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Application of TLC-densitometry, first-derivative UV-spectrophotometry and ratio derivative spectrophotometry for the determination of dorzolamide hydrochloride and timolol maleate

Lories I. Bebawy *

National Organization for Drug Control and Research, 6 Hussen Kamal el Deen, Ben-el-sariat, Dokki, Giza 12311, Egypt

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Abstract

Three methods are described for the simultaneous determination of dorzolamide hydrochloride (DORZ) and timolol maleate (TIM) in ophthalmic solutions. The first method is based on application of thin layer chromatographic separation of both drugs followed by the densitometric measurements of their spot areas. After separation on silica gel GF_{254} plates, using methanol–ammonia 25% (100:1.5 v/v) as the mobile phase, the chromatographic zones corresponding to the spots were scanned at 253 and 297 nm, respectively. The calibration function was established in the ranges of 2–18 μg for DORZ and 0.5–4.5 μg for TIM. The second method depends on first derivative ultraviolet spectrophotometry, with zero-crossing measurement method. The first derivative values D_1 at 250.2 and 312.5 nm were selected for the assay of DORZ and TIM, respectively. Calibration graphs follow Beer's law in the range 10–64 and 2.5–16 μg ml^{-1}, respectively. The third method is based on ratio first derivative spectrophotometry. The signals in the first derivative of the ratio spectra at 244 and 306.2 nm were selected to determine DORZ and TIM in the mixture and calibration graphs are linear in the range of 5–40 and 5.0–17.5 μg ml^{-1}, respectively. The proposed methods were successfully applied to the determination of these compounds in synthetic mixtures and in pharmaceutical preparations. The proposed methods are simple, rapid and suitable for quality control application. © 2002 Published by Elsevier Science B.V.

Keywords: Dorzolamide hydrochloride; Timolol maleate; Pharmaceutical formulations; Simultaneous determination; TLC-densitometry; First derivative/ratio-derivative spectrophotometry

1. Introduction

Dorzolamide hydrochloride (DORZ), (4S-trance)-4-(Ethylamino)-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide hydrochloride, is a carbonic anhydrase in-

* Tel.: +20-202-749-6077; fax: +20-202-305-9626.
E-mail address: a.c.afri@egyptonline.com (L.I. Bebawy).

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hibitor with action similar to those of acetazolomide. It is used topically in the management of open-angle glaucoma and ocular hypertension, either alone or as adjunctive therapy with a topical beta blocker [1].

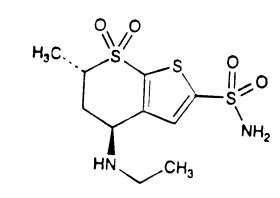
Timolol maleate (TIM), (S)-1-*tert*-Butylamino-3-(4-morphilino-1,2,5-thiadiazol-3-yloxy) propan-2-ol maleate, is a non-cardioselective beta blocker. It is used as the maleate in the management of glaucoma, hypertension, angina pectories and myocardial infarction. Eye drops containing timolol maleate equivalent to 0.25 and 0.5% of timolol are used to reduce raised intra-ocular pressure in open angle glaucoma and ocular hypertension [1].

A mixed formulation of both dorzolamide hydrochloride and timolol maleate is employed in the treatment of elevated intra ocular pressure in patients with open-angle glaucoma or pseudo exfoliative glaucoma when beta-blocker monotherapy is not sufficient.

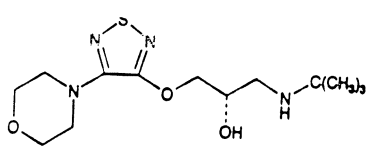
Their chemical structures are shown in Scheme 1.

Scientific literature reports HPLC methods for the individual determination of DORZ in biological fluids and ophthalmic solution [2–6].

Various methods have been used for the determination of timolol maleate either alone or in combination with pilocarpine including spectrophotometry [7–11], HPLC [12–14], polarography [15], potentiometry [16] and GC [17]. Recently, first-derivative electronic absorption spectroscopy was used for the determination of timolol maleate in a pharmaceutical ophthalmic solution [18].



Dorzolamide



Timolol

Scheme 1.

The British Pharmacopoeia [19] recommends an ultraviolet spectrophotometric method with a tedious previous extraction procedure for the analysis of timolol. The USP24 Pharmacopoeia [20], also describes a spectrophotometric method for the determination of timolol. Only one HPLC method has been reported for their simultaneous quantification in two component mixtures [21].

