

Research Article

Simultaneous Determination of Ternary Mixture of Carboxin, Chlorpyrifos, and Tebuconazole Residues in Cabbage Samples Using Three Spectrophotometric Methods

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Three simple precise and accurate spectrophotometric methods are developed for simultaneous determination of ternary mixtures of carboxin, chlorpyrifos, and tebuconazole residues in cabbage grown in the experimental field. The first method is a double divisor-ratio spectra derivative that relies on the derivative of ratio spectra and attained through dividing the absorption spectra of the ternary mixture by the sum of standard spectrum of a mixture of two from three components, using methanol as a solvent and measuring CAR at 242 nm, CHL at 236 nm, 276 nm, and 300 nm, and TEB at 226 nm. The second method is a successive derivative of ratio spectra which determined CAR at 256 nm and 258 nm, CHL at 290 nm and 292 nm, and TEB at 226 nm and 228 nm. The third method is a mean centering of ratio spectra where CAR, CHL, and TEB were measured at 306 nm, 280 nm, and 240 nm, respectively. These procedures do not involve any previous separation. The extraction of analytes was carried out by using acetonitrile, and the procedure of purification was fulfilled by dispersive solid-phase extraction with a primary-secondary amine (PSA). The proposed methods showed excellent linearity range for three spectrophotometric methods over the concentration ranges of 1–30 $\mu\text{g/mL}$, 1–50 $\mu\text{g/mL}$, and 1–45 $\mu\text{g/mL}$ for carboxin, chlorpyrifos, and tebuconazole, respectively. The analytical characteristics such as detection limit, determination limit, relative standard deviation, and accuracy of the three methods were performed. The limits of detection were in the range of 0.153–0.260 $\mu\text{g/mL}$ for carboxin, 0.137–0.272 $\mu\text{g/mL}$ for chlorpyrifos, and 0.109–0.205 $\mu\text{g/mL}$ for tebuconazole with limits of quantification lower than 0.790, 0.824, and 0.621 $\mu\text{g/mL}$ for CAR, CHL, and TEB, respectively. The recoveries ranged from 87.02% to 94.53% for carboxin, 92.32% to 108.53% for chlorpyrifos, and 87.19% to 98.00% for tebuconazole with relative standard deviations less than 5.91%, 5.99%, and 5.53% in all instances for carboxin, chlorpyrifos, and tebuconazole, respectively. The results obtained from the proposed methods were compared statistically by using one-way ANOVA, and the results revealed that there were no significant differences between three different spectrophotometric methods. The suggested methods can be applied with great success to the simultaneous estimation of carboxin, chlorpyrifos, and tebuconazole residues in cabbage samples.

1. Introduction

Pesticides are greatly applied to keep the crops away from a variety of pests. The main purpose of the use of pesticides is to gain high yield of the agricultural product, but successive and repeated use of certain pesticides have led to their accumulation in plants, animals, sediments, and soils, thus increasing the rate of spreading of contamination in the environment [1]. Recently, the application and production of pesticide through

worldwide increased for both agricultural and nonagricultural goals which cause soil, air, surface water, and groundwater pollution that in turn involves significant risk for both environment and human health either directly through exposure to it or indirectly via residues in food and drinking water. Globally, pesticides reached alarming levels in water, air, soil, food production, and biological stuff [2].

Carboxin (CAR) which is chemically known as 2, 3-dihydro-6-methyl-oxathiin-5-carboxanilide (Vitavax) is

considered a systematic fungicide that has been used to kill pathogenic fungi in the agriculture [3]. The chemical structure of carboxin is shown in Figure 1(a). Carboxin is a type of anilide fungicide that is used broadly at multiple stages of plant growth and during the storage of postharvest to protect the product against rotting. Although it has low mammalian toxicity, the levels of fungicide residues are generally regulated by legislation in order to diminish the risk of consumers' exposure to the deleterious or dispensable uptake of pesticides [4]. Different analytical techniques were used for the carboxin determination either alone or in combination with other pesticides in food, and environmental samples include spectrophotometric method [3], gas chromatography (GC) [5], gas-liquid chromatography (GLC) [6], thermospray liquid chromatography/mass spectrometry [7], high-performance liquid chromatography (HPLC) [8], and HPLC equipped with UV detector [9].

Chlorpyrifos (CHL) is a crystalline organophosphate insecticide. It is chemically defined as O, O-diethyl-O-3, 5, 6-trichloro-2-pyridyl phosphorothioate insecticide. In agriculture, it remains one of the most widely used organophosphate insecticides [10]. The chemical structure of chlorpyrifos is shown in Figure 1(b). Chlorpyrifos is analyzed by various methods such as spectrophotometry and derivative spectrophotometry [11–15] and spectrophotometry using multivariate calibration methods [16], partial least squares [17, 18], GC [19], GC equipped with electron capture detector (GC-ECD) [20], GC-MS [21], GC-MS/MS [22], and HPLC with UV detection [23–25] was also used for estimation of chlorpyrifos.

Tebuconazole (TEB) is chemically defined as ((RS)-1-p-chlorophenyl)-4, 4-dimethyl-3-(1H-1, 2, 4-triazol-1-ylmethyl)pentan-3-ol. It is utilized to treat pathogenic fungi on the plant in agriculture and on crops [26]. The chemical structure of tebuconazole is shown in Figure 1(c). It was determined by several methods including GC [27], gas chromatography-nitrogen phosphorus detection [28], gas chromatography-flame ionization detector [29], GC-MS [30, 31], and supercritical fluid chromatography (SFC)-MS/MS [32] and mostly determined by HPLC and HPLC-DAD method [33, 34], HPLC-MS/MS [35], liquid chromatography (LC) with UV detection [36], and LC-tandem mass spectrometric detection [37]. In addition, quantitative structure-property relationship (QSPR) models were developed using multiple linear regression, partial least squares, and neural network analyses for determining a number of pesticides including carboxin, chlorpyrifos, and tebuconazole [38].

Different spectrophotometric methods could be used for resolving and determining mixtures which contain two or more active compounds simultaneously without requiring a preliminary separation process such as classical derivative spectrophotometry [39–42]. Furthermore, UV-visible spectrophotometry method could not be used for the determination of chemicals with overlapped spectra, but it might be utilized for the estimation of certain chemicals in various samples [43].

In 1990, Salinas et al. [44] proposed a method for resolving binary mixtures. This method was based on the first

derivative of ratio spectra which was attained through dividing the absorption spectra of binary mixtures by the standard spectrum of one of the components. Then, Berzas Nevado et al. [45] expanded the method which was introduced by Salinas from the analysis of binary mixture to ternary mixture by derivative ratio spectra zero-crossing method. This method depends on the measuring of the signal at the zero-crossing point.

Another spectrophotometric method was introduced by Dinç et al. [46–48] for estimation of three compounds in ternary mixtures simultaneously. This method consists of two steps through dividing the absorption spectra of ternary mixtures by the sum of two spectra. Later, the ratio spectrum was derivatized. This method is termed as a double divisor-ratio spectra derivative method which is depending upon the use of the coincident spectra of the ratio spectra derivative gained by the use of a double divisor and measurements at maximum or minimum wavelengths.

Currently, other methods for the determination of ternary mixtures simultaneously were developed by Afkhami and Bahram that lack preceding steps of separation. These methods are successive derivatives of ratio spectra which consist of two steps and depend on the successive derivative of ratio spectra. The other method is mean centering of ratio spectra, this method could be applied successfully for the analysis of binary and ternary mixture simultaneously, and the mathematical clarification of the proposed methods was described in [49, 50]. Recently, pesticides could be determined in binary mixture using mean centering of ratio spectra [51]. Fast, easy, and inexpensive methods are widely used for the determination of pesticide residues in fruits and vegetables involving acetonitrile extraction partitioning and dispersive solid-phase extraction [52]. Hence, this paper tries to establish methods to resolve a ternary mixture of pesticides in cabbage samples and then to compare the results obtained by these three approaches.

2. Experimental

2.1. Apparatus. Double-beam UV-visible spectrophotometer (Shimadzu, Model UV-1800, Japan) with a fixed 1 nm bandwidth and 1 cm quartz cell was utilized for spectrophotometric measurement, and the computer was connected to a double-beam spectrophotometer in order to record zero-order spectra. All calculations were made using Matlab 6.5 and Microsoft Excel. ANOVA *F*-test was performed by SPSS.

2.2. Chemicals and Materials. Pesticide standards carboxin (99% purity), chlorpyrifos (99% purity), and tebuconazole (99.9% purity) were provided by Santa Cruz Biotechnology, USA. HPLC-grade solvents including acetonitrile and methanol were provided by Merck (Darmstadt, Germany). Primary-secondary amine (40 μ m) (PSA) was supplied by Sigma-Aldrich. Sodium chloride and anhydrous magnesium sulfate were provided by BDH (VWR Chemicals BDH, England); MgSO₄ was heated for activation at 200°C for 4 h and then cooled and stored in a desiccator prior to use.

