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Research Article

Three Different Spectrophotometric Methods for Simultaneous Determination of Pyriproxyfen and Chlorothalonil Residues in Cucumber and Cabbage Samples

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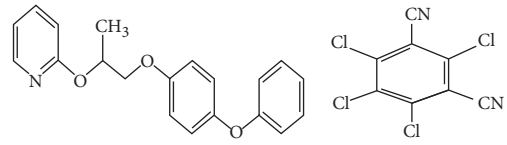
In this study, three simple and accurate spectrophotometric methods for simultaneous determination of pyriproxyfen and chlorothalonil residues in cucumbers and cabbages grown in experimental greenhouse were studied. The first method was based on the zero-crossing technique measurement for first and second derivative spectrophotometry. The second method was based on the first derivative of the ratio spectra. However, the third method was based on mean centering of ratio spectra. These procedures lack any previous separation steps. The calibration curves for three spectrophotometric methods are linear in the concentration range of 1–30 $\mu g \cdot mL^{-1}$ and 0.5–7 $\mu g \cdot mL^{-1}$ for pyriproxyfen and chlorothalonil successively. The recoveries ranged from 82.12–97.40% for pyriproxyfen and 81.51–97.04% for chlorothalonil with relative standard deviations less than 4.95% and 5.45% in all instances for pyriproxyfen and chlorothalonil, respectively. The results obtained from the proposed methods were compared statistically by using one-way ANOVA, and the results revealed there were no significant differences between ratio spectra and mean centering methods with the zero-crossing technique. The proposed methods are successfully applied for the simultaneous estimation of the residue of both pesticides in cucumber and cabbage samples.

1. Introduction

Pesticides are widely used to protect agriculture products like fruits and vegetables in addition to foods. However, pesticide residues may enter the food chain and cause harmful toxic effects on human health. Therefore, the determination of pesticides in such products is of great concern in recent years [1]. Chemical ingredients are also used to eliminate or remove pests which damage agricultural products during production, consumption, and storage of nutrients, causing product loss [2, 3]. In addition, pesticides have not only helped in protecting crops from pests, diseases, and weeds but also helped in keeping public health from various disease-carrying, nuisance pests. Pesticides have also helped the agricultural district to achieve international standards for food quality and safety [4]. Pyriproxyfen, chemically known as 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)-propyl ether is a vital insecticide applied to protect crops against whitefly [5]. The chemical structure of pyriproxyfen is shown in Figure 1(a). The classical manners for determining pyriproxyfen along with another pesticide in agriculture products like fruits and vegetables are gas chromatography-mass spectrometry [6] and liquid chromatography-mass spectrometry [7]. Moreover, many papers were published for the estimation of pyriproxyfen with other pesticides by high-performance liquid chromatography and HPLC with UV detection [8, 9]. Ultraperformance liquid chromatography coupled with tandem mass spectrometry is also used for determination of pyriproxyfen [10].

Chlorothalonil (tetra chloroisophtalo nitrile class) is an effective pesticide that can act against a wide range of plants pathogens that attack fruits, vegetables, crops, and cereals. It is used through out the world to minimize sporulation of fungi and decrease the growth of it [11]. The chemical structure of fungicide chlorothalonil is demonstrated in Figure 1(b). Chlorothalonil has moderate acute toxicity [12]. Many analytical methods are available to determine



(a) (b)

Figure 1: The chemical structure of (a) pyriproxyfen and (b) chlorothalonil.

chlorothalonil residues in various matrices, but the well-known methods are high-performance liquid chromatography-diode array detection [13, 14] and high-performance liquid chromatography with UV detection [15–18]. Gas chromatography with electron capture detection [19–21] and by headspace solid-phase microextraction was coupled to gas chromatography with electron capture detection [22]. Gas chromatography-mass spectrometry is used in a few cases [23]. As well as, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is a novel methodology used for preparing samples for pesticide multiresidue analysis [24]. Also, the QuEChERS method was utilized for the analysis of pesticides with various polarities, from vegetable and fruit matrices [25].

In 1990, Salinas et al. [26] proposed a method for resolution of a binary mixture. This method relies on the derivative of ratio spectra for a binary mixture obtained by dividing the absorption spectrum of the mixture to the standard spectrum of one compound.

Currently, another spectrophotometric method was introduced by Afkhami and Bahram [27] for quantification of binary mixtures simultaneously that lack preceding steps of separation. This method is called mean centering of ratio spectra that could apply successfully for the analysis of binary and ternary mixture simultaneously; the mathematical clarification of the proposed methods was also mentioned. Hence, this paper tries to establish methods to resolve a binary mixture of pesticides in cucumber and cabbage samples and then compare the results obtained by these three approaches.

2. Experimental

2.1. Apparatus. A Shimadzu UV-visible double beam spectrophotometer (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 and gather the data of the absorption spectra for each one of pyriproxyfen and chlorothalonil with their mixture solutions.

Software UV Probe program was used for transforming data from zero-order spectra to various orders (^{1}D and ^{2}D) of spectra for each one of pyriproxyfen and chlorothalonil with their mixture solutions. All calculations were performed using Matlab 6.5 and Microsoft Excel. ANOVA and *F*-test were performed by SPSS.

2.2. Chemical and Materials. Pesticide standards pyriproxyfen (99% purity) and chlorothalonil (99% purity) were provided by Santa Cruz Biotechnology, USA. HPLC-grade solvents including methanol and acetonitrile were provided from Merck (Darmstadt, Germany). Primary secondary amine (40 μm) (PSA) was supplied by Sigma-Aldrich. Anhydrous magnesium sulfate and anhydrous sodium acetate were provided by BDH (VWR Chemicals BDH, England); $MgSO_4$ was heated for activation at 400°C for 4 h to remove phthalates and was then cooled and stored in a desiccator prior to use.

2.3. Preparation of Standard Stock Solution. Standard solutions of pyriproxyfen and chlorothalonil pesticide (100 μg·mL^{-1}) were prepared by dissolving 10 mg of each pesticide in 100 mL of methanol and then stored in a freezer at −18°C in stained glass-stopper bottles. Both stock solutions were stored for less than three months. Working standard solution is prepared daily by diluting the stock solutions in methanol.