The simultaneous determination of both compounds by conventional spectrophotometry without prior separation procedures is not possible due to the overlapping of their UV spectra and the presence of interfering excipients. However, densitometry and derivative spectrophotometry allows the quantification of the two compounds in a simple way. For routine pharmaceutical quality control programs, they are more rapid and of lower cost when compared with HPLC.

The aim of this work was to demonstrate the capability of the TLC, first derivative and ratio derivative methods to resolve and overcome the problem of overlapping spectral bands. The proposed methods allow the determination of DORZ and TIM without the need for prior separation.

2. Experimental

2.1. Apparatus

- The SHIMADZU 1601 PC double beam UV–VIS spectrophotometer with 1 cm quartz cuvettes, a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software was equipped with a Hewlett Packard printer was used for all the absorbance measurements and treatment of data.
- Densitometer-SHIMADZU dual-wavelength flying SC 9301.
- TLC plates-20 × 20 cm with 0.25 mm thickness, silica gel GF_{254} (E. Merck. Darmstatd, Germany).

2.2. Materials

- Dorzolamide hydrochloride, kindly supplied by Global Napi pharmaceuticals, 6 October City, Egypt. The purity of the sample was found to be $100.25 \pm 0.46\%$ according to the reference method [21].
- Timolol maleate, kindly supplied by Global Napi pharmaceuticals 6 October City, Egypt. Its purity was found to be $99.96 \pm 0.51\%$ according to the reference method [21].
- Cosopt 2% ophthalmic solutions, Global Napi, batch No. HJ2040. Each 1 ml is claimed to contain 20.00 mg dorzolamide, 5.00 mg timolol 2.94 mg sodium citrate dihydrate, 0.075 mg benzalkonium chloride, 4.75 mg hydroxyethylcellulose and 16.00 mg mannitol.

2.3. Reagent and standard stock solutions

All chemicals and solvents used were of analytical grade.

- Methanol (E. Merck).
- Methanol–ammonia 25%(100:1.5 v/v)—mobile phase for densitometric method.
- Sodium hydroxide, 0.1 M aqueous solution.

2.3.1. Standard stock solutions

- Dorzolamide stock solutions 4 mg ml^{-1} and timolol 1 mg ml^{-1} in methanol for densitometric method. Their solutions were found to be stable for at least one week if they had been stored in a refrigerator and also stable during the actual analysis.
- Aliquots of the above methanol solutions were diluted separately with 0.1 M sodium hydroxide to contain 200 μg ml^{-1} DORZ and 50 μg ml^{-1} TIM for first-derivative and ratio derivative methods. Their solutions in 0.1 M sodium hydroxide were freshly prepared.

2.4. Sample solutions

Pharmaceuticals: 5 ml of commercial ophthalmic solution (100 mg DORZ and 25 mg TIM) was transferred into a 25 ml volumetric flask and diluted to volume with methanol for densitometric method.

5 ml of methanol solution was diluted to 100 ml with 0.1 M sodium hydroxide (200 μg ml^{-1} DORZ and 50 μg ml^{-1} TIM) for first derivative and ratio derivative method.

Laboratory mixtures: synthetic mixtures containing the same amounts of DORZ and TIM as in the pharmaceutical samples were dissolved in methanol. They were further diluted to yield suitable concentrations.

All these solutions were used to establish the validity of the methods.

3. Procedures

3.1. Calibration curves for densitometric method

Different amounts ranging from 0.5 to 4.5 ml of standard stock solutions of DORZ and TIM were transferred into separate 5-ml volumetric flasks and the volume was made up with methanol. Aliquots of 5 μl of each solution were applied to a separate precoated thin layer chromatographic plates (20×20 cm) using 50 μl micropipette. The plates were placed in chromatographic jar previously saturated with the mobile phase methanol–ammonia 25% (100:1.5 v/v) for 30 min. The plates were developed at room temperature by ascending migration over a distance 16 cm. After elution, the plates were removed, air dried and the spots were visualized under UV lamp at 254 nm. The chromatograms were scanned with the spectrodensitometer at 253 and 297 nm for DORZ and TIM, respectively. The calibration curves were plotted representing the relationship between the recorded area under the peak and the corresponding concentrations and the regression equations were calculated.

3.2. Calibration curves for spectrophotometric measurements

3.2.1. First derivative method

Working standard solutions of DORZ and TIM mixtures in 0.1 M sodium hydroxide (con-

taining 5 μg ml^{-1} of TIM and increasing concentrations of DORZ ranging from 10.0 to 64.0 μg ml^{-1}) were prepared from standard stock solutions of DORZ (200 μg ml^{-1}) and TIM (50 μg ml^{-1}) in 0.1 M sodium hydroxide. The first order spectra (D_1)of these solutions were recorded over the wavelength range 220–350 nm against 0.1 M sodium hydroxide as a blank. The derivative values at 250.2 nm $D_{1(250.2)}$ were measured for the determination of DORZ in the presence of TIM.