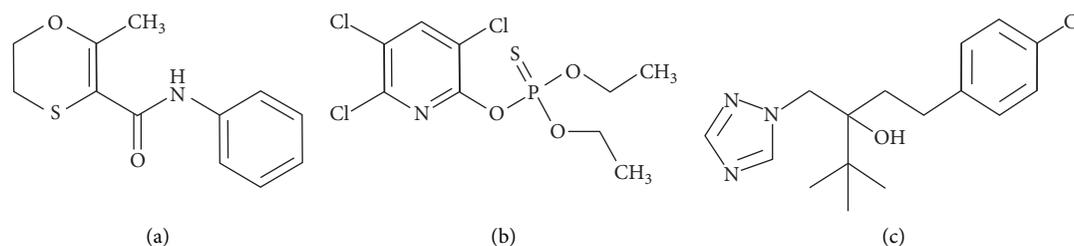


FIGURE 1: The structure of (a) carboxin, (b) chlorpyrifos, and (c) tebuconazole.

2.3. Standard Stock Solution Preparation. Stock solutions of carboxin, chlorpyrifos, and tebuconazole pesticide (100 $\mu\text{g}/\text{mL}$) were prepared through dissolving 10 mg of each pesticide in 100 mL methanol and then put in a freezer at -18°C in stained glass-stopper bottles. All stock solutions were stored for less than two months. Working standard solution is prepared daily through diluting the stock solutions in methanol.

2.4. Field Experiment. The field experiment was performed from 20 October 2017 to 10 April 2018 at the Grdarash field which belongs to the College of Agriculture, Salahaddin University, Erbil. For this purpose, cabbage seeds were mixed with carboxin (Vitavax) fungicide in the recommended dose of 3 g/kg seeds and then planted in pots with 5 cm diameter containing sterile soil in greenhouse for 8 weeks and later transplanted to outdoor field. After the plant reaches a certain stage of growth, chlorpyrifos (insecticide) in the recommended dose of 6 mL/5 L water and tebuconazole (fungicide) in the recommended dose of 1.5 mL/5 L water were sprayed foliarly under the supervision of pesticide expert at regular intervals, taken safety period into consideration. The cabbage sampling was conducted on 10 April 2018. The samples were put in sterile plastic bags and transported directly to the laboratory then homogenized and kept in the refrigerator at 4°C for further analysis.

2.5. Sample Preparation. QuEChERS method described by [27] was followed for the extraction of carboxin, chlorpyrifos, and tebuconazole residues from cabbage samples. About 500 g of chopped sample was balanced and homogenized, then weighed 10 g of the prior chopped fresh sample and transferred into a 50 mL Teflon centrifuge tube. Then 10 mL of acetonitrile as extraction solvent was added by using a transfer pipette, and the mixture was shaken for 1 min in an air bath at 22°C to confirm the solvent and entire samples interacted well, and then 4 g of anhydrous MgSO_4 and 1 g sodium chloride were added and vortexed for 1 min instantly; later, centrifugation of the extracts was carried out for 5 min at 5000 rpm; 10 mL aliquot of the higher layer was transported into a 15 mL Teflon centrifuge tube that contains 300 mg PSA and 1.5 g anhydrous MgSO_4 and then vortexed for 1 min and centrifugation at 7000 rpm for 5 min was performed. After that, filtration of the resulting solution was done by using a filter (0.45 μm). Then, the filtrate was transported into a 15 mL tube and cautiously under a stream

of nitrogen concentrated to near dryness. Eventually, 5 mL methanol used to redissolve the residues.

3. Application of Methods

3.1. Double Divisor-Ratio Spectra Derivative Method. For CAR to be determined, the stored spectra which contain a different concentrations of CAR in a ternary mixture were divided by the standard spectrum of a double divisor CHL and TEB (10 $\mu\text{g}/\text{mL}$ each in methanol) to obtain the ratio spectra. Later, calculation of the first derivative of the ratio spectra using $\Delta\lambda = 6 \text{ nm}$ was performed. The amount of CAR was determined by measuring the amplitude at 242 nm corresponding to a maximum in the first derivative of the ratio spectra in the spectral region selected 220–290 nm and relies merely on the concentration of CAR but not depending on the concentration of CHL and TEB in a ternary mixture.

Similarly, for determination of the CHL, the stored spectra which contain various concentrations of CHL in a ternary mixture were divided by the standard spectrum of a double divisor CAR and TEB (5 $\mu\text{g}/\text{mL}$ each in methanol) to obtain the ratio spectra. Next, calculation of the first derivative of the ratio spectra was performed at $\Delta\lambda = 4 \text{ nm}$. The wavelengths at 236 nm, 276 nm, and 300 nm were selected for the estimation of the amount of CHL in the first derivative of the ratio spectra in the spectral region selected 200–320 nm and rely on the concentration of CHL but not depending on the concentration of CAR and TEB in the ternary mixture.

The same idea was applied to determine the TEB; the stored spectra which contain various concentrations of TEB in a ternary mixture were divided by the standard spectrum of a double divisor CAR and CHL (5 $\mu\text{g}/\text{mL}$ each in methanol) to obtain the ratio spectra. After that, calculation of the first derivative of the ratio spectra was performed at $\Delta\lambda = 2 \text{ nm}$. The amount of TEB was determined by measuring the amplitude at 226 nm corresponding to a minimum in the first derivative of the ratio spectra in the spectral region selected 200–250 nm and only dependent on the concentration of TEB but not depending on the concentration of CAR and CHL in the ternary mixture.

3.2. Successive Derivative Ratio Spectra Method. For CAR to be determined, the first derivative of the ratio spectra was attained through division of the recorded absorption spectra of the ternary mixture which contains different

concentrations of CAR in the range of 200–350 nm by the standard spectrum of 15 $\mu\text{g}/\text{mL}$ of CHL by using $\Delta\lambda = 2$ nm. Next, the second ratio spectra were obtained through division of these vectors (the first derivative of the ratio spectra) by the first derivative of the ratio spectra (TEB/CHL) 15 $\mu\text{g}/\text{mL}$ for each of TEB and CHL. Then the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2$ nm.

Likewise, to determine the concentration of CHL, the first derivative of the ratio spectra was attained through division of the recorded absorption spectra of the ternary mixture which contains different concentrations of CHL in the range of 200–350 nm by the standard spectrum of 15 $\mu\text{g}/\text{mL}$ CAR by using $\Delta\lambda = 2$ nm. Later, the second ratio spectra were obtained through division of these vectors (the first derivative of the ratio spectra) by the first derivative of the ratio spectra (TEB/CAR) 15 $\mu\text{g}/\text{mL}$ for each of TEB and CAR. Then the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2$ nm.

In the same way to determine the concentration of TEB, the first derivative of the ratio spectra was attained through division of the recorded absorption spectra of the ternary mixture which contains different concentrations of TEB in the range of 200–350 nm by the standard spectrum of 15 $\mu\text{g}/\text{mL}$ CHL by using $\Delta\lambda = 2$ nm. Next, the second ratio spectra were obtained through division these vectors (the first derivative of the ratio spectra) by the first derivative of the ratio spectra (CAR/CHL) 15 $\mu\text{g}/\text{mL}$ for each of CAR and CHL. Then the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2$ nm. The calibration graphs were gained by plotting the amplitudes at 256 nm and 258 nm for CAR, at 290 nm and 292 nm for CHL, and at 226 nm and 228 nm for TEB versus the corresponding concentration of each pesticide.

3.3. Mean Centering of Ratio Spectra Method. For the CAR to be determined, the stored spectra of the ternary mixture which contains different concentrations of CAR were divided by the absorption spectrum of TEB (10 $\mu\text{g}/\text{mL}$), and the obtained first ratio spectra were mean-centered. Next, the second ratio spectra could be attained by dividing these vectors to mean centering of 15 $\mu\text{g}/\text{mL}$ CHL/10 $\mu\text{g}/\text{mL}$ TEB and then by mean-centering.

Also for the determination of CHL, the stored spectra of the ternary mixture which contains different concentrations of CHL were divided by the absorption spectrum of TEB (10 $\mu\text{g}/\text{mL}$), and the obtained first ratio spectra were mean-centered. After that, the second ratio spectra could be attained by dividing these vectors to mean centering of 10 $\mu\text{g}/\text{mL}$ CAR/10 $\mu\text{g}/\text{mL}$ TEB and then by mean-centering.