2.4. Limit of Detection (LOD) and Limit of Quantification (LOQ). Limits of detection were defined as the lowest concentration that could be determined with acceptable accuracy and precision. The limits of detection and limits of quantification of the proposed methods are calculated according to LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the reagent blank and S is the slope of the calibration curve [28].

2.5. Accuracy and Precision. The accuracy of the proposed methods was studied by calculating the percentage recoveries at three different concentrations 3.0, 20.0, and 30.0 μg·mL^{-1} for pyriproxyfen and 1.0, 4.0, and 7.0 μg·mL^{-1} for chlorothalonil with five replication measurements for each concentration. However, the precision of the proposed methods was checked by the signal for eleven replications containing 10 μg·mL^{-1} of pyriproxyfen and 3 μg·mL^{-1} of chlorothalonil. The results rely on the value of the relative standard deviation.

2.6. Calibration Graph

2.6.1. Zero-Crossing Method. Accurate aliquots of pyriproxyfen and chlorothalonil were transferred from their respective working standard solutions into two series of 10 ml calibration flasks, and then they were made up to the volume with methanol. The first sequences include a fixed concentration of chlorothalonil (3 μg·mL^{-1}) and varying concentrations of pyriproxyfen (1–30 μg·mL^{-1}). The second sequences

include a fixed concentration of pyriproxyfen ($10\,\mu g{\cdot}mL^{-1}$) and varying concentrations of chlorothalonil ($0.5–7\,\mu g{\cdot}mL^{-1}$). The absorption spectra of the sample were registered between 200 and 350 nm in which methanol was used as blank. The zero-order spectra of pyriproxyfen and chlorothalonil in binary mixtures were changed to corresponding first and second derivative spectra in the range 200–350 nm by the computer via SHIMADZU UV Probe data system program (Version 2.43). The overlap spectra (zero, first, and second orders) of both pesticides in the binary mixture are illustrated in Figures 2–4.

2.6.2. Ratio Spectra Derivative Method. The absorption spectra of the solution of pyriproxyfen at different concentrations in the binary mixture were divided with the standard spectrum of chlorothalonil ($1.5\,\mu g{\cdot}mL^{-1}$) in methanol; as a result, the ratio spectra were obtained when the first derivative was calculated from ratio spectra and traced with an interval of $\Delta\lambda = 4$ nm in the same solvent. The amplitude at 272 nm ($^1DD_{272}$) and 286 nm ($^1DD_{286}$) was selected for the quantification of pyriproxyfen in a binary mixture. In the same manner, the absorption spectra of the solution of chlorothalonil at different concentrations in the binary mixture were divided with the standard spectrum of pyriproxyfen ($15\,\mu g{\cdot}mL^{-1}$) in methanol; as a result, the ratio spectra were attained, and then the first derivative was calculated from ratio spectra and traced with an interval of $\Delta\lambda = 4$ nm of the solution in methanol. The amplitude at 228 nm ($^1DD_{228}$) and 262 nm ($^1DD_{262}$) was selected for the quantification of chlorothalonil in a binary mixture.

2.6.3. Mean Centering of the Ratio Spectra Method. For pyriproxyfen to be determined, the stored spectra of the binary mixture which contains a different concentration of pyriproxyfen were divided by the absorption spectrum ($1.5\,\mu g{\cdot}mL^{-1}$) of chlorothalonil, and then the obtained ratio spectra were mean centered.

A similar idea was used for chlorothalonil determination, the stored spectra of the binary mixture which contains a different concentration of chlorothalonil were divided by the absorption spectrum $15\,\mu g{\cdot}mL^{-1}$ of pyriproxyfen, and the obtained ratio spectra were mean centered. The calibration curve for both pyriproxyfen and chlorothalonil was constructed by plotting the mean centering values at 278 nm and 254 nm, respectively.

2.6.4. Sample Preparation. The modified QuEChERS method proposed by Hou et al. [29] was followed for the extraction of pyriproxyfen and chlorothalonil residues from cucumber and cabbage samples. About 500 g of the chopped sample was balanced and homogenized, and then 10 g of the prior chopped fresh sample was weighed and placed in a 50 mL Teflon centrifuge tube, fortified by the addition of the standard stock solution at three levels. 10 mL extraction solvent of acetonitrile containing 5% acetic acid (HOAc)/toluene (1 : 1, v/v) was added by using a transfer pipette, and the mixture was shacked for 1 min in an air bath at 22°C; then, 4 g of anhydrous $MgSO_4$ and 1 g of anhydrous sodium acetate were added and vortexed for 1 min instantly; later, centrifugation of the extracts for 5 min at 2500 rpm was done; 10 mL aliquot of the higher layer was transported into a 15 mL Teflon centrifuge tube that contains 300 mg PSA and $1.5\,g{\cdot}MgSO_4$. The samples were vortexed for 1 min, and centrifugation for 5 min at 8800 rpm was performed. The resulting solution was filtered using a $0.45\,\mu m$ filter; after that, 10 mL of the filtrate was transported into a 15 mL tube cautiously under a stream of nitrogen concentrated to near-dryness. The extract was dissolved in methanol to be used for determination of the residue of the pesticides with the aid of the standard addition method using the proposed methods.

3. Results and Discussion

Pyriproxyfen and chlorothalonil are two pesticides, and their normal UV absorption spectra are completely overlapped in the wavelength range between 200 and 350 nm (Figure 2). As a result, the quantification of the two pesticides in the binary 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 resolution of the overlapped spectra.