Working standard solutions of DORZ and TIM mixtures in 0.1 M sodium hydroxide (containing 20.0 μg ml^{-1} of DORZ and increasing concentrations of TIM ranging from 2.5 to 16 μg ml^{-1}) were prepared using the same standard stock solutions. The D_1 spectra of these solutions were also recorded between 220 and 350 nm and the derivative values at 312.5 nm $D_{1(312.5)}$ were measured for the determination of TIM in the presence of DORZ.

3.2.2. Ratio spectra first derivative spectrophotometry

Samples were prepared in 10 ml volumetric flasks containing 5–40 μg ml^{-1} of DORZ and 5–17.5 μg ml^{-1} of TIM in 0.1 M sodium hydroxide. Absorption spectra were recorded and divided by the spectrum of the standard solution of TIM (17.5 μg ml^{-1} in 0.1 M sodium hydroxide). All spectra were stored in the IBM-PC. The first derivatives of the ratio spectra were calculated with $\Delta\lambda = 8$ nm. In the binary mixture, the amount of DORZ can be determined by measuring the first derivative value at 244 nm in the range of 220–340 nm.

A similar procedure was followed for different concentrations of TIM when DORZ was 40 μg ml^{-1}. In the same way as described above, the content of TIM was determined by selecting the first derivative of the ratio spectrum $\Delta\lambda = 4$ nm in the range 200–340 nm and by measuring the value at 306.2 nm.

3.3. Sample preparation

The methods described above were applied to the prepared solutions as under Section 2.4 sample solutions.

4. Results and discussion

4.1. TLC-densitometric method

Instrumental planar chromatography with precise application of the samples and computer controlled evaluation and quantification of the developed chromatograms, has been considered as reliable for purity control and quantitative drug testing [22]. Experimental conditions, such as mobile phase composition, scan mode and speed and wavelength of detection, were optimized to provide accurate, precise and reproducible results for both DORZ and TIM. The chosen scan mode was the zigzag mode and the wavelengths of scanning were chosen to be 253 and 297 nm for DORZ and TIM, respectively. The differences between the R_f values of the investigated drugs (0.67 and 0.56 for DORZ and TIM, respectively) were obtained by the system containing methanol–ammonia 25% (100:1.5 v/v). The equilibration time required before development is important to achieve homogeneity of the atmosphere thus minimizes the evaporation of the solvent from the TLC plate during the development, therefore, the saturation time of the tank has been optimized and found to be 30 min.

By applying this technique, a linear correlation was obtained between the area under the peak and the concentration; the analytical data of the calibration curves are summarized in (Table 1).

The proposed TLC method is very simple, rapid and uses a minimal volume of solvents, compared with the other separation techniques. Further, an extremely large number of samples can be analyzed at the same time without comprising accuracy, the proposed method is thus suitable for quality control laboratories, where economy and time is essential.

4.2. First derivative spectrophotometry

Fig. 1 shows the absorption zero-order UV spectra of DORZ with a maximum at 258.8 nm,

Table 1
Analytical data for the calibration graphs ($n = 5$) for the determination of dorzolamide hydrochloride and timolol maleate by the proposed methods

Parameters	Dorzolamide hydrochloride			Timolol maleate		
	TLC	First derivative	Ratio spectra	TLC	First derivative	Ratio spectra
Concentration range	2.0–18.0	10.0–64.0	5.0–40.0	0.5–4.5	2.5–16.0	5.0–17.5
Regression equation (Y)[a]						
Slope (b)	2.112	6.02×10^{-4}	0.178	4.156	8.71×10^{-4}	0.65
RSD of slope	5.82×10^{-4}	6.45×10^{-7}	1.74×10^{-4}	6.85×10^{-4}	1.25×10^{-6}	2.45×10^{-3}
Intercept (a)	0.84	-1×10^{-4}	0.042	1.121	-1×10^{-4}	0.14
RSD of intercept	1.31×10^{-3}	1.64×10^{-6}	8.2×10^{-4}	8.16×10^{-4}	3.70×10^{-6}	4.08×10^{-3}
Correlation coefficient (r)	0.9995	1.00	0.9983	0.9999	0.9989	0.9990

[a] $Y = a + bc$ where 'c' is the concentration in μg for TLC and in μg ml^{-1} for first derivative and ratio spectra derivative. 'y' is the absorbance.

and of TIM with a maximum at 294.8 nm. Due to the extensive overlap of the spectral bands of the two drugs, conventional UV spectrophotometry cannot be used for their individual determination in a binary mixture. However, zero-crossing first-order derivative spectrophotometry permits a more selective identification and determination of the two drugs in a mixture. The zero-crossing method involves measurements of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the individual component.