A similar idea was used for TEB determination, the stored spectra of the ternary mixture which contains different concentrations of TEB were divided by the absorption spectrum of CAR (10 $\mu\text{g}/\text{mL}$), the obtained first ratio spectra were mean centered. After that, the second ratio spectra could be attained by dividing these vectors to mean centering of 15 $\mu\text{g}/\text{mL}$ CHL/10 $\mu\text{g}/\text{mL}$ CAR and then by mean-centering.

To create the calibration graph for CAR, CHL, and TEB, mean-centered value was plotted at 306 nm, 280 nm, and 240 nm for three pesticides, respectively, against the corresponding concentration.

4. Results and Discussion

CAR, CHL, and TEB are three pesticides, and their normal UV absorption spectra are completely overlapped in the wavelength range between 200 and 350 nm (Figure 2). As a result, determination of three pesticides in ternary mixture simultaneously is impossible by classical spectrophotometry to resolve a mixture. Therefore, three different techniques of derivative spectrophotometry have been used to reduce interference and resolve the overlapped spectra.

4.1. Double Divisor-Ratio Spectra Derivative Method. In this method, for estimation of the active constituents, the major instrumental parameter conditions required optimization is as follows.

4.1.1. Selection of the Working Wavelength. The selection of the working wavelength in the applied method depends on the first derivative of the ratio spectra of pure compound and its ternary mixture would be coincided in the spectral region corresponding to a maximum point or a minimum point of the wavelength. These coinciding points of the derivative of the ratio spectra can be chosen as working wavelengths for the estimations of the compounds in the ternary mixture. For CAR, the coincident point in the spectral region 220–290 nm corresponding to a maximum (242 nm) was selected as a working wavelength for the determination of CAR in its ternary mixtures (Figure 3). But for CHL we observed that three coincident points in the spectral region 200–320 nm corresponding to a minimum (236 nm), a maximum (276 nm), and a minimum (300 nm) wavelength were suitable for the determination of CHL in its ternary mixtures (Figure 4). Also for TEB the coincident point in the spectral region 200–250 nm corresponding to a minimum (226 nm) wavelength was selected as a working wavelength for the determination of TEB in its ternary mixtures (Figure 5).

4.1.2. Effect of Double Divisor Concentrations. The double divisor was found either by the sum of the absorption spectra of the same concentration of the two compounds in the same ternary mixture or it was gained by preparing the mixed solution of two compounds of the same concentration in the ternary mixture. For this purpose, different concentrations of double divisor were studied. As a result, 10 $\mu\text{g}/\text{mL}$ for each of CHL and TEB as a double divisor for determining CAR, 5 $\mu\text{g}/\text{mL}$ for each of CAR and TEB for determining CHL, and 5 $\mu\text{g}/\text{mL}$ for each of CAR and CHL for determining TEB were found to be proper for the determination of three pesticides.

4.1.3. Effect of the Value of $\Delta\lambda$. For the first derivative of the ratio spectra, $\Delta\lambda$ influence was studied. It was noticed that

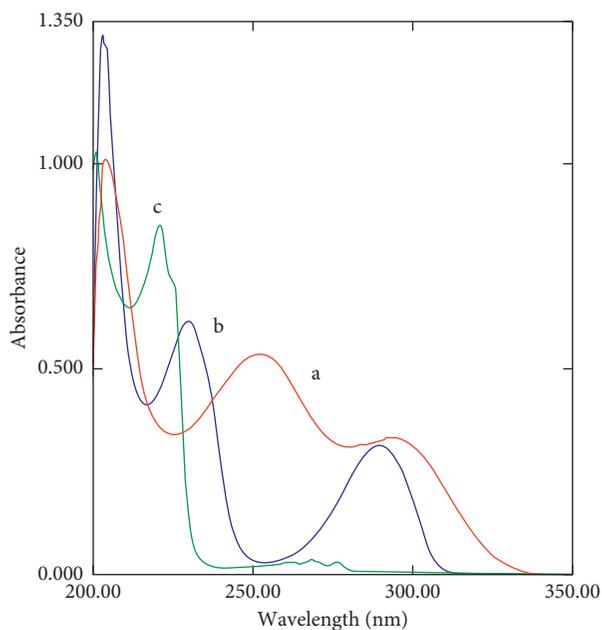


FIGURE 2: Zero-order spectra of (a) 10 $\mu\text{g/mL}$ carboxin (CAR), (b) 20 $\mu\text{g/mL}$ chlorpyrifos (CHL), and (c) 20 $\mu\text{g/mL}$ tebuconazole (TEB) against methanol as a blank.

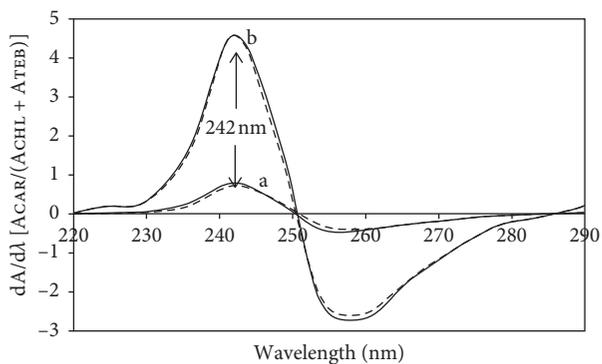


FIGURE 3: The coincident spectra of the first derivative of the ratio spectra of (a) 5 $\mu\text{g/mL}$ pure CAR (—) and 5 $\mu\text{g/mL}$ CAR in a ternary mixture (-----), and (b) 30 $\mu\text{g/mL}$ pure CAR (—) and 30 $\mu\text{g/mL}$ CAR in a ternary mixture (-----) in methanol using 10 $\mu\text{g/mL}$ CHL + 10 $\mu\text{g/mL}$ TEB as a double divisor at $\Delta\lambda = 6$.

the most relevant value of $\Delta\lambda$ which gives optimum recovery is at $\Delta\lambda = 6, 4,$ and 2 nm for the determination of CAR, CHL, and TEB, respectively.

The stored spectra of the ternary mixture which contains different concentrations of CAR in methanol in the range of 220–290 nm were divided by the standard spectrum of the double divisor (CHL and TEB, 10 $\mu\text{g/mL}$ for each in methanol) to obtain the ratio spectra (Figure 6(a)). Next the first derivatives of the ratio spectra were plotted with $\Delta\lambda = 6$ nm as displayed in Figure (6(b)). The concentration of CAR was estimated through measuring the signal at 242 nm corresponding to the maximum wavelength.

In a similar way, the stored spectra of the ternary mixture which contains different concentrations of CHL in methanol

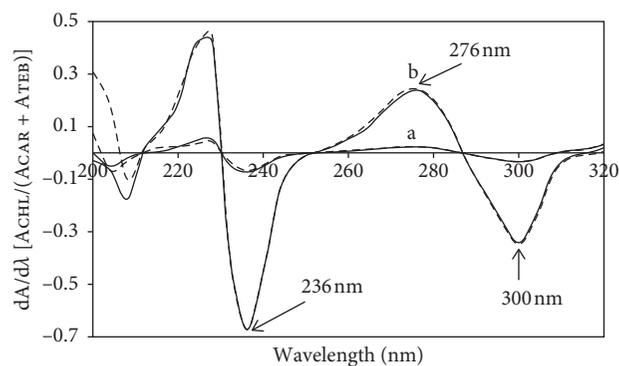


FIGURE 4: The coincident spectra of the first derivative of the ratio spectra of (a) 5 $\mu\text{g/mL}$ pure CHL (—) and 5 $\mu\text{g/mL}$ CHL in a ternary mixture (-----), and (b) 50 $\mu\text{g/mL}$ pure CHL (—) and 50 $\mu\text{g/mL}$ CHL in a ternary mixture (-----) in methanol using 5 $\mu\text{g/mL}$ CAR + 5 $\mu\text{g/mL}$ TEB as a double divisor at $\Delta\lambda = 4$.