3.1. Zero-Crossing Method. Normal UV absorption spectra of pyriproxyfen are completely overlapped with the spectrum of chlorothalonil. Figure 2 displays the zero-order absorption spectra of pyriproxyfen and chlorothalonil and their mixture in which methanol was employed as a reagent blank. A strong overlapping of the spectrum is very obvious. As a result, the simultaneous determination of chlorothalonil in the occurrence of pyriproxyfen or vice versa will not be able by direct absorbance measurements. It is challenging by classical spectrophotometry to resolve mixtures. Therefore, derivative spectrophotometry can solve this issue in a satisfying manner. Figure 3 shows the first-derivative absorption spectra of a solution of both pesticides and their mixture. The zero-crossing method could be appropriate to avoid this problem. To determine pyriproxyfen in the presence of chlorothalonil, the measurements were made at the zero-crossing point of chlorothalonil at 281.80 nm. However, the quantitation of chlorothalonil in the occurrence of pyriproxyfen could be made by the peak to baseline at 329.20 nm. Also, the second-derivative absorption spectra of pyriproxyfen and chlorothalonil in Figure 4 produce some zero-crossing points for both pesticides that could permit their quantitation, and the corresponding calibration graphs were at 281.40 nm and 285.60 nm for the quantitation of pyriproxyfen and 262.60 nm and 266 nm for the quantitation of chlorothalonil, as demonstrated in Figures 5–8.

3.2. Ratio Spectra Derivative Method. The absorption spectra of the solution of pyriproxyfen at different concentrations from $1–30\,\mu g{\cdot}mL^{-1}$ in the binary mixture were recorded in the wavelength range of 200–350 nm and then divided by the standard spectrum of chlorothalonil ($1.5\,\mu g{\cdot}mL^{-1}$) in

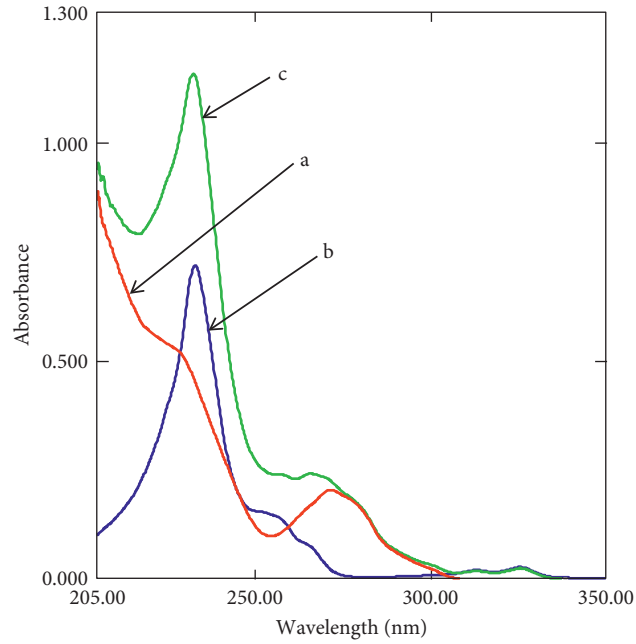


Figure 2: Zero-order spectra of (a) 10.0 μg·mL^{-1} of pyriproxyfen, (b) 3.0 μg·mL^{-1} of chlorothalonil, and (c) their mixture in methanol.

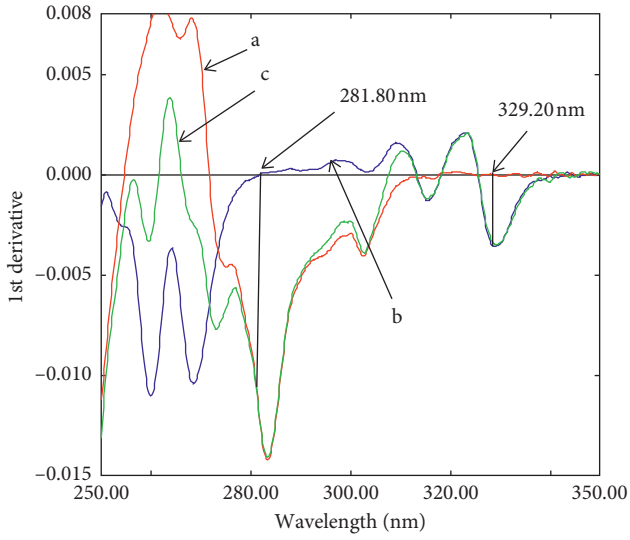


Figure 3: First-order derivative spectra of (a) 10.0 μg·mL^{-1} of pyriproxyfen, (b) 3.0 μg·mL^{-1} of chlorothalonil, and (c) their mixture in methanol.

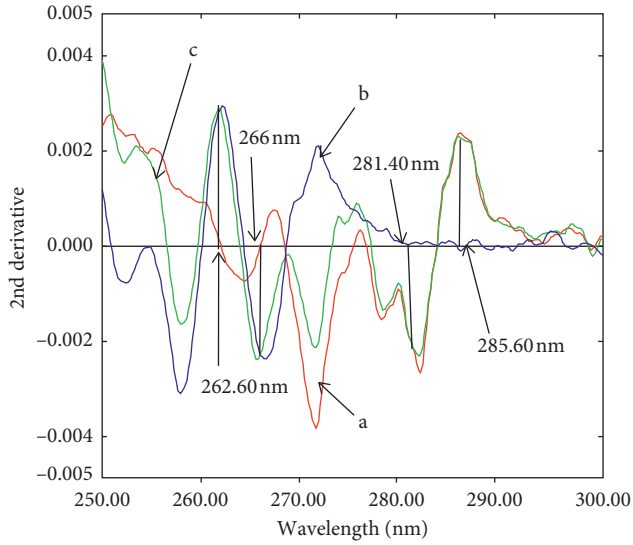


Figure 4: Second-order derivative spectra of (a) 10.0 μg·mL^{-1} of pyriproxyfen, (b) 3.0 μg·mL^{-1} of chlorothalonil, and (c) their mixture in methanol.

