peak-to-baseline amplitude at 225.20nm over the concentration range of (0.5 - 7) µg/mL with a detection limit of 0.066 g/mL and a r2 value of 0.9998. Figure shows the calibration curves (9).

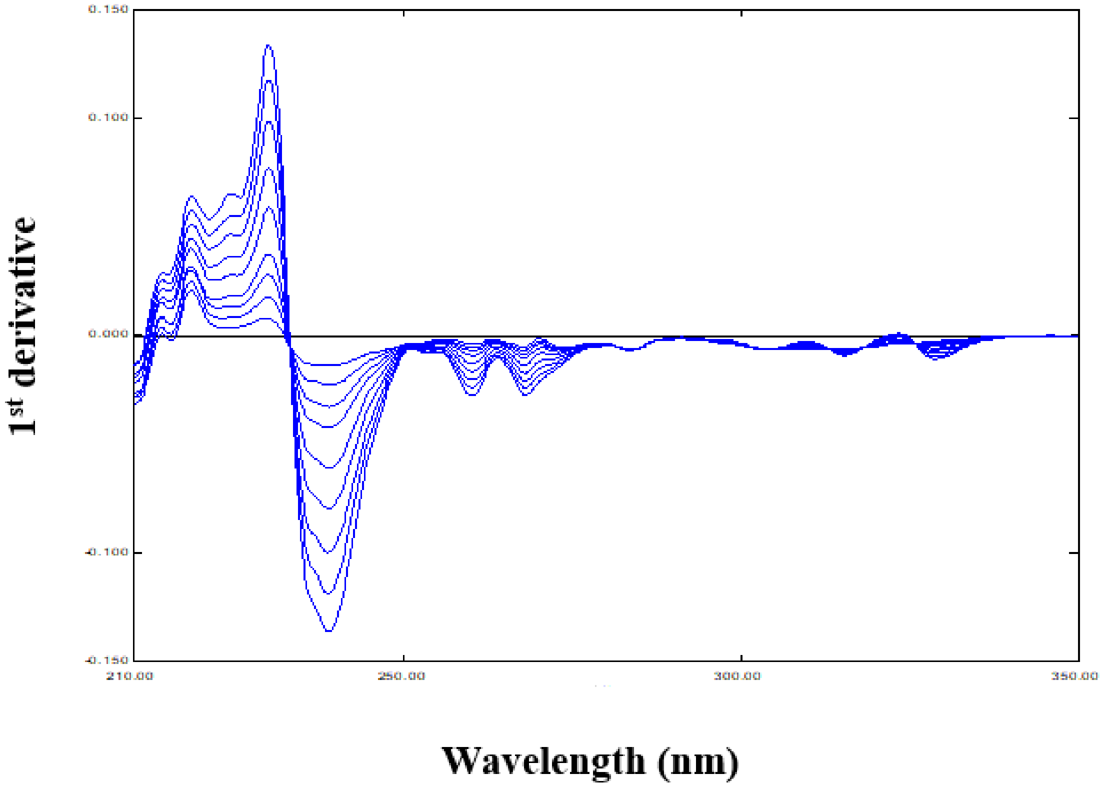


Figure 5: First derivative spectra of ternary mixture (0.5, 1, 1.5, 2, 3, 4, 5, 6 and 7 µg/mL) of CHL in the presence of 5 µg/mL of PYR and 5 µg/mL CAR

Several mixed solutions of CAR, CHL and PYR were generated using the first derivative spectrophotometric technique, with constant concentrations of CAR and CHL (5µg/mL) and (1µg/mL), respectively and variable amounts of PYR. First derivative spectra of the produced combination in the range of (210 - 350) nm were recorded to analyse PYR in the presence of CAR and CHL in the same solution, as shown in Fig (6). The results reveal that within a concentration range of (1-30) µg/mL, CAR concentration is linearly proportional to peak-to-baseline amplitude at 279.40nm and 283.60nm, with detection limits of 0.173 µg/mL and 0.150 µg/mL, respectively, r2 = 0.9998 and 0.9999. The calibration graph is shown in Figure (10). (11).