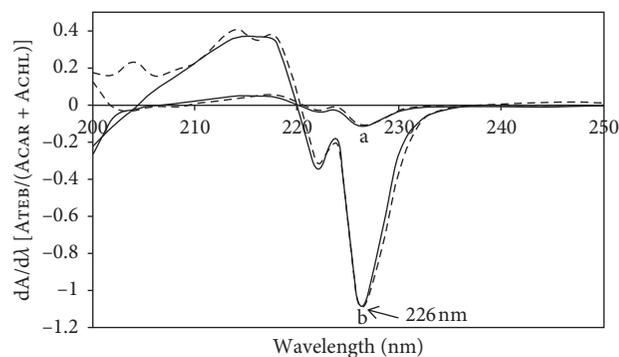


FIGURE 5: The coincident spectra of the first derivative of the ratio spectra of (a) 5 $\mu\text{g/mL}$ pure TEB (—) and 5 $\mu\text{g/mL}$ TEB in a ternary mixture (-----), and (b) 45 $\mu\text{g/mL}$ pure TEB (—) and 45 $\mu\text{g/mL}$ TEB in a ternary mixture (-----) in methanol using 5 $\mu\text{g/mL}$ CAR + 5 $\mu\text{g/mL}$ CHL as a double divisor at $\Delta\lambda = 2$.

in the range of 200–320 nm were divided by the standard spectrum of the double divisor (CAR and TEB, 5 $\mu\text{g/mL}$ for each in methanol) to obtain the ratio spectra (Figure 7(a)). After that, the first derivatives of the ratio spectra were obtained with interval $\Delta\lambda = 4$ nm. The CHL concentration was estimated through measuring the signal at 236 nm, 276 nm, and 300 nm as demonstrated in Figure 7(b).

The stored spectra of the ternary mixture which contains different concentrations of TEB in methanol in the range of 200–250 nm were divided by the standard spectrum of the double divisor (CAR and CHL, 5 $\mu\text{g/mL}$ for each in methanol) to obtain the ratio spectra (Figure 8(a)). Next the first derivatives of the ratio spectra were obtained using $\Delta\lambda = 2$ nm. The TEB concentration was estimated through measuring the signal at 226 nm corresponding to the minimum wavelength as shown in Figure (8(b)).

4.2. Successive Derivative Ratio Spectra Method. The stored spectra of the ternary mixture which contains different concentrations of CAR in the range between 200 and 350 nm were divided by a standard spectrum of 15 $\mu\text{g/mL}$ of CHL,

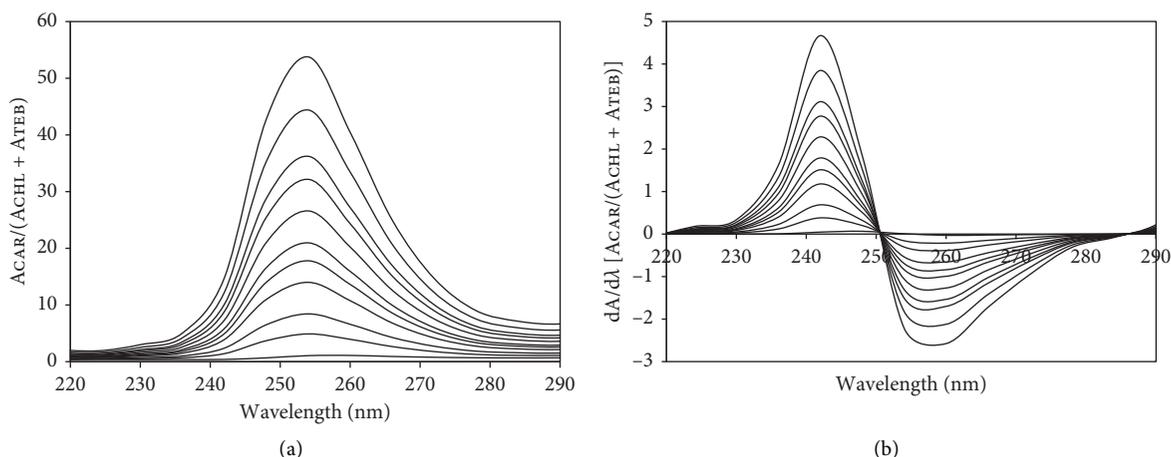


FIGURE 6: (a) Ratio spectra and (b) first derivative of the ratio spectra of CAR (1, 3, 5, 8, 10, 12, 15, 18, 20, 25, and 30 $\mu\text{g/mL}$) using 10 $\mu\text{g/mL}$ CHL + 10 $\mu\text{g/mL}$ TEB as a double divisor in methanol at $\Delta\lambda = 6$.

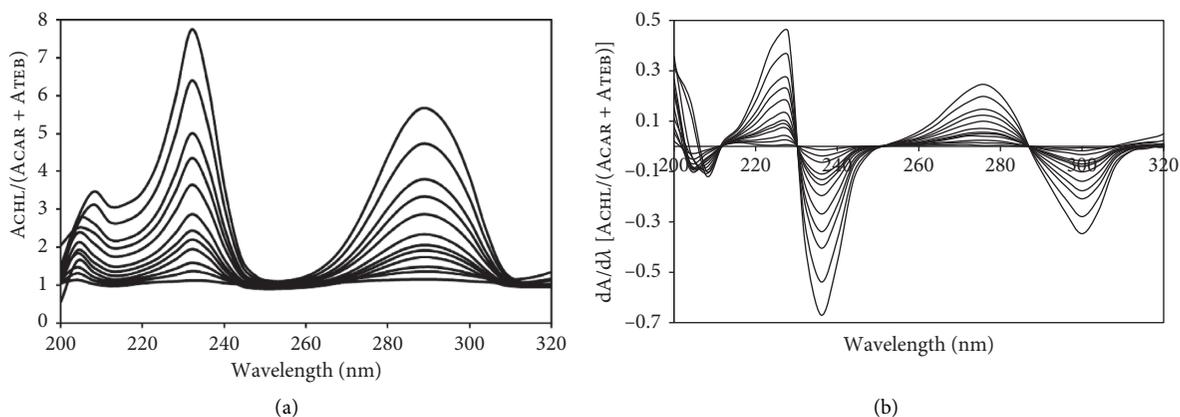


FIGURE 7: (a) Ratio spectra and (b) first derivative of the ratio spectra of CHL (1, 3, 5, 8, 10, 12, 15, 20, 25, 30, 40, and 50 $\mu\text{g/mL}$) using 5 $\mu\text{g/mL}$ CAR + 5 $\mu\text{g/mL}$ TEB as a double divisor in methanol at $\Delta\lambda = 4$.

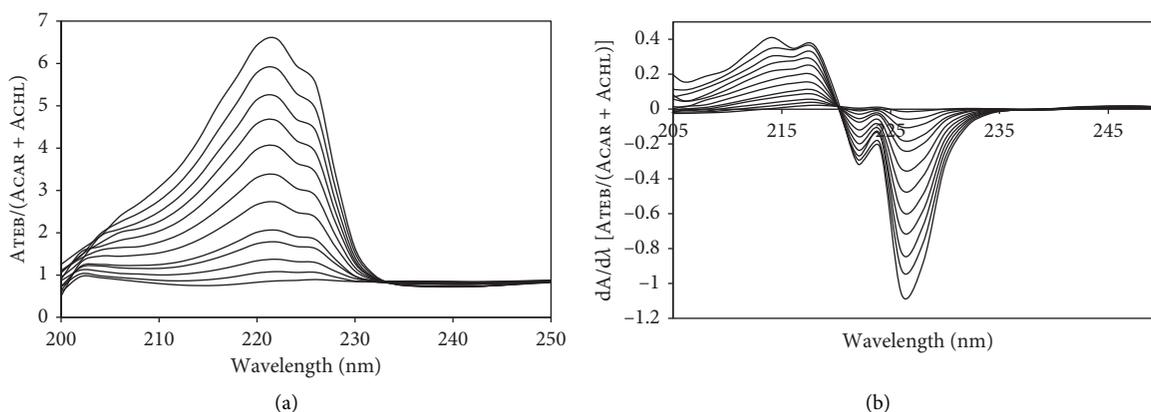


FIGURE 8: (a) Ratio spectra and (b) first derivative of the ratio spectra of TEB (1, 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, and 45 $\mu\text{g/mL}$) using 5 $\mu\text{g/mL}$ CAR + 5 $\mu\text{g/mL}$ CHL as a double divisor in methanol at $\Delta\lambda = 2$.

and the ratio spectra were obtained. Then the first derivative of these ratio spectra was attained by using $\Delta\lambda = 2$ nm. Next, the second ratio spectra were obtained through dividing these vectors (the first derivative of the ratio spectra) by the

first derivative of the ratio spectra (TEB/CHL) 15 $\mu\text{g/mL}$ for each of TEB and CHL. Then the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2$ nm (Figure 9). The CAR concentration was estimated through measuring the signal

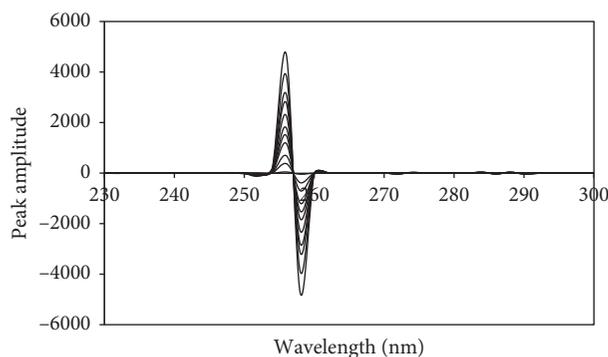


FIGURE 9: Successive derivative ratio spectra of 1, 3, 5, 8, 10, 12, 15, 18, 20, 25, and 30 $\mu\text{g/mL}$ of carboxin in methanol at $\Delta\lambda = 2\text{ nm}$.

at 256 nm and 258 nm of CAR in the presence of CHL and TEB.