methanol; as a result, the ratio spectra were obtained, as shown in Figure 9(a). Later, the first derivative was calculated from ratio spectra and traced with an interval of $\Delta\lambda = 4$ nm of the solution in methanol, as demonstrated in Figure 9(b). The amplitude at 272 nm ($^1DD_{272}$) and 286 nm ($^1DD_{286}$) was used for measuring the concentration of pyriproxyfen in a binary mixture. Also Figure 10(a) indicates the ratio spectra of chlorothalonil gained by dividing the absorption spectra of chlorothalonil at different concentrations from 0.5 to 7 μg·mL^{-1} in a binary mixture with the standard spectrum of pyriproxyfen (15 μg·mL^{-1}) in methanol to obtain the ratio spectra; then, the first derivative was calculated from ratio spectra and traced with an interval of $\Delta\lambda = 4$ nm of the solution in methanol, as shown in Figure 10(b). The amplitude at 228 nm ($^1DD_{228}$) and 262 nm ($^1DD_{262}$) was used for measuring the concentration of chlorothalonil in a binary mixture. The main important parameters that need to be optimized are the concentrations of the divisor; various divisor concentrations were tested, it was noticed that the standard solution (1.5 μg·mL^{-1}) of chlorothalonil gave the optimum signal-to-noise ratio, and it is relevant for estimation of pyriproxyfen in a binary mixture. Also, standard solution (15 μg·mL^{-1}) of pyriproxyfen was selected as the divisor for the determination of chlorothalonil in a binary mixture. In addition, the first derivative of the ratio spectra was affected by $\Delta\lambda$. As the $\Delta\lambda$ values

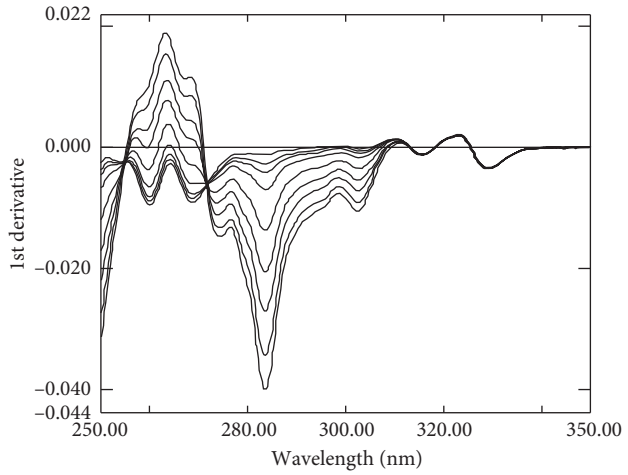


Figure 5: First derivative spectra of mixture containing 1, 2, 3, 5, 10, 15, 20, 25, and 30 $\mu g \cdot mL^{-1}$ pyriproxyfen and $3.0\,\mu g \cdot mL^{-1}$ chlorothalonil.

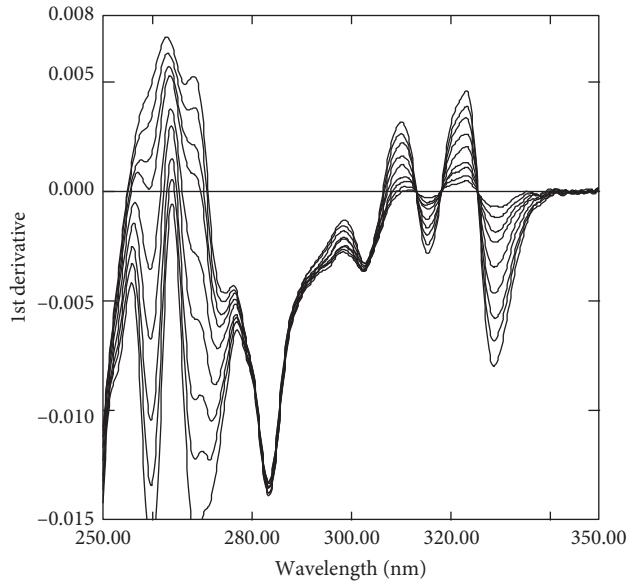


Figure 6: First derivative spectra of mixture containing 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 $\mu g \cdot mL^{-1}$ chlorothalonil and $10.0\,\mu g \cdot mL^{-1}$ pyriproxyfen.

increase, the noise level declines slightly. Therefore, testing at different $\Delta\lambda$ values and $\Delta\lambda = 4$ nm was regarded to be suitable.

3.3. Mean Centering of the Ratio Spectra Method. The stored spectra of the binary mixture which contain various concentrations of pyriproxyfen were divided by the standard spectrum ($1.5\,\mu g \cdot mL^{-1}$) of chlorothalonil; the ratio spectra were attained and then mean centered. Mean centering of the ratio spectra was gained in the wavelength range between 210 and 310 nm as clarified in Figure 11. The concentration of pyriproxyfen was estimated through measuring the signal at 278 nm corresponding to the maximum point (Figure 11).

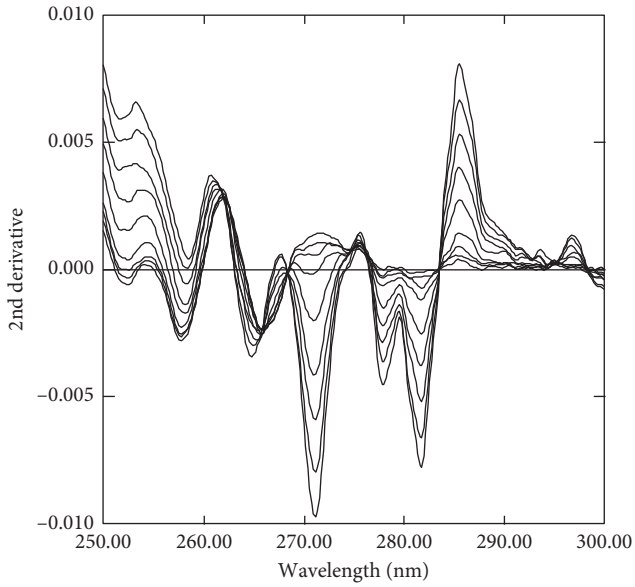


Figure 7: Second derivative spectra of mixture containing 1, 2, 3, 5, 10, 15, 20, 25, and 30 $\mu g \cdot mL^{-1}$ pyriproxyfen and $3.0\,\mu g \cdot mL^{-1}$ chlorothalonil.

