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A novel simple method for resolving overlapped spectral data with wider range of applicability

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ABSTRACT

Smart simple spectrophotometric method was developed for simultaneous detemination of compunds with interfering spectra in binary mixtures without previous separation, showing significant advantages over the conventional methods regarding minimum data manipulation and applicability. The new method is based on a modification for the ratio subtraction and derivative ratio methods. This modification enabled wider range of application. The proposed method was applied for the determination of brimonidine and timolol in laboratory prepared mixtures with mean percentage recoveries 100.40 ± 2.29 and 101.23 ± 1.30 respectively, and in their pharmaceutical formulation with mean percentage recoveries 101.08 ± 0.44 and 100.66 ± 0.52 respectively. The suggested method was validated according to USP 2005 guidelines and can be applied for routine quality control testing.

KEYWORDS

Spectrophotometry;
Binary mixtures;
Brimonidine;
Timolol and ratio difference method.

INTRODUCTION

Resolving drug mixtures has always been an extremely important issue in analysis of pharmaceuticals, since a large number of dosage forms are formulated as mixtures.

Many methods have been introduced for the analysis of binary mixtures among which the spectrophotometric based methods were the most simple, fast and applicable in almost all laboratories. Several manipulations were performed on the raw overlapping spectral data to enable mixture resolution for example, using different order derivatives,[1-6] derivatives of the ratio spectrum[7-10] and ratio subtraction technique[11].

The aim of the present work was to develop a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and wider range of application over the previously mentioned methods.

To prove the ability of the newly described method in resolving the overlapping spectral data and simultaneous determination of each component, it was applied for the analysis of a mixture of brimonidine and timolol recently introduced into the markets.

Timolol (Ti) is a potent, non-subtype selective β-receptor antagonist. It is used to treat hypertension, congestive heart failure, migraine prophylaxis, and has been widely used in the treatment of open-angle glaucoma and intraocular hypertension.

Brimonidine (Br) is another clonidine derivative that is administered ocularly to lower intraocular pressure in

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patients with ocular hypertension or open-angle glaucoma. The efficacy of brimonidine in reducing intraocular pressure is similar to that of the β-receptor antagonist timolol[11].

Brimonidine and timolol are formulated together in the form of ophthalmic solution used for the treatment of glaucoma.

Theory

The method is based on the fact that upon dividing the absorption spectrum of a compound by another spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. While upon dividing the absorption spectrum of a compound by the absorption spectrum of another compound, a new spectrum (ratio spectrum) will result, (figure 1 and 2). This represents the same basis of derivative ratio and ratio subtraction methods. The difference will be in the manipulation of the resulting data.

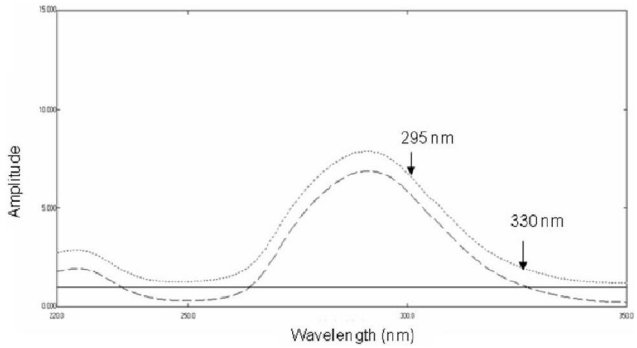

Figure 1 : The ratio spectrum of 20 µg/ml Br (——), 50 µg/ml Ti (----) and a mixture containing 20 µg/ml and 50 µg/ml Ti (·····) using a divisor of 50 µg/ml Ti in distilled water.

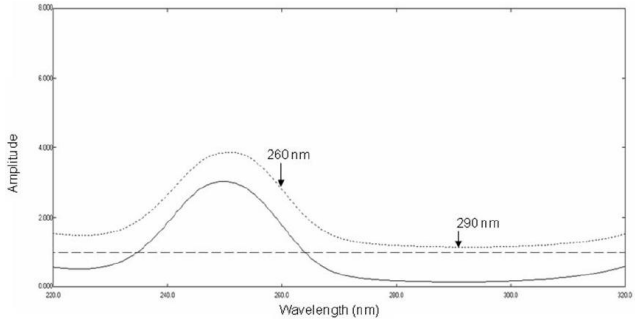

Figure 2 : The ratio spectrum of 20 µg/ml Br (——), 50 µg/ml Ti (---) and a mixrure containing 20 µg/ml and 50 µg/ml Ti (·····) using a divisor of 20 µg/ml Br in distilled water.

In derivative ratio technique the following step would be applying a certain order derivative, while in ratio subtraction technique it will be followed by subtraction of a constant at a certain wavelength then multiplication with the same divisor. The developed method has the advantage of being simpler, as the following step will simply be calculating the difference between any two points in the ratio spectrum.