In a similar way, the stored spectra of the ternary mixture which contains different concentrations of CHL in the range between 200 and 350 nm were divided by a standard spectrum of 15 $\mu\text{g/mL}$ of CAR, and the ratio spectra were obtained. Then the first derivative of these ratio spectra was attained by using $\Delta\lambda = 2\text{ nm}$. Later, the second ratio spectra were obtained through dividing these vectors (the first derivative of the ratio spectra) by the first derivative of the ratio spectra (TEB/CAR) 15 $\mu\text{g/mL}$ for each of TEB and CAR. Then, the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2\text{ nm}$ (Figure 10). The CHL concentration was estimated through measuring the signal at 290 nm and 292 nm of CHL in the presence of CAR and TEB.

The stored spectra of the ternary mixture which contains different concentrations of TEB in the range between 200 and 350 nm were divided by the standard spectrum of 15 $\mu\text{g/mL}$ of CHL, and the ratio spectra were obtained. Then the first derivative of the ratio spectra was attained by using $\Delta\lambda = 2\text{ nm}$. Next, the second ratio spectra were obtained through dividing these vectors (the first derivative of the ratio spectra) by the first derivative of the ratio spectra (CAR/CHL) 15 $\mu\text{g/mL}$ for each of CAR and CHL. Then the first derivative of the second ratio spectra was attained at $\Delta\lambda = 2\text{ nm}$ (Figure 11). The TEB concentration was estimated by measuring the signal at 226 nm and 228 nm of TEB in the presence of CAR and CHL.

The most important factor needed for optimization of reliable determination of the three pesticides in the ternary mixture is as follows.

4.2.1. Effect of Divisor Concentration. The influence of divisor concentration on the selectivity of the method and analytical parameters such as slope, intercept, detection limit, and correlation coefficient of the calibration equation was studied. For this purpose, various concentrations of divisors have been examined (1, 3, 5, 10, 15, 20, and 30 $\mu\text{g/mL}$) for each of TEB and CHL for carboxin determination, CAR and TEB for chlorpyrifos determination, and CAR and CHL for tebuconazole determination. Markedly altering the divisors concentration had a considerable impact on the

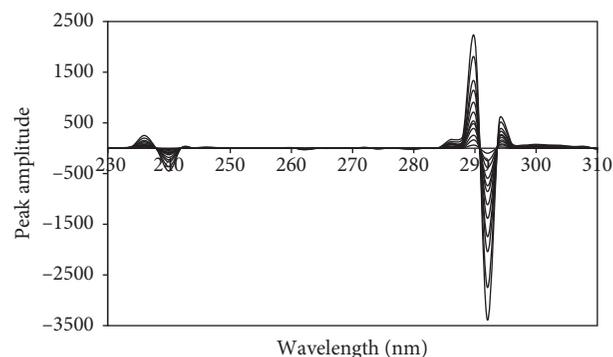


FIGURE 10: Successive derivative ratio spectra of 1, 3, 5, 8, 10, 12, 15, 20, 25, 30, 40, and 50 $\mu\text{g/mL}$ of chlorpyrifos in methanol at $\Delta\lambda = 2\text{ nm}$.

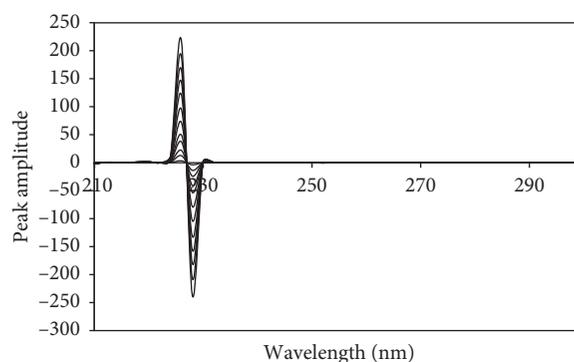


FIGURE 11: Successive derivative ratio spectra of 1, 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, and 45 $\mu\text{g/mL}$ of tebuconazole in methanol at $\Delta\lambda = 2\text{ nm}$.

method selectivity. Hence, 15 $\mu\text{g/mL}$ of each of CAR, CHL, and TEB was used as divisors.

4.2.2. Selection of the Working Wavelength. The minimum or maximum of the successive derivative ratio spectra with respect to wavelength was used for the construction of calibration graph.

Another parameter that should be optimized is the amount of $\Delta\lambda$ and its impact on the derivation of ratio spectra. For this purpose, different $\Delta\lambda$ was studied, and it was noticed that the value of $\Delta\lambda$ has no significant effect on the ratio spectra derivative method; therefore, $\Delta\lambda = 2\text{ nm}$ was used.

4.3. Mean Centering of Ratio Spectra Method. The stored spectra of the ternary mixture which contains different concentrations of carboxin were divided by the standard spectrum (10 $\mu\text{g/mL}$) of TEB, and the first ratio spectra were attained and then mean-centered. Mean centering of the ratio spectra was gained in the range between 220 and 320 nm. After that, the second ratio spectra were attained through dividing these vectors to mean centered of (15 $\mu\text{g/mL}$ CHL/10 $\mu\text{g/mL}$ TEB) and then mean-centered as clarified in Figure (12). The concentration of CAR was

estimated through measuring the signal at 306 nm corresponding to the maximum point.

In the similar way, the stored spectra of the ternary mixture which contains various concentrations of chlorpyrifos were divided by the standard spectrum (10 $\mu\text{g}/\text{mL}$) of TEB, and the first ratio spectra were attained then and mean-centered. Mean centering of the ratio spectra was gained in the range between 250 and 320 nm. Later, the second ratio spectra were attained through dividing these vectors to mean centering of 10 $\mu\text{g}/\text{mL}$ CAR/10 $\mu\text{g}/\text{mL}$ TEB and then mean-centered as shown in Figure (13). The concentration of CHL was estimated through measuring the signal at 280 nm corresponding to the maximum point.

On the other hand, the stored spectra of the ternary mixture which contains various concentrations of tebuconazole were divided by the standard spectrum (10 $\mu\text{g}/\text{mL}$) of CAR, and the first ratio spectra were attained and then mean-centered. Mean centering of the ratio spectra was gained in the range of 220–320 nm. Next, the second ratio spectra were attained through dividing these vectors to mean centering of 15 $\mu\text{g}/\text{mL}$ CHL/10 $\mu\text{g}/\text{mL}$ CAR and then mean-centered as shown in Figure (14). The concentration of TEB was estimated by measuring the signal at 240 nm corresponding to the maximum point.

The main parameter that should be studied for successful application of the mean centering of ratio spectra method could be summarized as a follows.

4.3.1. Effect of Divisor Concentration. The study of divisor concentration that has a considerable impact on the method selectivity and analytical parameters such as slope, intercept, detection limit, and correlation coefficient of the calibration equation was tested. To achieve that, various concentrations for each of CAR, CHL, and TEB (3, 5, 10, 15, 20, 25, and 30 $\mu\text{g}/\text{mL}$) were examined. The best results were obtained upon using 10 $\mu\text{g}/\text{mL}$ of TEB and 15 $\mu\text{g}/\text{mL}$ of CHL for estimation of CAR and 10 $\mu\text{g}/\text{mL}$ each of CAR and TEB for quantification of CHL. For determination of TEB, 10 $\mu\text{g}/\text{mL}$ of CAR and 15 $\mu\text{g}/\text{mL}$ of CHL were found to be the best divisor.

4.3.2. Selection of the Working Wavelength. To estimate the wavelength, zero-crossing point technique can be used where the interference of the other two components was eliminated, although the maximum and minimum of peaks could be used as a working wavelength. Furthermore, in this method, the signal-to-noise ratio was enhanced through removing the derivative steps.