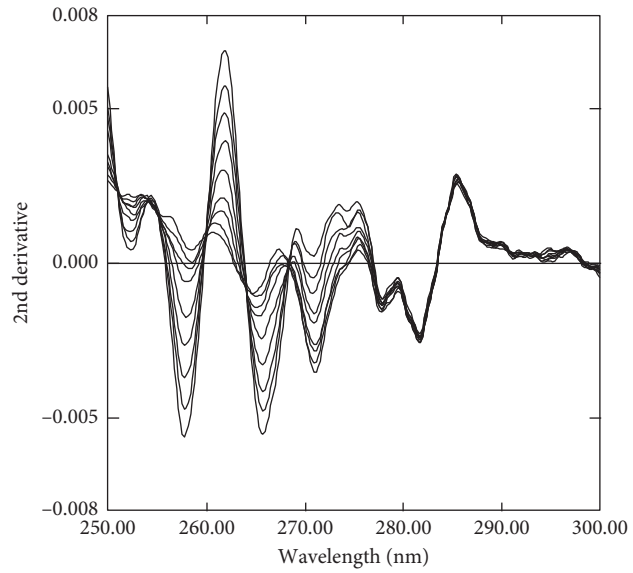


Figure 8: Second derivative spectra of mixture containing 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 $\mu g \cdot mL^{-1}$ chlorothalonil and $10.0\,\mu g \cdot mL^{-1}$ pyriproxyfen.

In a similar way, the stored spectra of the binary mixture which contain various concentrations of chlorothalonil were divided by the standard spectrum ($15\,\mu g \cdot mL^{-1}$) of pyriproxyfen, and the ratio spectra were attained and then mean centered. Mean centering of the ratio spectra was gained in the wavelength range between 210 and 300 nm, as shown in Figure 12. The concentration of chlorothalonil was estimated through measuring the signal at 254 nm corresponding to the maximum point (Figure 12).

In order to optimize the mean centering of ratio spectra and get the best results, the most important

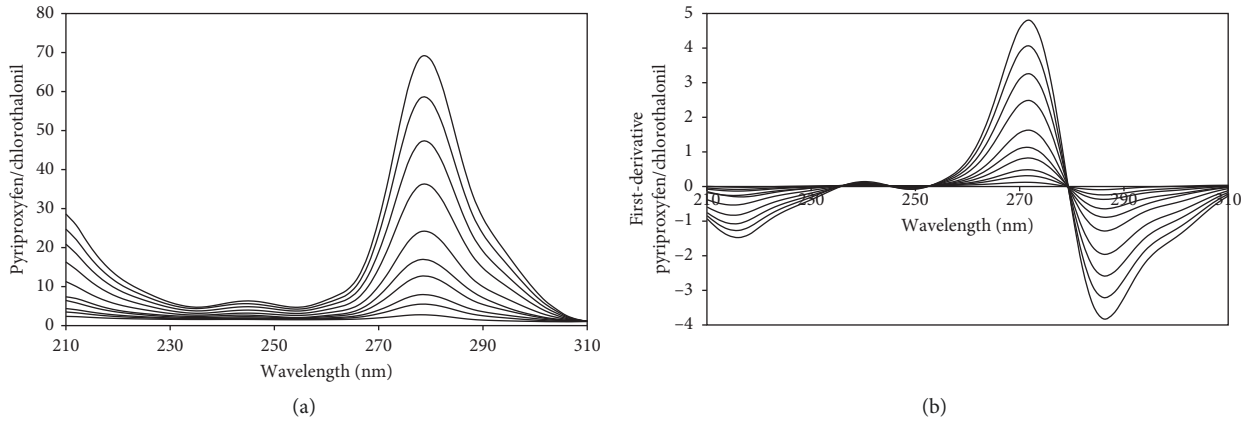


Figure 9: Ratio spectra (a) and first derivative ratio spectra (b) of pyriproxyfen (1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 $\mu g \cdot mL^{-1}$) when 1.5 $\mu g \cdot mL^{-1}$ of chlorothalonil was used as the divisor in methanol ($\Delta\lambda = 4$ nm).

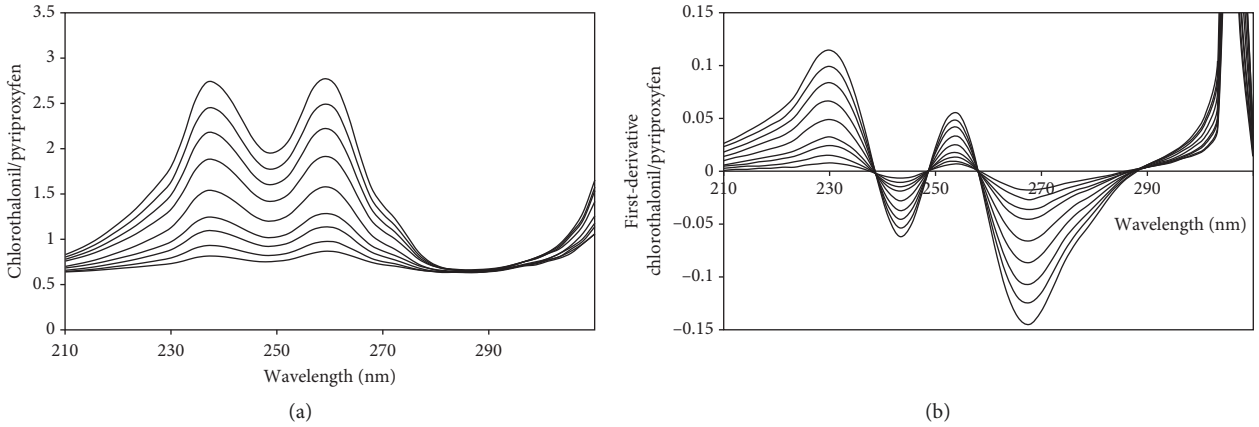


Figure 10: Ratio spectra (a) and first derivative ratio spectra (b) of chlorothalonil (0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 $\mu g \cdot mL^{-1}$) when 15 $\mu g \cdot mL^{-1}$ of pyriproxyfen was used as divisor in methanol ($\Delta\lambda = 4$ nm).

