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Spectrophotometric determination of trifluoperazine HCl and isopropamide iodide in binary mixture using second derivative and second derivative of the ratio spectra methods

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Abstract

Two methods are presented for the simultaneous determination of trifluoperazine hydrochloride and isopropamide iodide in binary mixture. The first method depends on second derivative (^{2}D) ultraviolet spectrophotometry, with zero crossing and peak to base measurement. The second derivative amplitudes at 270.4 and 230.2 nm were selected for the assay of trifluoperazine hydrochloride and isopropamide iodide, respectively. The second method depends on second derivative of the ratio spectra by division of the absorption spectrum of the binary mixture by a normalized spectrum of one of the components and then calculating the second derivative of the ratio spectrum. The second derivative of the ratio amplitudes at 257 and 228 nm were selected for the determination of trifluoperazine hydrochloride and isopropamide iodide, respectively. The two proposed methods were successfully applied to the determination of the two drugs in laboratory prepared mixtures and in commercial tablets.

Keywords: Isopropamide iodide; Trifluoperazine hydrochloride; Second derivative spectrophotometry; Second derivative of the ratio spectra

1. Introduction

Trifluoperazine HCl, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride, (I) is a phenothiazine tranquilizer with anti-emetic effect. The official method for the determination of (I) is non aqueous titration with perchloric acid, determining the end point potentiometrically [1] or using crystal violet indicator [2]. Various spectrophotometric methods have been reported for

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the determination of (I) by measurement the UV absorption at 256 nm [1] and using different reagents including bromocresol purple [3], potassium chlorate [4,5] and formaldehyde [6]. First and fourth derivatives ultra violet spectrophotometry have been reported for simultaneous determination of (I) and tranylcypromine sulphate in tablets [7].

Isopropamide iodide, γ-(Aminocarbonyl)-N-methyl-N,N-bis (1-methylethyl)- γ -phenylbenzenepropanaminium iodide, (II) is a quaternary ammonium anticholinergic. The official method for the determination of (II) is non aqueous titration with perchloric acid [2]. Various spectrophotometric methods have been reported for the determination of (II) by measurement of the UV absorption at 225 nm [8], first and second derivatives spectrophotometry [9], ion pair formation with methyl orange [10] and charge transfer complexation with iodine [11]. The official method for determination of (II) in tablet is ion exchange chromatographic-spectrophotometric read out procedure [2]. HPLC method using CROWN PAK column has been reported for the simultaneous determination of (II) and phenylpropanolamine HCl in capsule [12].

The binary mixture of (I) and (II) is used for their anti-emetic and anti-spasmodic effect. No spectrophotometric derivative ratio method has been reported for their simultaneous determination in two component mixture. Only one method has been reported for simultaneous determination of this mixture based on measurement of second derivative spectrophotometric values at 248–264 nm in methanolic solution for (I) and 232 nm in 0.1 N sodium hydroxide after chloroformic extraction procedure for (II) with linearity range of 2.5–12.5 μg ml^{-1} for (I) and 20–80 μg ml^{-1} for (II) [13].