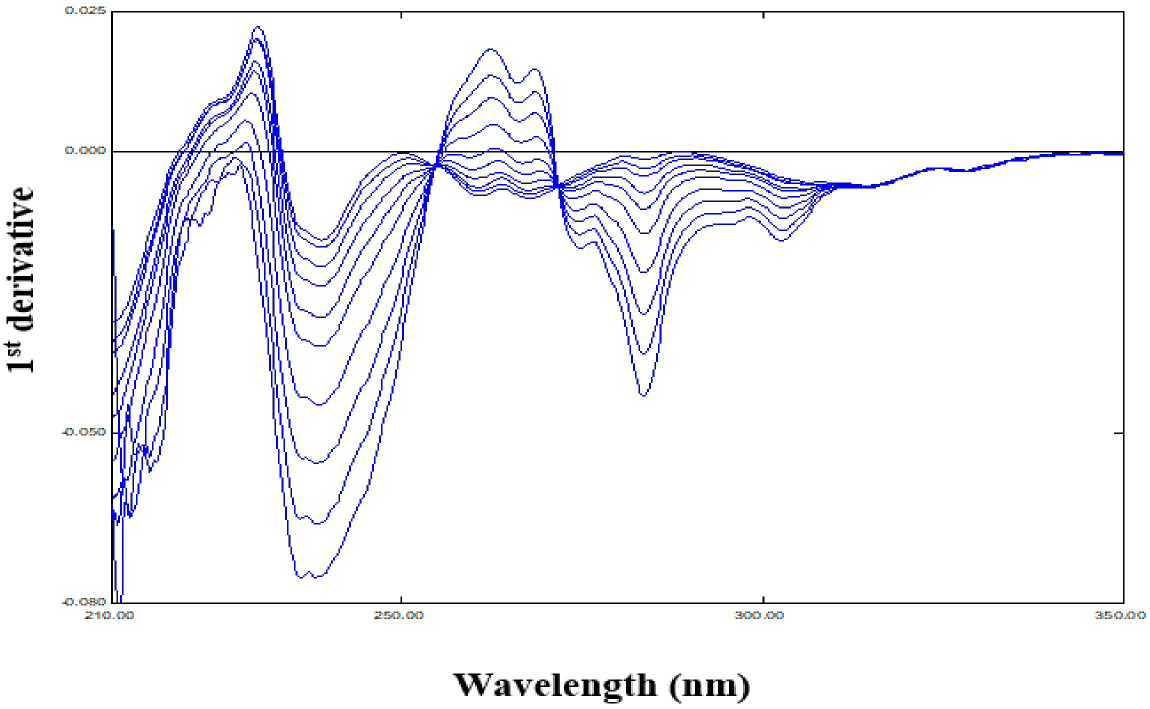


Figure 6: First derivative spectra of ternary mixture (1, 2, 3, 5, 7, 10, 15, 20, 25 and 30) µg/mL of PYR in the presence of 5 µg/mL of CAR and 1 µg/mL CHL

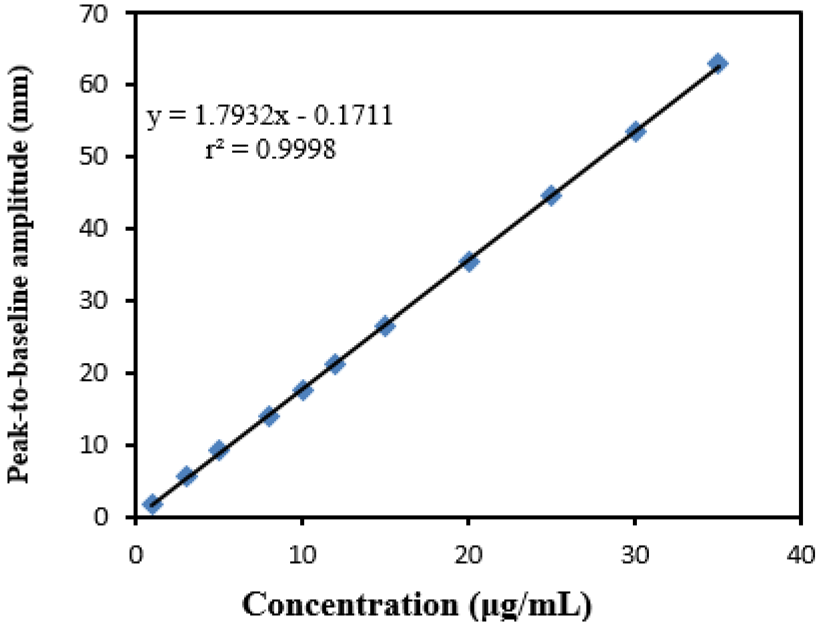


Figure 7: Calibration curves of first derivative spectrophotometric determination of Carboxin at 313.20nm, using peak-to-baseline technique