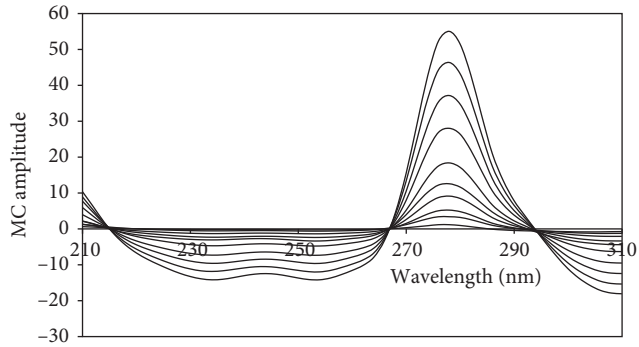


Figure 11: Mean-centered ratio spectra (1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 $\mu g \cdot mL^{-1}$) of pyriproxyfen in methanol as a blank.

parameter that is studied is divisor concentration that has a considerable impact on the method selectivity, for that various concentrations of chlorothalonil (0.5, 1, 1.5, 2, 3, 5, and 6 $\mu g \cdot mL^{-1}$) was tested as divisors for quantification of pyriproxyfen. The best results obtained were upon using 1.5 $\mu g \cdot mL^{-1}$ of chlorothalonil, and different concentrations (3, 5, 10, 15, 20, 25, and 30 $\mu g \cdot mL^{-1}$) of pyriproxyfen were tested as divisors for estimation of chlorothalonil, the concentration (15 $\mu g \cdot mL^{-1}$) of pyriproxyfen was selected for quantification of chlorothalonil. Also, the wavelength range has a great effect on the achieved mean centering ratio spectra, different wavelength ranges were tested, and the best result was attained when using the wavelength range from 210 to 310 nm for both pesticides. Furthermore, in this method, the signal-to-noise ratio enhanced through removing the derivative steps.

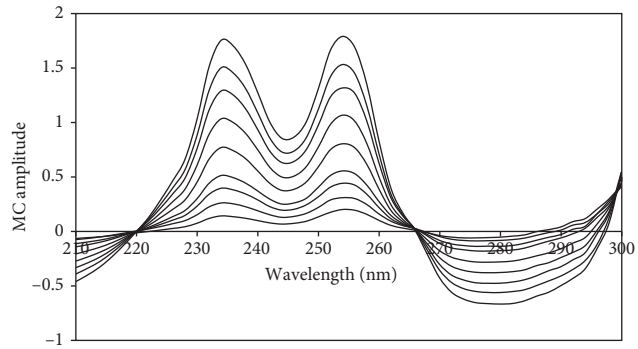


Figure 12: Mean-centered ratio spectra (0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 $\mu g \cdot mL^{-1}$) of chlorothalonil in methanol as a blank.

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

Methods	Compounds	λ_{max} (nm)	Linearity ($mg \cdot kg^{-1}$)	Regression equation	r^2	LOD ($mg \cdot kg^{-1}$)	LOQ ($mg \cdot kg^{-1}$)	RSD (%)
Zero crossing	Pyriproxyfen	$^1D_{281.80}$	1.0–30.0	$y = 2.209x + 0.5061$	0.9996	0.312	0.946	1.52
		$^2D_{281.40}$		$y = 1.7735x - 0.8171$	0.9996	0.254	0.772	1.38
		$^2D_{285.60}$		$y = 1.7656x + 0.1967$	0.9998	0.256	0.776	2.04
	Chlorothalonil	$^1D_{329.20}$	0.5–7.0	$y = 6.5735x + 0.6716$	0.9998	0.112	0.340	0.98
		$^2D_{262.60}$		$y = 4.1633x + 0.6333$	0.9994	0.141	0.427	1.85
		$^2D_{266}$		$y = 3.3325x + 1.5971$	0.9992	0.154	0.469	1.80
RSD	Pyriproxyfen	$^1DD_{272}$	1.0–30.0	$y = 0.1592x - 0.0294$	0.9997	0.270	0.818	0.83
		$^1DD_{286}$		$y = 0.1268x + 0.017$	0.9998	0.302	0.917	0.52
	Chlorothalonil	$^1DD_{228}$	0.5–7.0	$y = 0.0162x + 0.0016$	0.9996	0.144	0.436	1.59
		$^1DD_{262}$		$y = 0.0192x + 0.0047$	0.9997	0.153	0.465	1.33
MCRS	Pyriproxyfen	278	1.0–30.0	$y = 1.8125x - 0.1802$	0.9998	0.211	0.641	1.73
	Chlorothalonil	254	0.5–7.0	$y = 0.2224x + 0.0935$	0.9997	0.131	0.398	1.26

RSD: ratio spectra derivative method; MCRS: mean centering of the ratio spectra method; RSD%: percentage relative standard deviation; LOD: limits of detection; LOQ: limits of quantification.

Table 2: The accuracy of the proposed methods for simultaneous determination of pyriproxyfen and chlorothalonil in a binary mixture.

Compound	Methods of analysis	Concentration ($\mu g \cdot mL^{-1}$)	Recovery (%)	RSD (%)
Pyriproxyfen	Zero-crossing technique at $^1D_{281.80\,nm}$	1.0	95.69	4.18
		20.0	102.97	1.38
		30.0	99.28	1.26
	Peak-to-baseline technique at $^2D_{281.40\,nm}$	1.0	97.95	4.86
		20.0	101.12	1.91
		30.0	100.86	1.40
	Zero-crossing technique at $^2D_{285.60\,nm}$	1.0	95.34	4.45
		20.0	102.09	1.68
		30.0	101.76	0.83
	Ratio spectra derivative $^1DD_{272\,nm}$	1.0	95.10	4.05
		20.0	101.99	2.32
		30.0	101.05	0.63
	Ratio spectra derivative $^1DD_{286\,nm}$	1.0	95.26	3.92
		20.0	101.60	1.07
		30.0	99.60	0.77
	Mean centering of ratio spectra at 278 nm	1.0	95.24	4.51
		20.0	102.39	2.08
		30.0	100.41	1.48

Table 2: Continued.