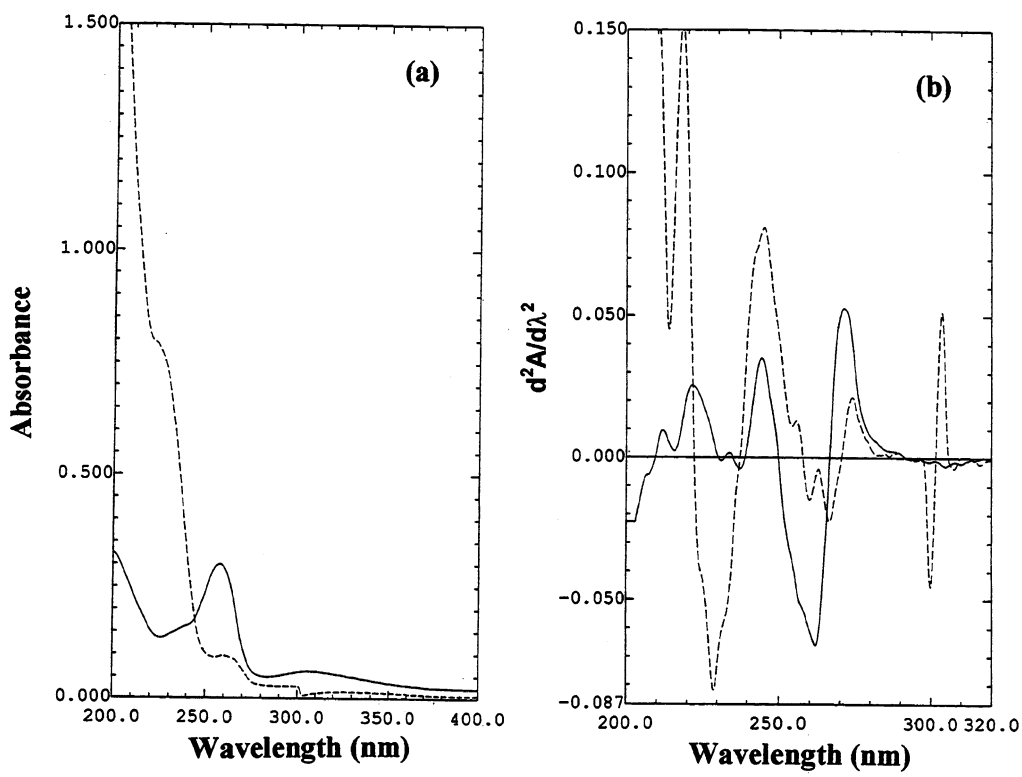

Fig. 1. UV absorption spectra (a) and second derivative spectra (b) of 4 μg ml^{-1} of trifluoperazine HCl (–) and 20 μg ml^{-1} of isopropamide iodide (---) in distilled water.

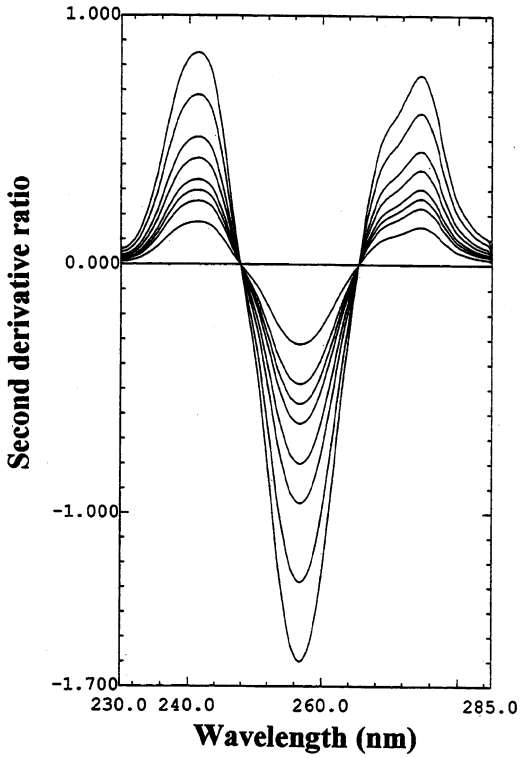

Fig. 2. Second derivative of the ratio spectra for different concentrations (2, 3, 3.5, 4, 5, 6, 8, 10 μg ml^{-1}) of trifluoperazine HCl, using normalized spectrum of isopropamide iodide as a devisor.

The application of derivative techniques to spectroscopy is very useful when signal overlap or interferences exist and it offers a powerful tool for both qualitative and quantitative analysis of mixtures in pharmaceutical analysis [14,15] and biomedical analysis [16]. The main advantage of the derivative of the ratio spectra method may be the chance of doing measurements in correspondence of peaks, hence a potential greater sensitivity and accuracy were obtained. The aim of this work was to demonstrate the capability of the second derivative (^{2}D) and second derivative of the ratio spectra (^{2}DD) methods to resolve and overcome the problem of overlapping spectral bands and allows the simultaneous determination of (I) and (II) without the need for prior separation.

By comparing the proposed ^{2}D method with published ^{2}D method [13], the proposed ^{2}D method was found to be more rapid, simple, sensitive and does not need prior separation of the two drugs.

The proposed ^{2}DD method was found to be more simple and sensitive than the published methods and not need any chemical derivatization or measuring the UV spectra of the drugs at short critical wavelengths. With these two proposed methods, one can gain the advantages of the speed, lower cost and environment protecting without sacrificing accuracy.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzue (Japan) UV-Visible spectrophotometer, model UV-1601 PC connected

