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SHORT COMMUNICATION

A smart simple spectrophotometric method for simultaneous determination of binary mixtures

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Spectrophotometry;
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Abstract A new simple spectrophotometric method was developed for the simultaneous determination of drugs with interfering spectra in binary mixtures without previous separation. The new method is based on a simple modification for the ratio subtraction method. This modification enabled wider range of application. The proposed ratio difference 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 ratio difference method was validated according to USP guidelines and can be applied for routine quality control testing.

1. Introduction

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].

A new method ratio difference has been developed having the advantages of minimal data processing and wider range of application. The method was applied for the analysis of a mixture of brimonidine tartarate (Br) and timolol maleate (Ti) recently introduced into the markets.

2. Theory of the ratio difference method

Upon dividing the absorption spectrum of a compound by a spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. However, upon dividing the absorption spectrum of a compound by the absorption spectrum of another compound, a new spectrum (ratio spectrum) will result (Figs. 1 and 2).

Mathematically it can be explained as follows:

In the ratio spectrum of a laboratory mixture of X and Y divided by a divisor Y′

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

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

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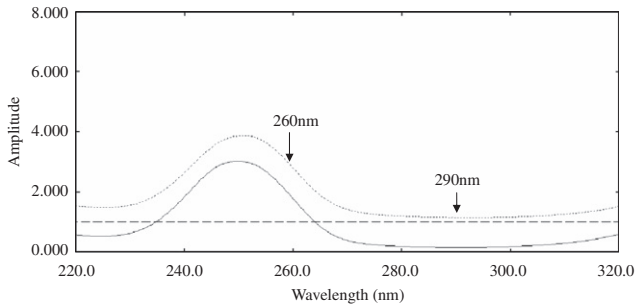

Figure 1 The ratio spectrum of 20 μg/mL Br (——), 50 μg/mL Ti (- - -) and a mixture containing 20 μg/mL Br and 50 μg/mL Ti (···) using a divisor of 50 μg/mL Ti in distilled water.

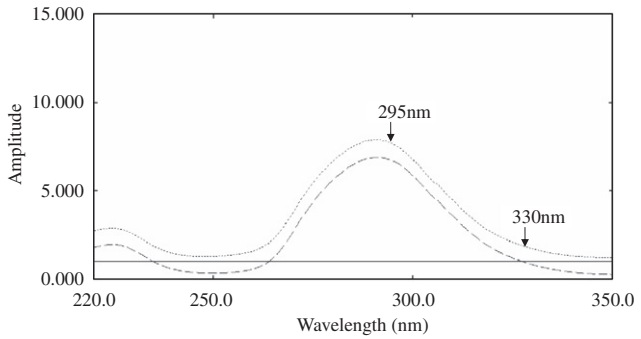

Figure 2 The ratio spectrum of 20 μg/mL Br (——), 50 μg/mL Ti (- - -) and a mixture containing 20 μg/mL Br and 50 μg/mL Ti (···) using a divisor of 20 μg/mL Br in distilled water.

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, and K is the constant resulting from Y/Y′.

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

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

Component X in a binary mixture can be determined from a calibration curve that relates the difference in amplitudes (ΔP_{1-2}) 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. Similarly component Y can be obtained by using a certain concentration of X as a divisor.

3. Materials and method

SHIMADZU UV-1601 dual beam UV–visible spectrophotometer (Kyoto/Japan), Br and Ti reference standards (kindly supplied by Sigma Pharmaceutiacal Co. Cairo, Egypt), stock standard solution of 0.1 mg/mL Br and 0.1 mg/mL Ti in distilled water.

3.1. Procedures

3.1.1. Construction of calibration curves

The zero order spectra of different solutions of Br and Ti were divided by the spectra of 50 μg/mL Ti and 20 μg/mL Br, respectively. The difference in the peak amplitudes ΔP at the ratio spectra was measured at 260 and 290 nm ($\Delta P_{260-290\ nm}$) for Br and at 295 and 330 nm ($\Delta P_{295-330\ nm}$) for Ti. Calibration graphs relating ΔP at the chosen wavelength couples to the corresponding concentrations of Br and Ti were constructed, and the corresponding regression equations were computed.

3.1.2. Analysis of laboratory prepared mixtures

The zero order spectra of different laboratory prepared mixtures were divided by the spectrum of 50 μg/mL Ti ($\Delta P_{260-290}$ was recorded) and the spectrum of 20 μg/mL Br ($\Delta P_{295-330\ nm}$ was recorded) for determination of Br and Ti, respectively.