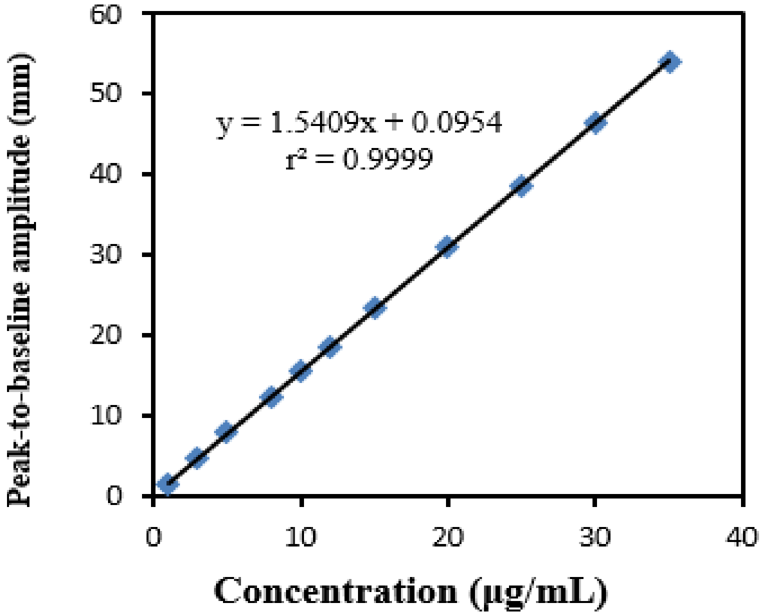


Figure 8: Calibration curves of first derivative spectrophotometric determination of Carboxin at 318.20nm, using peak-to-baseline technique

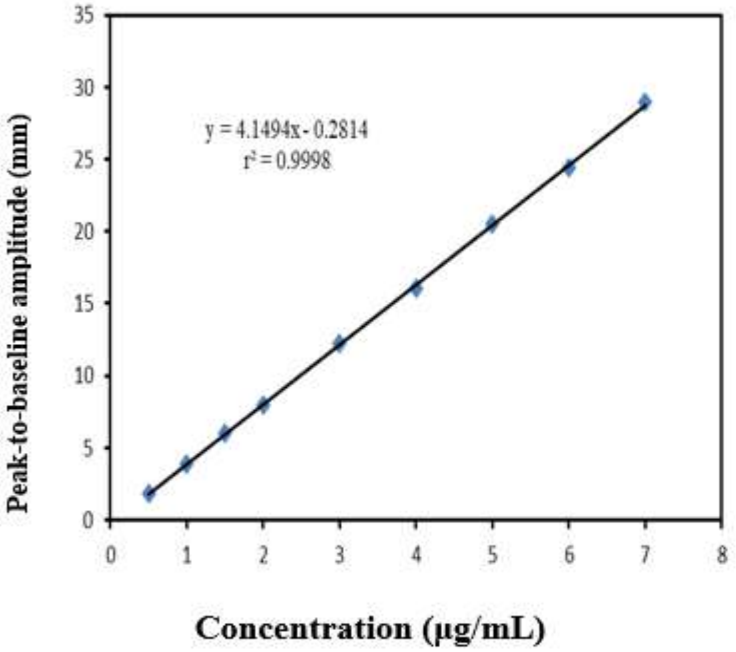


Figure 9: Calibration curves of first derivative spectrophotometric determination of Chlorothalonil at 225.20nm, using peak-to-baseline technique