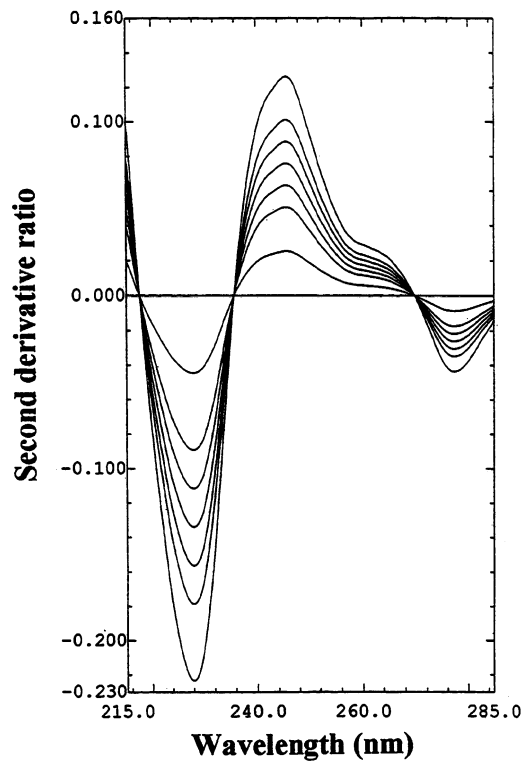

Fig. 3. Second derivative of the ratio spectra for different concentrations (5, 10, 12.5, 15, 17.5, 20, 25 μg ml^{-1}) of isopropamide iodide, using normalized spectrum of trifluoperazine HCl as a devisor.

Table 1
Characteristic parameters for the regression equations of second derivative (^{2}D) and second derivative of the ratio spectra (^{2}DD) methods for determination of trifluoperazine HCl (I) and isopropamide iodide (II)

Parameters	^{2}D		^{2}DD	
	I	II	I	II
Linearity (μg ml^{-1})	2–10	5–25	2–10	5–25
Regression equation(Y)[a]: Slope (b)	13.12×10^{-3}	3.50×10^{-3}	16.00×10^{-2}	8.90×10^{-3}
Standard deviation of the slope (S_b)	6.33×10^{-5}	1.90×10^{-5}	4.56×10^{-4}	2.08×10^{-5}
Relative standard deviation of the slope (%)	0.48	0.54	0.28	0.23
Confidence limit of the slope[b]	12.98×10^{-3}	3.46×10^{-3}	15.90×10^{-2}	8.85×10^{-3}
	-13.26×10^{-3}	-3.54×10^{-3}	-16.10×10^{-2}	-8.95×10^{-3}
Intercept (a)	5.46×10^{-5}	2.4×10^{-4}	3.18×10^{-3}	2.86×10^{-4}
Standard deviation of the intercept (S_a)	6.68×10^{-5}	3.33×10^{-4}	2.63×10^{-3}	3.65×10^{-4}
Confidence limit of the intercept[b]	(-8.84×10^{-5})	(-4.86×10^{-4})	(-2.45×10^{-3})	(-5.10×10^{-4})
	-1.98×10^{-4}	-9.66×10^{-4}	-8.81×10^{-3}	-1.08×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999

[a] $Y=a+bC$,where C is the concentration of drug in μg ml^{-1} and Y is the amplitude at the specified wavelength.
[b] 95% confidence limit.

to an IBM compatible computer and a HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzue). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. The absorption spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–400 nm. The second derivative of the measured spectra was obtained using the accompanying software with $\Delta\lambda=4$ nm and scaling factor of 20.

2.2. Materials and reagents

Pharmaceutical grade of (I) and (II) were kindly supplied by Kahira pharmaceutical and Chemical Industries Company (Cairo, Egypt) and certified to contain 100%.

The commercial Stellamide tablets used (Batch No. 910064) was manufactured by Kahira Pharmaceutical and Chemical Industries Company (Cairo, Egypt). Each tablet contains 1 mg of (I) and 5 mg of (II), in addition to tablet excipients consisting of lactose, cellulose, crosscarmellose sodium, gelatin, magnesium stearate, F.D.&C. yellow, titanium dioxide, and hydroxy propyl methyl cellulose.

2.3. Standard solutions and calibration

Stock standard solutions of each of (I) and (II) were prepared separately by dissolving 100 mg of each drug in 100 ml distilled water.

The standard solutions were prepared by dilution of the stock standard solutions with distilled water to reach concentration range of 2–10 μg ml^{-1} for (I) and 5–25 μg ml^{-1} for (II).

2.3.1. For ^{2}D method

The values of the ^{2}D amplitudes were measured at 270.4 nm (zero-crossing of II) and 230.2 nm (zero-crossing of I) for the determination of (I) and (II), respectively.