Mathematically it can be explained as follows:

In the ratio spectrum of a lab mixture of X and Y divided by a divisor Y'

$$P_1 = P_{1X} + K \quad (1)$$

$$P_2 = P_{2X} + K \quad (2)$$

Where, P_1 and P_2 are the amplitudes of the mixture spectrum at λ_1 and λ_2, respectively. P_{1X} and P_{2X} are the amplitudes of X at λ_1 and λ_2, respectively. K is the constant resulting from Y/Y'

$$\Delta P = P1 - P2 = (P_{1X} + K) - (P_{2X} + K) = P_{1X} - P_{2X} \quad (3)$$

So the component Y will be completely cancelled and the difference will represent the X component only.

A calibration curve constructed relating the difference in amplitudes (ΔP) in the ratio spectrum at λ_1 and λ_2 using a certain concentration of Y as a divisor to the corresponding concentration of X, regression equation is computed for the determination of X in the unknown samples of the binary mixture.

Similarly component Y can be obtained by using certain concentration of X as a divisor.

EXPERIMENTAL

(A) Apparatus

SHIMADZU dual beam (Kyoto/ Japan) UV-visible spectrophotometer model UV-1601 PC connected to IBM compatible and an hp1020 laserjet printer. The bundle software, UV. PC personal spectroscopy software version 3.7 (SHIMADZU) was used to process absorption and ratio spectra, the spectral band width was 2 nm and scanning speed was 2800 nm/min.

(B) Reference samples

Brimonidine tartarate and timolol maleate reference standards were kindly supplied by Sigma pharmaceutiacal Co. (Cairo, Egypt).

(C) Pharmaceutical formulation

Combigan® ophthalmic solution manufactured by Allergan (Mayo, Ireland). Batch no. E62201 labeled to contain 2 mg/ml of brimonidine tartarate and 5 mg/ml of timolol.

(D) Materials and reagents

All solvents used were of analytical grade, distilled water was used.

(E) Standard solutions

(1) Stock standard solution 0.1 mg/ml brimonidine in distilled water.
(2) Stock standard solution 0.1 mg/ml timolol in distilled water.

(F) Procedures

(a) Construction of calibration curves

Calibration curve of brimonidine tartarate

Aliquots (0.5-3.0 ml) of Br stock solution (0.1 mg/ml) were transferred into a series of 10 ml volumetric flasks, and the volume was completed with distilled water. The zero order spectra of the prepared solutions were divided by the spectrum of 50 µg/ml timolol. The peak amplitudes of the ratio spectra were measured at 260 and 290 nm.

Calibration graphs relating the differences in the peak amplitudes at the chosen wavelength couple to the corresponding concentrations of Br were constructed, and the corresponding regression equation was computed.

Calibration curve of timolol maleate

Aliquots (1.0-6.0 ml) of Ti stock solution (0.1 mg/ml) were transferred into a series of 10-ml volumetric flasks, and the volume was completed with distilled water. The zero order spectra of the prepared solutions were divided by the spectrum of 30 µg/ml brimonidine. The peak amplitudes of the ratio spectra were measured at 295 and 330 nm.

Calibration graphs relating the differences in the peak amplitudes at the chosen wavelength couple to the corresponding concentrations of Ti were constructed, and the corresponding regression equation was computed.

(b) Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of Br and Ti were prepared, the zero order spectrum of each laboratory prepared mixture was first divided by the spectrum of 50µg/ml timolol and the difference in amplitude between 260 and 290 nm was calculated to determine brimonidine, after substitution in the corresponding regression equation.

The zero order spectra were then divided by the spectrum of 30µg/ml Br and the difference in amplitude between 295nm and 330 nm was calculated to determine timolol, after substitution in the corresponding regression equation.

(c) Application of the proposed method for the simultaneous determination of Br and Ti in combigan ophthalmic solution

0.5 ml of the solution was transferred to 10 ml measuring flask and the volume was completed with distilled water. 1ml of that solution was transferred to another 10ml measuring flask. The procedure was completed as described under 2.6.2. The validity of the method was assessed by applying the standard addition technique.

RESULTS AND DISCUSSION

A simple spectrophotometric method was developed for the simultaneous determination of the components of binary mixtures with overlapping spectra without previous separation. As an example for the application of the new suggested method the binary mixture of Br and Ti was chosen.

The absorption spectra of Br and Ti show high degree of interference as shown in figure 3, that the application of the direct spectrophotometry failed to determine either of them in their mixture.

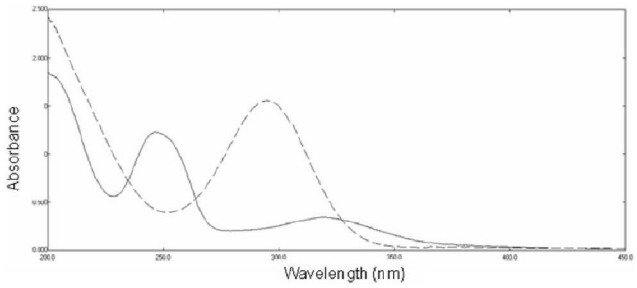

Figure 3 : The absorption spectra of 20 µg/ml Br (——) and 50 µg/ml Ti (---) in distilled water.