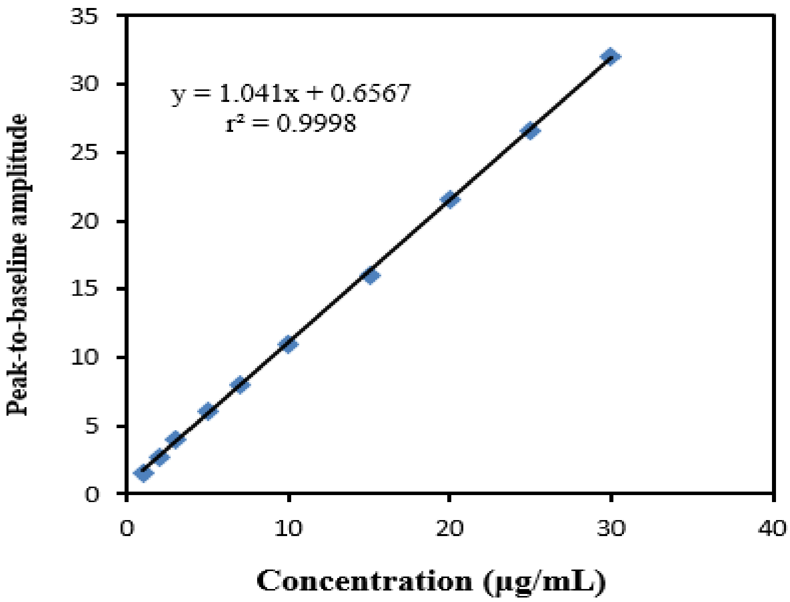


Figure 10: Calibration curves of first derivative spectrophotometric determination of Pyriproxyfen at 279.40nm, using peak-to-baseline technique

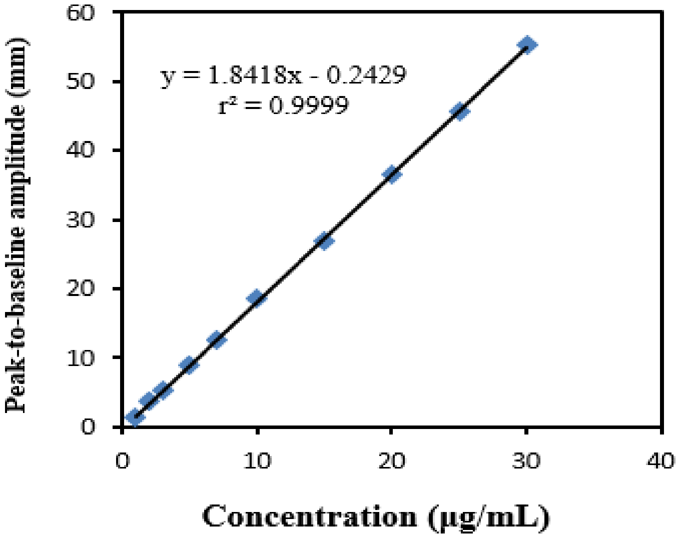


Figure 11: Calibration curves of first derivative spectrophotometric determination of Pyriproxyfen at 283.60nm, using peak-to-baseline technique

Application of the methods

The suggested method was employed in this study to determine the CAR, CHL and PYR in genuine cabbage samples. Five replications of each concentration level were injected into the genuine fresh cabbage samples: (1.0, 20, 35) µg/mL for CAR, (0.5, 4.0, 7.0) µg/mL for CHL and (1.0, 15, 30) µg/mL for PYR. The relative standard deviation of three pesticides in real cabbage samples was less than 5% for CAR, CHL and PYR, indicating the accuracy of the proposed technique. The European Union (EU) food safety authority has established a maximum residual limit (MRL) for some pesticides in order to guarantee that food is safe for consumer health. The quantity of CAR, CHL and PYR residues in cabbage samples that were either grown or procured from the market were lower than the EU-MRL (1 mg/kg), and PYR was not found in the samples of cabbage that were procured from the neighbourhood market . Therefore, none of these three pesticides pose a risk to the health of consumers. The residue of each CAR, CHL and PYR was simultaneously determined using the spectrophotometric technique and the standard addition method in actual cabbage sample. The main conclusions are provided in (Table 3).

Table 3: Determination the residues (mg/kg) of carboxin, chlorothalonil and pyriproxyfen in cabbage samples.

Compound	Planted	Local market
Carboxin	0.202	0.258
Chlorothalonil	0.318	0.232
Pyriproxyfen	0.246	Not detected

Conclusions

The developed approach for CAR, CHL and PYR determination was proven to be easy, quick, precise and accurate. The derivative technique is faster than chromatographic methods and has been successful in resolving the overlapping spectra. It also doesn't require any expensive or specialized equipment. Furthermore, the QuEChERS approach has been utilised to extract actual cabbage samples since it is very simple and affordable, has fewer steps, takes less time than other extraction methods and yields high recoveries and accuracy. As a result, the suggested approach is normally suitable for estimating CAR, CHL and PYR residues in cabbage samples.

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