2.3.2. For ^{2}DD method

For (I): the UV absorption spectra of standard solutions of (I) were divided by a normalized spectrum of (II) [a spectrum of unit concentration]. The second derivative was calculated for the obtained spectra with $\Delta\lambda=4$ nm. The second derivative of the ratio spectra obtained were

smoothed with 16 experimental points. The amplitudes at 257 nm were measured and found to be proportional to the concentration of (I).

For (II): the UV absorption spectra of standard solutions of (II) were divided by a normalized spectrum of (I). The second derivative was calculated for the obtained spectra with $\Delta\lambda = 4$ nm. The second derivative of the ratio spectra obtained were smoothed with 16 experimental points. The amplitudes at 228 nm were measured and found to be proportional to the concentrations of (II).

2.4. Sample preparation

Ten tablets were weighed and finely powdered. A portion of the powder equivalent to about 1 mg of (I) and 5 mg of (II) was weighed accurately, dissolved and diluted to 100 ml with distilled water. The sample solution was filtered. Further dilution was carried out with distilled water to provide a solution of 4 μg ml^{-1} of (I) and 20 μg ml^{-1} of (II). The general procedures for ^{2}D and ^{2}DD described under calibration were followed and the concentrations of (I) and (II) were calculated.

Table 2
Determination of trifluoperazine HCl (I) and isopropamide iodide (II) in synthetic mixtures and commercial tablets using the proposed second derivative (^{2}D), second derivative of the ratio spectra (^{2}DD) and reference ^{2}D methods

	Mean found ± S.D.[a]		
	^{2}D	^{2}DD	Reference ^{2}D
Synthetic mixtures			
For (I)	100.0 ± 0.79	99.8 ± 0.43	
For (II)	100.1 ± 0.31	99.9 ± 0.89	
Commercial tablets			
For (I)	99.7 ± 0.43	100.3 ± 0.59	100.1 ± 0.51
	$t = 1.31$	0.57	(2.31)[b]
	$F = 1.28$	1.34	(6.39)[b]
For (II)	100.3 ± 0.44	100.1 ± 0.56	100.2 ± 0.53
	$t = 0.32$	0.29	(2.31)[b]
	$F = 1.45$	1.12	(6.39)[b]
Recovery[c]			
For (I)	99.8 ± 0.45	100.1 ± 0.60	
For (II)	100.1 ± 0.39	100.0 ± 0.42	

[a] Mean and S.D. for percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content ($n = 5$).

2.5. Percent recovery study

This study was performed by addition of known amounts of (I) and (II) to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained were compared with expected results (Table 2).

3. Results and discussion

3.1. ^{2}D method

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing. These parameters need to be optimized to give a well-resolved large peak and to give good selectivity and larger sensitivity in the determination. Generally, the noise level decreases with an increase in $\Delta\lambda$ thus decreasing the fluctuation in the derivative spectrum. However if the value of $\Delta\lambda$ is too large, the spectral resolution is very poor. Therefore, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level and the resolution of the spectrum. Some values of $\Delta\lambda$ were tested. $\Delta\lambda = 4$ nm and wavelength scanning speed = 2800 nm min^{-1} were selected for the ^{2}D method as the optimal conditions to give a satisfactory signal to noise ratio.

The UV absorption of spectra of (I) and (II) were produced in Fig. 1(a). The two spectra clearly display considerable overlap. The direct UV absorption measurement for assaying binary mixture seems to be impossible. The second derivative (^{2}D) spectra present spectral features which can be used for the simultaneous determination of (I) and (II) (Fig. 1(b)). The zero crossing

method is the most common procedure for the preparation of analytical calibration graph [17]. (I) was determined by measurement of its second derivative amplitude at the zero crossing point of (II) (at 270.4 nm). While (II) was determined by measurement of its second derivative amplitude at the zero crossing point of (I) (at 230.2 nm). The plots of the absolute values of second derivative at 270.4 and 230.2 nm against concentrations of (I) and (II), respectively, showed linear relationship.

3.2. ^2DD method

The main advantage of the derivative of the ratio spectra method may be the chance of doing measurements in correspondence of peaks, hence a potential greater sensitivity and accuracy. While the main disadvantages of the zero crossing method in derivative spectrophotometry for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and circumstance that the working wavelengths generally do not fall in correspondence of peaks of the derivative spectrum. This may be particularly dangerous when the slope of the spectrum is very high with consequent loss of accuracy and precision, and the working wavelength is proximity of the base of the spectrum, which causes poor sensitivity [18]. Fortunately, in the present case, the above circumstances did not occur.