Compound	Methods of analysis	Concentration ($\mu g \cdot mL^{-1}$)	Recovery (%)	RSD (%)
Chlorothalonil	Peak-to-baseline technique at $^1D_{329.20\,nm}$	0.5	104.30	3.33
		4.0	101.01	1.33
		7.0	100.96	1.10
	Zero-crossing technique at $^2D_{262.60\,nm}$	0.5	104.08	3.99
		4.0	102.18	2.37
		7.0	102.13	1.89
	Zero-crossing technique at $^2D_{266\,nm}$	0.5	104.60	4.54
		4.0	102.42	2.32
		7.0	101.82	1.32
	Ratio spectra derivative $^1DD_{228\,nm}$	0.5	101.23	4.56
		4.0	100.92	2.36
		7.0	99.65	1.32
	Ratio spectra derivative $^1DD_{262\,nm}$	0.5	103.12	3.75
		4.0	100.65	1.93
		7.0	98.58	1.4
	Mean centering of ratio spectra at 254 nm	0.5	104.58	4.22
		4.0	100.77	1.44
		7.0	100.64	1.22

Table 3: Percentage mean recovery of pyriproxyfen and chlorothalonil in real cucumber samples by proposed methods.

Pyriproxyfen

Fortification level ($\mu g \cdot mL^{-1}$)	1st and 2nd derivatives						Ratio spectra derivative				Mean centering of ratio spectra 278 nm	
	$^1D_{281.80\,nm}$		$^2D_{281.40\,nm}$		$^2D_{285.60\,nm}$		$^1DD_{272\,nm}$		$^1DD_{286\,nm}$			
	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD
3.0	88.32	3.83	86.85	4.59	88.50	4.03	88.06	4.95	89.28	3.62	88.00	4.56
20.0	94.72	2.78	93.41	2.73	94.16	3.13	94.40	3.19	95.24	2.48	93.87	2.17
30.0	96.27	2.18	96.42	2.20	94.86	2.44	94.73	2.32	96.39	1.92	95.14	2.15

Chlorothalonil

Fortification level ($\mu g \cdot mL^{-1}$)	1st and 2nd derivatives						Ratio spectra derivative				Mean centering of ratio spectra 254 nm	
	$^1D_{329.20\,nm}$		$^2D_{262.60\,nm}$		$^2D_{266\,nm}$		$^1DD_{228\,nm}$		$^1DD_{262\,nm}$			
	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD
1.0	90.47	4.38	88.04	4.81	82.24	5.11	81.51	5.45	90.32	4.63	87.37	4.43
4.0	95.39	2.19	94.84	3.41	94.06	3.09	91.32	4.43	95.30	2.70	95.93	3.10
7.0	97.04	2.24	95.15	3.76	95.88	2.10	91.36	3.17	96.85	2.23	96.44	3.00

Table 4: Percentage mean recovery of pyriproxyfen and chlorothalonil in real cabbage samples by proposed methods.

Pyriproxyfen

Fortification level ($\mu g \cdot mL^{-1}$)	1st and 2nd derivatives						Ratio spectra derivative				Mean centering of ratio spectra 278 nm	
	$^1D_{281.80\,nm}$		$^2D_{281.40\,nm}$		$^2D_{285.60\,nm}$		$^1DD_{272\,nm}$		$^1DD_{286\,nm}$			
	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD
3.0	85.27	4.23	82.12	4.08	85.57	4.59	83.51	4.37	85.29	4.66	88.69	4.06
20.0	95.50	2.09	95.36	3.19	94.84	3.15	93.88	4.36	96.10	2.84	95.34	2.93
30.0	97.40	2.04	96.33	2.05	95.61	2.21	91.32	3.50	96.98	2.08	95.95	2.37

Chlorothalonil

Fortification level ($\mu g \cdot mL^{-1}$)	1st and 2nd derivatives						Ratio spectra derivative				Mean centering of ratio spectra 254 nm	
	$^1D_{329.20\,nm}$		$^2D_{262.60\,nm}$		$^2D_{266\,nm}$		$^1DD_{228\,nm}$		$^1DD_{262\,nm}$			
	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD	%*R*	%RSD
1.0	90.89	4.58	86.24	5.14	85.00	5.37	84.33	5.03	91.14	4.60	89.17	4.02
4.0	96.65	2.42	92.10	3.71	95.79	3.02	92.16	3.03	95.86	2.52	95.17	2.67
7.0	94.95	2.24	93.08	2.90	94.03	2.53	91.87	2.39	96.30	2.18	95.75	2.04

Table 5: One-way ANOVA statistical analysis results of pyriproxyfen and chlorothalonil by three proposed methods in laboratory prepared mixtures.

		Sum of squares	Degrees of freedom	Mean squares	F	Significance
Pyriproxyfen	Between groups	5.659	2	2.830	1.358 ($F_{\text{theor.}} = 3.68$)	0.287
	Within groups	31.246	15	2.083		
	Total	36.905	17			
Chlorothalonil	Between groups	1.169	2	0.585	0.387 ($F_{\text{theor.}} = 3.68$)	0.686
	Within groups	22.670	15	1.511		
	Total	23.839	17			

4. Calibration Graph and Statistical Data

The analytical characteristics and statistical data of the calibration curves of the proposed methods including the linear range of the calibration graph, correlation coefficients, LOD, LOQ, and relative standard deviation for simultaneous estimation of pyriproxyfen and chlorothalonil by using three different spectrophotometric methods were calculated for each pesticide, as shown in Table 1. The table shows that the high values of the correlation coefficients which is greater than 0.999 point to good linearity of the calibration curves. The lower values of LOD and LOQ as obtained by the proposed methods denote that these methods are sensitive. Also the relative standard deviation for both pesticides is less than 2.04% using the three different spectrophotometric methods which indicate the acceptable precision of the methods. Results of recovery studies are shown in Table 2. For both pesticides, the mean recovery was greater than 95.10%, which indicates that the proposed methods have acceptable accuracy. All three methods are fast, non-devastating, cheap, and need no costly solvent and reagents; also poison and ozone-harming organic solvents and pollutant reagents are not required [30].