3.1.3. Application of the proposed ratio difference method for the simultaneous determination of Br and Ti in Combigan ophthalmic solution

0.5 mL of the solution was transferred to another 100 mL measuring flask, and the volume was completed with distilled water. The concentrations of Br and Ti were obtained as in Section 3.1.2.

4. Results and discussion

The proposed ratio difference method can be applied for resolving absorption spectra of two components with high degree of overlap as in the case of Br and Ti (Fig. 3), where the application of the direct spectrophotometry failed to determine either of them in their mixture.

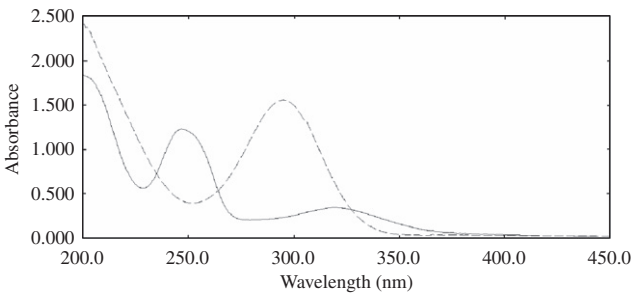

Figure 3 The absorption spectra of 20 μg/mL Br (——) and 50 μg/mL Ti (- - -) in distilled water.

Table 1 Determination of Br and Ti in laboratory prepared mixtures by the proposed ratio difference method.

Concentration (μg/mL)		Recovery (%)	
Br	Ti	Br $\Delta P_{260-290\ nm}$	Ti $\Delta P_{295-330\ nm}$
10.00	50.00	96.52	101.06
20.00	20.00	100.75	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

Table 2 Determination of Br and Ti in Combigan® eye drops by the proposed ratio difference method and application of standard addition technique.

Product in Combigan® eye drop[a]	Standard addition				Recovery (mean ± SD, %)	
	Taken (μg/mL)	Added (μg/mL)	Found (μg/mL)	Recovery (%)	Proposed method	Standard addition
Br	10.00	5.00	4.93	98.60	101.08 ± 0.44	100.10 ± 1.41
		10.00	10.14	101.40		
		20.00	20.06	100.30		
Ti	25.00	20.00	20.15	100.75	100.66 ± 0.52	100.42 ± 0.68
		25.00	24.91	99.64		
		30.00	30.26	100.87		

[a]Combigan® eye drops, batch no. E62201 labeled to contain 2 mg/mL of brimonidine tartarate and 5 mg/mL of timolol.

Table 3 Assay validation sheet of the proposed ratio difference method 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[a]	100.21 ± 0.73	100.06 ± 0.32
Intermediate precision[b]	100.07 ± 0.40	100.09 ± 0.39
Linearity		
Slope	0.0762	0.0811
Intercept	0.1105	0.0780
Correlation coefficient r	0.9999	0.9998
Range (μg/mL)	5–30	10–60

[a]The intraday ($n=3$), average of three concentrations (10, 15 and 20 μg/mL) for Br and (20, 30 and 40 μg/mL) for Ti repeated three times within the day.

[b]The interday ($n=3$), average of three concentrations (10, 15 and 20 μg/mL) for Br and (20, 30 and 40 μg/mL) for Ti repeated three times on three consecutive days.

The method comprises two critical steps, the first is the choice of the divisors, the selected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentrations 50 μg/mL Ti and 20 μg/mL Br gave the best results regarding the average percentage recovery when used for the prediction of Br and Ti concentrations, respectively. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and a good linearity is present at each wavelength individually.

Linear correlation was obtained between the differences in amplitude at 260 and 290 nm ($\Delta P_{260-290\ nm}$) for Br and at 295 and 330 nm ($\Delta P_{295-330\ nm}$) for Ti, against the corresponding concentrations of Br and Ti, respectively.

The proposed ratio difference method was applied for the determination of brimonidine and timolol in laboratory prepared mixtures (Table 1), and in their pharmaceutical formulations (Table 2). The suggested ratio difference method was validated according to USP guidelines [12] and can be applied for routine quality control testing, as shown in Table 3.

The suggested ratio difference method showed a wider range of application over the ratio subtraction method; it was able to determine both components in the binary mixture without limitations, whereas the ratio subtraction method [11] failed to determine the component with the more extended spectrum (Br in this case).

Regarding simplicity, the proposed ratio difference method showed minimal data manipulation; instead of applying a certain order derivative in the derivative ratio method [7–10] or subtraction of a constant and re-multiplication by the divisor in the ratio subtraction method, simply the following step will be calculating ΔP at any two wavelengths in the ratio spectrum.

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2012.04.004.

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