To optimize the simultaneous determination of the (I) and (II) by using the ^{2}DD method, it is necessary to test the influence of the variables: divisor standard concentration, $\Delta\lambda$ and smoothing function. All these variables were studied. The influence of the $\Delta\lambda$ for obtaining the second derivative of the ratio spectra was tested and $\Delta\lambda = 4$ nm was selected as optimum value. A correct choice of the divisor standard concentration is fundamental for several reasons. Among these, in the wavelength range where the absorbance of the standard spectrum used as divisor is zero or below the base line, the noise of ratio spectra is greatly increased. Hence, a certain overlap of spectra in the working wavelength region is actually desirable, to avoid an increase of the error. If the concentration of divisor is increased or decreased, the resulting derivative ratio values are proportionality decreased or increased with consequent variation of both sensitivity and linearity range. From several tests, the best results in terms of signal to noise ratio, sensitivity and repeatability followed using normalized spectra as divisor. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and 16 experimental points were considered as suitable.

The second derivative of the ratio spectra was preferred than the first derivative for a better resolution of the ratio spectra and more accurate and precise results. In this method, the UV absorption spectra of (I) were divided by a normalized spectrum [19] of (II) (obtained by dividing the spectra for several standards of different concentrations by their corresponding concentrations and subsequently averaging them, in order to obtain a spectrum of unit concentration). The second derivative was calculated for the ratio spectra obtained with $\Delta\lambda = 4$ nm. These spectra were smoothed with 16 experimental points due to the high noise of the signals obtained [19], (Fig. 2). The concentration of (I) was proportional to the amplitude at 257 nm, in the concentration range 2–10 μg ml^{-1}. Similarly for the determination of (II), the UV absorption spectra of (II) were divided by a normalized spectrum of (I). From the ratio spectra obtained, second derivative was calculated with $\Delta\lambda = 4$ nm. These spectra were also smoothed with 16 experimental points (Fig. 3). the concentration of (II) was proportional to the amplitude at 228 nm, in the concentration range 5–25 μg ml^{-1}.

For the ^{2}D and ^{2}DD methods, the characteristic parameters of regression equations and correlation coefficients are given in Table 1.

The accuracy of ^{2}D and ^{2}DD methods were checked by analyzing six laboratory-prepared mixtures of (I) and (II) at various concentrations within the linearity range. Satisfactory recoveries with small standard deviations were

obtained (Table 2), which indicated the high repeatability and accuracy of the two methods.

3.3. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels: 50%, 75%, 100%, 125% and 150% of label claim for each individual drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for each drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy and precision of the proposed methods for determination of each drug. The recoveries ranging from 99.7% to 100.6% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.5% the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [2] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [2], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the proposed methods were found to be 0.4 and 0.3 μg ml^{-1} for (I), and 0.9 and 0.5 μg ml^{-1} for (II), detected by ^2D and ^2DD methods, respectively. While the quantitation limits of the proposed methods were found to be 0.9 and 0.8 μg ml^{-1} for (I), and 2 and 1.2 μg ml^{-1} for (II), determined by ^2D and ^2DD methods, respectively.

The stability of (I) and (II) during the analytical procedures were studied and found to be stable. The two analytes were stable for at least 10 h in solution when they are protected from light.

3.4. Tablets analysis

The two proposed method were applied to the determination of (I) and (II) in commercial tablets. Five replicates determinations were made. Satisfactory results were obtained for both drugs and were in a good agreement with the label claims (Table 2). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding (I) and (II) to the previously analyzed tablets. The recovery of each drug was calculated by comparing the concentrations obtained from the spiked mixtures with those of the pure drug. The results of analysis of the commercial tablets and the recovery study (standard addition method) of both drugs (Table 2) suggested that there is no interference from any excipients, which are present in tablets. The results of determination of (I) and (II) in tablets obtained from the proposed ^2D and ^2DD methods were compared with those of the reference ^2D method [13]. Statistical comparison of the results was performed with regard to accuracy and precision using Student's *t*-test and the F-ratio at 95% confidence level (Table 2). There is no significant difference between the two proposed methods and the reference method with regard to accuracy and precision.

4. Conclusion

The two proposed methods (^2D and ^2DD) can be used for simultaneous determination of trifluoperazine HCl and isopropamide iodide in tablets. The ^2D method is more rapid and simple than the ^2DD method, While the ^2DD method has greater sensitivity and accuracy. The two proposed methods are suitable for routine determination of trifluoperazine HCl and isopropamide iodide in their formulations,

but they can not be considered as stability indicating assays.

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