The suggested method starts by scanning zero order spectra of the prepared standard solutions of Br and Ti in distilled water. Different divisor concentrations of Ti and Br were tried, for the determination of Br and Ti, respectively, followed by the careful choice of the wavelength couple to correlate their differences in amplitudes to their corresponding concentrations.

Careful choice of the divisor is mandatory; the se-

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lected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentrations 50 µg/ml Ti and 30 µg/ml Br gave the best results regarding average recovery percent when used for the prediction of Br and Ti concentrations, respectively.

Different wavelengths were chosen on the ratio spectra of the two drugs and the linearity at those wavelengths singly was assessed. A good linearity at 260 and 290 nm for Br, and at 295 and 330 nm for Ti was obtained, (TABLE 1).

TABLE 1 : Correlation coefficients (r) at different wavelengths on the ratio spectrum of Br and Ti.

Wavelength (nm)		260	290	295	330
r	Br	0.9999	0.9997		
	Ti			0.9998	0.9993

A linear correlation was obtained between the difference in amplitude of the ratio spectra at 260 and 290 nm for Br. and at 295 and 330 nm for Ti, against the corresponding concentration of Br and Ti, respectively, (TABLE 2).

TABLE 2 : The regression equations and correlation coefficients of the difference in amplitude at different wavelengths in the ratio spectrum of Br and Ti.

Component	Wavelength	Equation	r
Br	260-290nm	y=0.0762x+0.1105	0.9999
Ti	295-330nm	y=0.0811x+0.078	0.9998

The proposed method was successfully applied for the simultaneous determination of Br and Ti in laboratory prepared mixtures containing different ratios of Br and Ti. The mean percentage recoveries and standard deviations were shown in TABLE 3.

TABLE 3 : Determination of Br and Ti in laboratory prepared mixtures by the proposed method.

Concentration µg/ml		Recovery % of Br	Recovery % of Ti
Br	Ti	260 - 290 nm	295-330 nm
10.00	50.00	96.52	101.06
20.00	20.00	100.74	101.42
20.00	40.00	100.43	102.53
20.00	50.00	100.82	101.23
25.00	30.00	103.33	102.26
30.00	50.00	99.58	98.87
Mean		100.40	101.23
Std		2.29	1.30
RSD		2.28	1.28

The suggested method was found to be applicable and valid for the analysis of Combigan® Ophthalmic solution with no interference of the excipients. The validity of the proposed procedure was assessed by applying the standard addition technique (TABLE 4).

TABLE 4 : Determination of Br and Ti in combigan® eye drops by the proposed method and application of standard addition technique.

Product	Proposed Method	Standard addition			
		Taken	Added	Found	Recovery %
		µg/ml	µg/ml	µg/ml	
Br in Combigan® eye drops 2 µg/ml Br and 5 µg/ml Ti B.No. E62201	101.08±0.44	10.00	5.00	4.93	98.60
			10.00	10.14	101.40
			20.00	20.06	100.30
		Mean		100.10	
		SD		1.41	
		RSD%		1.41	
Ti in Combigan® eye drops 2 µg/ml Br and 5 µg/ml Ti B.No. E62201	100.66±0.52	25.00	20.00	20.15	100.75
			25.00	24.91	99.64
			30.00	30.26	100.87
		Mean		100.42	
		SD		0.68	
		RSD%		0.68	

The validation parameters according to USP 2005 guidelines[12] of accuracy, repeatability and intermediate precision are presented in TABLE 5. The data showed that the results obtained by the new method are accurate, precise, robust and specific over the specified range.

TABLE 5 : Assay validation sheet of the proposed methods for the determination of Br and Ti.

Parameter	Br	Ti
Accuracy (mean ± SD)	100.16±1.32	100.17±1.66
Specificity	100.40±2.29	101.23±1.30
Precision		
Repeatability *	100.21 ± 0.73	100.06 ± 0.32
Intermediate precision**	100.07 ± 0.40	100.09 ± 0.39
Linearity		
Slope	0.0762	0.0811
Intercept	0.1105	0.078
Correlation coefficient (r)	0.9999	0.9998
Range	5 – 30 µg/ml	10 – 60 µg/ml

*** The intraday (n = 3), average of three concentrations (10, 15, 20 µg/ml) for Br and (20, 30, 40 µg/ml) for Ti repeated three times within the day; ** The interday (n = 3), average of three concentrations (10, 15, 20 µg/ml) for Br and (20, 30, 40 µg/ml) for Ti repeated three times in three successive days.**

The suggested method was able to determine either component in the binary mixture without limitations, whereas the ratio subtraction method[11] can only determine the component with the less extended spectrum, therefore enabling a wider range of application.

CONCLUSION

The present work described a simple method for manipulating overlapped spectral data for simultaneous determination of compounds in binary mixtures. The method was successfully applied for simultaneous determination of brimonidine and timolol in pure powder form and in pharmaceutical formulation. The method was validated and showed that it can be used for the regular quality control testing due to its simplicity and wide range of applicability.

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