5. Validation of Proposed Methods

5.1. Calibration Graph and Statistical Data. The statistical data of different calibration graphs were obtained by three different spectrophotometric methods, including double divisor-ratio spectra derivative (DDRSD), successive derivative of ratio spectra (SDRS), and mean centering of ratio spectra (MCRS) methods. The linearity range of the three proposed methods over the concentration ranges was 1–30 $\mu\text{g}/\text{mL}$ for CAR, 1–50 $\mu\text{g}/\text{mL}$ for CHL, and 1–45 $\mu\text{g}/\text{mL}$ for TEB in the ternary mixture which was assessed by analyzing various mixtures of CAR, CHL, and TEB. However, the high value of

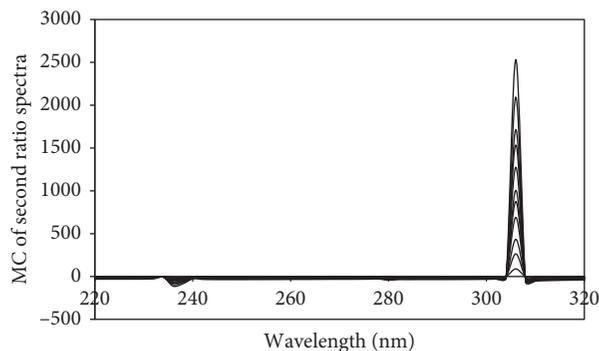


FIGURE 12: Mean centering of the second ratio spectra of 1, 3, 5, 8, 10, 12, 15, 18, 20, 25, and 30 $\mu\text{g}/\text{mL}$ of carboxin in methanol as a blank.

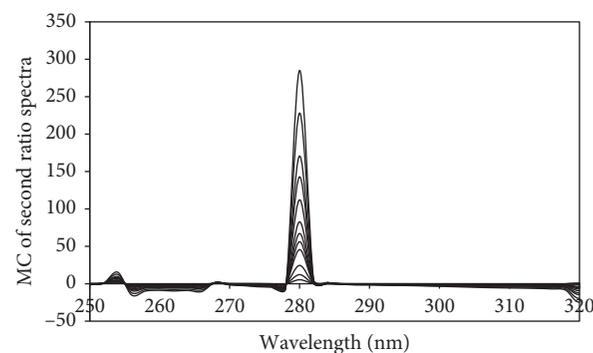


FIGURE 13: Mean centering of the second ratio spectra of 1, 3, 5, 8, 10, 12, 15, 20, 25, 30, 40, and 50 $\mu\text{g}/\text{mL}$ of chlorpyrifos in methanol as a blank.

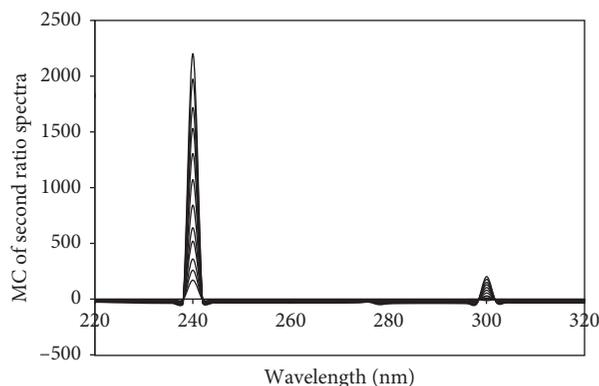


FIGURE 14: Mean centering of the second ratio spectra of 1, 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, and 45 $\mu\text{g}/\text{mL}$ of tebuconazole in methanol as a blank.

the regression coefficients which is larger than 0.999 indicates that the calibration graphs had a good linearity. The limit of detection and limit of quantification of the proposed methods were found and calculated according to the equation mentioned in [53] and the main results are shown in Table 1. The accuracy and precision of the proposed methods were evaluated at three different concentrations of each pesticide in the ternary

TABLE 1: Calibration data for the determination of carboxin, chlorpyrifos, and tebuconazole by the proposed methods.

Methods	Compounds	λ_{\max} (nm)	Linear range ($\mu\text{g/mL}$)	Regression equation	r^2	Sa	Sb	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
DDRSD	CAR	242	1–30	$Y = 0.159x - 0.0991$	0.9999	0.1021	0.000638	0.178	0.541
	CHL	236	1–50	$Y = -0.0135X + 0.0026$	0.9998	0.0015	0.000066	0.160	0.485
	CHL	276	1–50	$Y = 0.005X - 0.0012$	0.9996	0.0007	0.000030	0.137	0.416
	CHL	300	1–50	$Y = -0.007X + 0.0016$	0.9997	0.0009	0.000038	0.227	0.688
	TEB	226	1–45	$Y = -0.0237x + 0.0122$	0.9997	0.0030	0.000123	0.109	0.332
SDRS	CAR	256	1–30	$Y = 159.9X - 110.38$	0.9999	9.9812	0.623717	0.153	0.463
	CAR	258	1–30	$Y = -161.84x + 112.3$	0.9999	10.073	0.629442	0.206	0.626
	CHL	290	1–50	$Y = 42.148X + 28.653$	0.9996	6.1303	0.261535	0.228	0.691
	CHL	292	1–50	$Y = -66.768X - 54.876$	0.9995	10.632	0.453597	0.272	0.824
	TEB	226	1–45	$Y = 4.8322X - 0.9684$	0.9996	0.7400	0.030216	0.127	0.385
	TEB	228	1–45	$Y = -5.2282X + 1.2232$	0.9996	0.7585	0.030967	0.121	0.367
MCRS	CAR	306	1–30	$Y = 83.991x + 15.029$	0.9997	7.8579	0.491032	0.260	0.790
	CHL	280	1–50	$Y = 5.7606x - 2.4007$	0.9997	0.7027	0.029981	0.159	0.483
	TEB	240	1–45	$Y = 45.877x + 142.7$	0.9992	11.134	0.437175	0.205	0.621

Sa = standard error of the intercept, Sb = standard error of the slope, DDRSD: double divisor-ratio spectra derivative, SDRS: successive derivative of ratio spectra, and MCRS: mean centering of ratio spectra.

TABLE 2: Accuracy and precision for simultaneous determination of carboxin, chlorpyrifos, and tebuconazole in the ternary mixture by the proposed methods.

Compound	Methods of analysis	Concentration ($\mu\text{g/mL}$)	% recovery	% RSD
Carboxin	Double divisor-ratio spectra derivative at 242 nm	1.0	95.03	4.30
		15.0	98.49	1.57
		30.0	97.50	0.89
	Successive derivative ratio spectra at 256 nm	1.0	96.53	3.95
		15.0	99.14	1.84
		30.0	100.42	0.62
	Successive derivative ratio spectra at 258 nm	1.0	96.70	4.63
		15.0	99.55	0.44
		30.0	100.81	1.07
	Mean centering of ratio spectra at 306 nm	1.0	95.72	3.55
		15.0	100.37	1.30
		30.0	99.56	1.54
Chlorpyrifos	Double divisor-ratio spectra derivative at 236 nm	1.0	104.44	4.34
		30.0	100.88	0.95
		50.0	99.67	1.61
	Double divisor-ratio spectra derivative at 276 nm	1.0	95.2	4.26
		30.0	98.27	2.23
		50.0	97.04	1.33
	Double divisor-ratio spectra derivative at 300 nm	1.0	96.0	4.45
		30.0	102.28	1.21
		50.0	99.94	0.55
	Successive derivative ratio spectra at 290 nm	1.0	102.84	4.05
		30.0	99.28	0.85
		50.0	101.61	1.78
Successive derivative ratio spectra at 292 nm	1.0	104.13	4.43	
	30.0	101.20	2.38	
	50.0	100.47	0.89	
Mean centering of ratio spectra at 280 nm	1.0	104.16	4.81	
	30.0	102.83	2.32	
	50.0	99.23	1.68	

TABLE 2: Continued.

Compound	Methods of analysis	Concentration ($\mu\text{g/mL}$)	% recovery	% RSD
Tebuconazole	Double divisor-ratio spectra derivative at 226 nm	1.0	104.89	4.69
		20.0	99.2	1.82
		45.0	98.19	1.02
	Successive derivative ratio spectra at 226 nm	1.0	95.78	4.57
		20.0	97.02	1.59
		45.0	101.16	1.64
	Successive derivative ratio spectra at 228 nm	1.0	95.31	4.45
		20.0	101.01	1.74
		45.0	99.21	0.71
	Mean centering of ratio spectra at 240 nm	1.0	95.60	4.32
		20.0	102.58	0.80
		45.0	99.55	1.48

TABLE 3: Percentage mean recovery of carboxin, chlorpyrifos, and tebuconazole in real cabbage samples by the proposed methods. (a)

Carboxin fortification level ($\mu\text{g/mL}$)	DDRS		SDRS				MCRS	
	242 nm		256 nm		258 nm		306 nm	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
1.0	90.70	5.28	87.02	5.90	87.22	5.01	88.76	5.91
15.0	90.95	2.47	90.98	5.34	89.79	3.33	91.78	2.18
30.0	94.53	4.01	88.41	4.68	88.42	4.61	92.45	2.66

(b)