5. Application of the Methods

All the three methods were applied successfully for the quantification of pyriproxyfen and chlorothalonil in cucumber and cabbage samples grown in the experimental greenhouse. The recoveries of the two pesticides were investigated by fortifying fresh cucumber and cabbage samples planted in the greenhouse. The percentage of recoveries was determined at three different spiked levels, by spiking with 3.0, 20.0, and 30.0 $\mu g{\cdot}mL^{-1}$ for pyriproxyfen and 1.0, 4.0, and 7.0 $\mu g{\cdot}mL^{-1}$ for chlorothalonil with five replications per level. Table 3 refers to the average recoveries of pyriproxyfen and chlorothalonil at three fortification levels in cucumbers. In all circumstance, the average recoveries were greater than 86.85% and 81.51% with a relative standard deviation less than 4.95% and 5.45% for pyriproxyfen and chlorothalonil, respectively. Table 4 summarizes the results of the estimation of both pesticides in cabbages. The average recoveries greater than 82.12% and 84.33% with a relative standard deviation less than 4.66% and 5.37% for pyriproxyfen and chlorothalonil, respectively; the good recoveries suggested that the proposed methods have a good accuracy.

In order to compare the ratio spectra derivative and mean centering methods with classical derivative spectrophotometry (zero-crossing technique) of the pyriproxyfen and chlorothalonil, the one-way ANOVA test was used. 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 5 shows that the calculated or experimental F-values is less than the tabulated values in the analysis of variance, indicating that there were no significant differences between the three methods.

Table 6: The residues ($mg{\cdot}kg^{-1}$) of pyriproxyfen and chlorothalonil in cucumber and cabbage.

Compound	Cucumber	Cabbage
Pyriproxyfen	Not detect	0.217
Chlorothalonil	0.203	0.292

To keep food safe and secure for consumers' health, the European Union (EU) Food Safety Authority has set the maximum residue limit (MRL) of these pesticides. For chlorothalonil residue in cucumbers and cabbages, the value obtained is much lower than the MRL established by EU-MRL 1 $mg{\cdot}kg^{-1}$ for cucumbers and 3 $mg{\cdot}kg^{-1}$ for cabbages [31]. Also the MRL for chlorothalonil in both vegetables established by Codex Alimentarius Commission is 5 $mg{\cdot}kg^{-1}$ [21, 29]. However, for pyriproxyfen, the MRL in cucumbers and cabbages established by Codex Alimentarius Commission is equal to 0.1 $mg{\cdot}kg^{-1}$ for cucumbers and 0.7 $mg{\cdot}kg^{-1}$ for cabbages [32]. The results showed that the pyriproxyfen residues are not detected in cucumber samples. Meanwhile, the residue of both pesticides in cabbage samples and chlorothalonil in cucumber samples was all below the LOQ and less than the maximum residue limits. So these two pesticides have no any risk for consumer health. The three spectrophotometric methods were successfully applied with the aid of the standard addition method for simultaneous determination of the residue of each pyriproxyfen and chlorothalonil in real cucumber and cabbage samples, and the main results are summarized in Table 6.

6. Comparison of the Methods

A comparison has been done between some of analytical variables obtained from the proposed methods for determination of pyriproxyfen and chlorothalonil in cucumber and cabbage samples with other chromatographic methods, and the main results are shown in Table 7. The table shows that the proposed methods have good linearity, accuracy, and precision for determination of the residues of both

Table 7: Comparison of the proposed methods with some other methods.

Pyriproxyfen					
Analytical parameters	Zero crossing	RSD	MCRS	Literature method [7]	Literature method [9]
Linearity range ($mg{\cdot}kg^{-1}$)	1–30	1–30	1–30	0.002–0.4	1–25
LOD ($mg{\cdot}kg^{-1}$)	0.254–0.312	0.270–0.302	0.211	0.001–0.05	0.217
Recovery (%)	82.12–97.40	83.51–96.98	88.00–95.95	60–64	86.03–94.55
RSD (%)	Below 4.59	Below 4.95	Below 4.56	9–13	1.28
Application	Cucumbers and cabbages	Cucumbers and cabbages	Cucumbers and cabbages	Oranges	Tomatoes
Chlorothalonil					
Analytical parameters	Zero crossing	RSD	MCRS	Literature method [19]	Literature method [29]
Linearity range ($mg{\cdot}kg^{-1}$)	0.5–7.0	0.5–7.0	0.5–7.0	0.005–5.0	0.5–10
LOD ($mg{\cdot}kg^{-1}$)	0.112–0.154	0.144–0.153	0.131	0.02	0.05
Recovery (%)	82.24–97.04	81.51–96.85	87.37–96.44	86.2–103.3	71–93
RSD (%)	Below 5.37	Below 5.45	Below 4.43	Below 10.5	Below 6
Application	Cucumbers and cabbages	Cucumbers and cabbages	Cucumbers and cabbages	Cucumbers and tomatoes	Cabbages

RSD: ratio spectra derivative method; MCRS: mean centering of the ratio spectra method; RSD%: percentage relative standard deviation.

pesticides indicating that the proposed methods are acceptable in this trend.

7. Conclusions

In this study, the determination of a binary mixture of pyriproxyfen and chlorothalonil in cucumbers and cabbages by using the zero-crossing method, ratio spectra derivative method, and mean centering ratio spectra method has been carried out. These three methods gave good results when applied to the resolution of a binary mixture and do not require complicated or expensive instruments; in addition, they are rapid compared to chromatographic methods. We used modified QuEChERS for sample preparation in order to obtain highest recoveries for chlorothalonil. The efficiency of the proposed methods can be demonstrated by the recovery values obtained from cucumbers and cabbages, which were between 81.51 and 97.40% with a relative standard deviation lower than 5.45%. There is no significant difference between the three proposed methods according to statistical analysis. Therefore, these three proposed methods are typically suited for the estimation of pyriproxyfen and chlorothalonil residues in cucumbers and cabbages.

Data Availability

All data are included within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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