Chlorpyrifos fortification level ($\mu\text{g/mL}$)	DDRS				SDRS				MCRS			
	236 nm		276 nm		300 nm		290 nm		292 nm		280 nm	
	%R	%RSD										
1.0	99.9	5.99	96.89	4.77	103.89	5.54	108.53	4.52	107.36	5.98	103.82	3.14
30.0	98.19	4.45	95.35	3.78	96.95	3.34	96.29	2.29	96.19	4.94	97.26	2.67
50.0	93.99	2.20	95.42	2.57	97.02	2.03	92.48	1.44	92.32	2.11	95.07	3.05

(c)

Tebuconazole fortification level ($\mu\text{g/mL}$)	DDRS		SDRS				MCRS	
	226 nm		226 nm		228 nm		240 nm	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
1.0	88.95	5.53	87.19	4.11	87.79	4.93	91.10	2.36
20.0	93.59	3.32	93.74	4.03	93.79	2.67	95.46	2.15
45.0	92.22	3.46	98.00	4.50	96.33	4.84	96.95	1.57

mixture (five replicate measurements), and then the percentage recovery and percentage relative standard deviations were calculated at all three concentration levels. The results rely on the value of the %R and %RSD, the percentage recovery was found to be in the range of 95.03% to 104.89%, and the good percentage recovery indicates a good accuracy, and the main results are shown in Table 2. It is clear that the three methods can be applied to resolve the three pesticides in the ternary mixture with good accuracy and precision. All the three methods are quick, non-devastating, and cheap, and they do not need costly solvent and reagents; also poison and ozone-harming organic solvents and polluting reagents are not required [54].

5.2. Application of the Methods. In this study, three proposed methods were applied for the estimation of CAR, CHL, and TEB in real cabbage samples. The real fresh cabbage samples spiked at three different concentration levels (1, 15, and 30 $\mu\text{g/}$

mL) for carboxin, (1, 30, and 50 $\mu\text{g/mL}$) for chlorpyrifos, and (1, 20, and 45 $\mu\text{g/mL}$) for tebuconazole with five replications per level. The mean percentage recoveries of three pesticides in real cabbage samples are shown in Table 3. In all circumstances, the mean percentage recoveries were greater than 87.02%, 92.32%, and 87.19% with a relative standard deviation less than 5.91%, 5.99%, and 5.53% for carboxin, chlorpyrifos, and tebuconazole, respectively, and the good recoveries suggested that the proposed methods have a good accuracy. One-way ANOVA test was used to compare the results between the three proposed methods statistically. For this purpose, Snedecor's F -values were computed and compared with the standard tabulated value ($p = 0.05$). The similar computation processes were repeated for each pesticide. Table 4 shows that the calculated or experimental F -values are less than the tabulated values F -values in the analysis of variance, indicating that there was no significant difference between the three methods. To keep food safe and secure for consumers health, maximum residue limit

TABLE 4: One-way ANOVA statistical analysis results of carboxin, chlorpyrifos, and tebuconazole in cabbage samples obtained by applying DDRSD, SDRS, and MCRC methods.

Compounds		Sum of squares	Degree of freedom	Mean squares	<i>F</i>	Sig
Carboxin	Between groups	12.078	2	6.039	1.008 (F _{theor.} = 3.68)	0.388
	Within groups	89.830	15	5.989		
	Total	101.908	17			
Chlorpyrifos	Between groups	2.978	2	1.489	0.202 (F _{theor.} = 3.68)	0.819
	Within groups	110.372	15	7.358		
	Total	113.350	17			
Tebuconazole	Between groups	12.685	2	6.343	1.021 (F _{theor.} = 3.68)	0.384
	Within groups	93.164	15	6.211		
	Total	105.849	17			

TABLE 5: Determination of the residues (mg/kg) of carboxin, chlorpyrifos, and tebuconazole in cabbage samples.

Compound	DDRSD	SDRS	MCRC
Carboxin	0.248	0.225	0.235
Chlorpyrifos	0.443	0.463	0.451
Tebuconazole	0.118	0.144	0.130

TABLE 6: Comparison of the published methods with the proposed methods for determination of carboxin, chlorpyrifos, and tebuconazole in real samples.

Analyte	Method	Linear range ($\mu\text{g}/\text{mL}$)	LOD ($\mu\text{g}/\text{mL}$)	LOQ ($\mu\text{g}/\text{mL}$)	%Recovery	%RSD	Application	Ref
CAR	GC	0.1–10	0.003–0.03	0.1	92–99	5–14	Fruits and vegetables	[5]
	DDRSD	1–30	0.178	0.541	90.70–94.53	2.47–5.28	Cabbage	This study
	SDRS	1–30	0.153–0.206	0.463–0.626	87.02–90.98	3.33–5.90	Cabbage	This study
	MCRC	1–30	0.260	0.790	88.76–92.45	2.18–5.91	Cabbage	This study
CHL	HPLC-DAD	15–60	0.5	1	Higher than 90	Less than 6	Mexican axolotl	[24]
	RSD	1–10	0.201	0.671	80–121	2.9–5.3	Water, soil	[13]
	GC-MS	0.5–50 ($\mu\text{g}/\text{kg}$)	0.01–0.2 ($\mu\text{g}/\text{kg}$)	0.05–1 ($\mu\text{g}/\text{kg}$)	80–105	0.1–13.37	Apple	[21]
	GC-MS/MS	2–2000 ($\mu\text{g}/\text{kg}$)	0.3 ($\mu\text{g}/\text{kg}$)	1 ($\mu\text{g}/\text{kg}$)	86.2–92.3	Less than 9.5	Duck muscle	[22]
	DDRSD	1–50	0.137–0.227	0.416–0.688	93.99–103.89	2.03–5.99	Cabbage	This study
	SDRS	1–50	0.228–0.272	0.691–0.824	92.32–108.53	1.44–5.98	Cabbage	This study
	MCRC	1–50	0.159	0.483	95.07–103.82	2.67–3.14	Cabbage	This study
TEB	GC-FID	1–1000	0.5	2	98.5–100.8	Less than 2	Formulation	[29]
	GC-MS	0.01–1	0.015	0.05	88.9–97.1	1.7–4.0	Gherkin fruits, soil	[30]
	GC-MS	0.01–1	0.003	0.01	88.2–105	1.52–7.65	Watermelon, soil	[31]
	DDRSD	1–45	0.109	0.332	88.95–93.59	3.32–5.53	Cabbage	This study
	SDRS	1–45	0.121–0.127	0.367–0.385	87.19–98.00	2.67–4.93	Cabbage	This study
	MCRC	1–45	0.205	0.621	91.10–96.95	1.57–2.36	Cabbage	This study

GC: gas chromatography. HPLC-DAD: high-performance liquid chromatography with diode array detection. RSD: ratio spectra derivative. GC-MS: gas chromatography-mass spectrometry. GC-MS/MS: gas chromatography tandem mass spectrometry. GC-FID: gas chromatography-flame ionization detector. DDRSD: double divisor-ratio spectra derivative. SDRS: successive derivative of ratio spectra. MCRC: mean centering of ratio spectra.

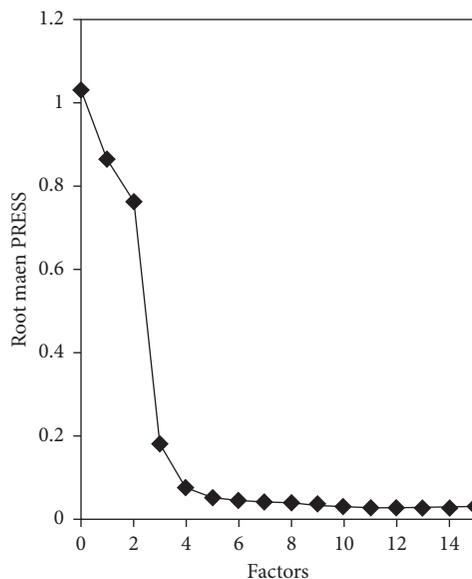


FIGURE 15: Plot of root mean PRESS against the number of factors for thirty-five CAR, CHL, and TEB mixtures for PLS-2 model.

(MRL) has been set by the European Union (EU) Food Safety Authority for these pesticides. The amount of carboxin, chlorpyrifos, and tebuconazole residues in cabbage samples was less than the EU-MRL (1 mg/kg) [55], so these three pesticides have no risk for consumer health. The three spectrophotometric methods were successfully applied with the aid of standard addition method for simultaneous determination of the residue of each CAR, CHL, and TEB in real cabbage samples, and the main results are summarized in Table 5.

6. Comparison of the Methods

A comparison has been made between the results obtained by the proposed methods for the determination of carboxin, chlorpyrifos, and tebuconazole in real cabbage samples in the standpoint of linearity, LOD, LOQ, percentage recovery, and percentage relative standard deviations with other reported methods (Table 6). The table illustrates that the proposed methods have a good linearity, % recovery, and %RSD, indicating that these methods could be successfully applied for determination of the residues of these three pesticides in cabbage samples. Furthermore, the derivative spectrophotometric results were compared to one of the multivariate techniques, namely, partial least squares regression (PLS-2). For this purpose, a calibration set for the ternary pesticides mixture CAR, CHL, and TEB was constructed using the collected thirty-five spectral data as independent variables and the concentrations of the pesticide in the mixture as dependent variables. The recorded spectra in the wavelength range of 200–350 nm with a sampling interval of 2 nm were arranged as row matrix. The matrix was organized into pairs, and each absorbance matrix is paired with its corresponding concentration matrix and was used as a training set to build up a PLS-2 model using Origin Pro 2018 SR1. By using the cross-validation technique for evaluating estimation or performance for calibration, the concentration of the sample left out was predicted. The process was repeated as the number of samples [56].

Figure (15) shows a plot of the root mean PRESS against the number of factors for PLS-2 model for the determination of pesticides in their mixture. The calibration model for PLS-2 was confirmed with the thirty-five mixtures of the training set. The predictive capability of PLS-2 was examined for simultaneous determination of CAR, CHL, and TEB in each mixture (Table 7). The statistical *t*-test for the difference between the two population means and *F*-test for the difference in the two variances were used to compare the result of the cited pesticides obtained by the suggested derivative techniques with those of thirty-five selected ternary-pesticide mixture samples analyzed via the established PLS-2 model in concentration ranges up to 30 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 45 $\mu\text{g/mL}$ for carboxin, chlorpyrifos, and tebuconazole, respectively (Table 8).

7. Conclusion

In this work, three sensitive and reliable spectrophotometric methods including (DDRS, SDRS, and MCRS) were used for the determination of three pesticide (carboxin, chlorpyrifos, and tebuconazole) residues simultaneously in a ternary mixture of cabbage samples without preliminary separation process. Standard addition technique can be easily used in the proposed methods and the matrix effects can be removed. Therefore, the three proposed methods can be applied successfully for resolution of the ternary mixture and do not require complicated or expensive instruments. In addition, it is rapid and low-cost method when compared with other methods such as chromatographic methods. Also, we used the QuEChERS method for extraction of real cabbage sample because it is very simple and inexpensive as well as it involves minimum steps with a very short time in comparisons with other extraction methods and provides good recoveries and precision. The methods developed showed satisfactory validation parameters in terms of linearity, LOD which is lower than 0.260, 0.272, and 0.205 $\mu\text{g/mL}$ for CAR, CHL, and TEB, respectively, and LOQ

TABLE 7: Composition of synthetic samples, their predictions by PLS-2 model, and statistical parameters for the system.

Mixture ($\mu\text{g/mL}$)	Prediction ($\mu\text{g/mL}$)					Recovery (%)		
	TEB	CAR	CHL	TEB	CAR	CHL	TEB	CAR
1.0	5.0	5.0	1.1538	4.8864	5.1665	115.384	97.7284	103.3292
3.0	5.0	5.0	2.9352	5.0309	4.8647	97.841	100.6188	97.2946
5.0	5.0	5.0	4.8985	5.0348	4.9533	97.9694	100.6962	99.0666
8.0	5.0	5.0	8.0142	5.0072	5.0041	100.1775	100.1446	100.0822
10.0	5.0	5.0	10.4588	5.0396	5.0815	104.5875	100.7914	101.6296
15.0	5.0	5.0	14.9976	5.0451	4.9865	99.9838	100.9014	99.7292
20.0	5.0	5.0	20.0810	5.0582	5.0307	100.4049	101.1638	100.6134
25.0	5.0	5.0	24.9038	5.0167	5.0803	99.61512	100.3348	101.0664
30.0	5.0	5.0	29.9612	4.8925	5.0327	99.87063333	97.8498	100.6548
35.0	5.0	5.0	34.9406	4.9169	5.0155	99.83025714	98.3370	100.3100
40.0	5.0	5.0	39.9352	5.0613	4.9577	99.837875	101.2268	99.1542
45.0	5.0	5.0	45.0852	5.0168	4.9507	100.1893111	100.3362	99.0144
5.0	1.0	5.0	5.0206	0.9791	5.0675	100.4118	97.9080	101.3494
5.0	3.0	5.0	4.8683	2.9711	4.9546	97.3662	99.0353	99.0916
5.0	5.0	5.0	4.7783	5.0556	4.7905	95.5654	101.1110	95.8098
5.0	8.0	5.0	5.0285	8.0553	5.1218	100.5702	100.6906	102.4360
5.0	10.0	5.0	5.0801	10.0092	4.9350	101.6022	100.0915	98.6992
5.0	12.0	5.0	4.7582	11.8771	4.8902	95.1634	98.9755	97.8038
5.0	15.0	5.0	5.0684	14.9575	5.0535	101.3678	99.7166	101.0694
5.0	18.0	5.0	5.0175	18.0244	4.9093	100.3492	100.1354	98.1852
5.0	20.0	5.0	5.1071	20.2095	5.1159	102.1426	101.0476	102.3174
5.0	25.0	5.0	4.8286	24.8066	4.9015	96.572	99.2264	98.0302
5.0	30.0	5.0	5.0737	30.0459	5.0720	101.4746	100.1530	101.4394
5.0	5.0	1.0	5.0518	5.0269	1.0148	101.035	100.5376	101.4770
5.0	5.0	3.0	5.0373	5.0372	3.0660	100.7464	100.7434	102.2013
5.0	5.0	5.0	4.9260	4.9755	4.9260	98.519	99.5098	98.5208
5.0	5.0	8.0	5.0191	4.8732	8.2167	100.3814	97.4644	102.7084
5.0	5.0	10.0	4.9644	5.0564	10.2209	99.2886	101.1288	102.2089
5.0	5.0	12.0	5.0038	5.0637	11.5764	100.0754	101.2744	96.4698
5.0	5.0	15.0	4.9029	4.9253	14.8613	98.0588	98.5050	99.0753
5.0	5.0	20.0	5.0941	5.0256	20.0511	101.8816	100.5116	100.2556
5.0	5.0	25.0	5.0098	4.9747	25.2086	100.1966	99.4942	100.8342
5.0	5.0	30.0	5.0380	5.0328	30.0165	100.7604	100.6568	100.0549
5.0	5.0	40.0	4.9562	5.0607	39.8884	99.1234	101.2142	99.7210
5.0	5.0	50.0	5.0025	4.9505	50.0176	100.049	99.0102	100.0353
Mean recovery						100.2397799	99.9506	100.0651
*R.S.E. (%) single						0.8004	0.7524	0.8780
+R.S.E. (%) total						0.8232		

*R.S.E. (%) single = $\sqrt{\sum_{i=1}^N (\hat{y}_i - y_i)^2 / \sum_{i=1}^N (y_i)^2} \times 100$. +R.S.E. (%) total = $\sqrt{\sum_{j=1}^M \sum_{i=1}^N (\hat{y}_{ji} - y_{ji})^2 / \sum_{j=1}^M \sum_{i=1}^N (y_{ji})^2} \times 100$.

TABLE 8: Presence of other pesticides.

Pesticide	<i>t</i> -and <i>F</i> -tests: Two-sample assuming unequal variances at 0.05 significance level					
	Tebuconazole		Carboxin		Chlorpyrifos	
Mean	13.9174	95962	18.2557	12.9532	21.8104	13.8493
Variance	143.9883	651189	303.6798	180.9091	385.3132	187.4866
Sample size	11	20	13	20	7	20
Degree of freedom	14		21		6	
Separate variance <i>t</i> -test statistic	1.0721		0.9315		0.9247	
<i>t</i> critical two-tail	2.1448		2.0796		2.4469	
<i>p</i> value	0.3018		0.3622		0.3908	
<i>F</i> -test	2.2812		1.6786		2.0721	
<i>F</i> critical value	2.3779		2.3080		2.7401	
<i>p</i> value	0.0585		0.1514		0.1140	

which is lower than 0.790, 0.824, and 0.621 $\mu\text{g}/\text{mL}$ for CAR, CHL, and TEB, respectively. The efficiency of the proposed methods can be illustrated by the mean percentage recovery values which were between 87.02% and 108.53% with %RSD lower than 6%. According to the statistical analysis, no significant differences between the three proposed methods were detected. As a result, all the three proposed methods have a good accuracy, precision, and sensitivity and are typically suited for the estimation of carboxin, chlorpyrifos, and tebuconazole residues in real cabbage samples.

Data Availability

All data are included within the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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