Fig. 2 shows the D_1 spectra of DORZ and TIM (the zero-crossing wavelength points are indicated). The selection of the optimum wavelength is based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration, it is not affected by the concentration of any other component and gives a near-zero intercept on the ordinate axis of the calibration curve. Therefore, 312.5 nm (zero-crossing wavelength point of DORZ) and 250.5 nm (zero-crossing wavelength point of TIM) were chosen as optimum working wavelengths for the simultaneous determination of DORZ and TIM in a binary mixture, respectively. Measurements of the absolute values of the derivative spectrum taken at these wavelengths gave the best linear response to the analyte concentration.

Fig. 3 shows a typical set of D_1 spectra of 5.0 μg ml^{-1} TIM plus increasing amounts of DORZ (10–64 μg ml^{-1}) and the D_1 spectrum of TIM alone (5.0 μg ml^{-1}). Analogously, Fig. 4 exhibits a series of D_1 spectra of mixtures of 20.0 μg ml^{-1}

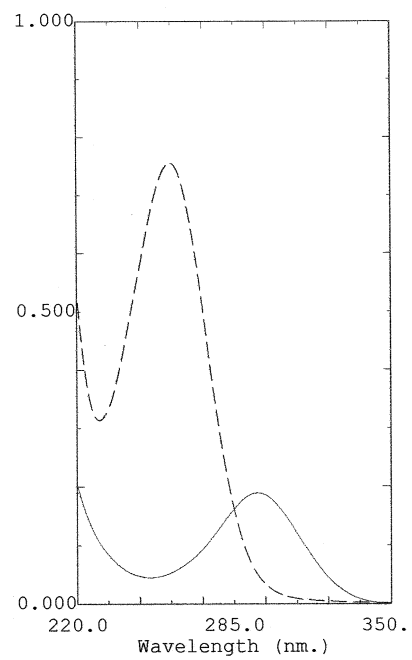


Fig. 1. Absorption (zero-order) UV spectra of 20.0 μg ml^{-1} dorzolamide (- - -), 5.0 μg ml^{-1} timolor (—), in 0.1 M sodium hydroxide.

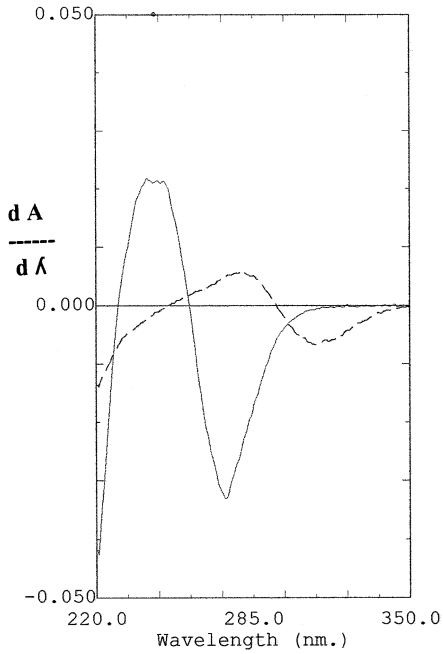


Fig. 2. First-order derivative of 20.0 μg ml^{-1} dorzolamide (—), 5.0 μg ml^{-1} timolol (- - -), in 0.1 M sodium hydroxide.

DORZ plus increasing amounts of TIM (2.5–16 μg ml^{-1}) and the D_1 spectrum of DORZ alone (20.0 μg ml^{-1}). It is interesting to note the distinct isosbestic points in Fig. 3 at 312.5 nm (zero-crossing wavelength points of DORZ) and in Fig. 4 at 250.5 nm (zero-crossing wavelength points of TIM) irrespective of their concentration.

Under the experimental conditions described above, linear relationships between selected derivative values from D_1 spectra of drugs tested and their concentrations were observed as shown in (Table 1).

The mutual interference between the two drugs was also investigated. A series of five working standard solutions containing 10–70 μg ml^{-1} of DORZ in 0.1 M sodium hydroxide and a series of five working standard solutions containing 2–18 μg ml^{-1} of TIM in the same solvent were analyzed by the proposed method. The derivative values D_1 at 250.2 and 312.5 nm, were measured for the determination of DORZ and TIM, respectively. The following linear regression equations were obtained through regression analysis of data:

$$D_{1(250.2)} = 2.75 \times 10^{-4}C - 0.95 \times 10^{-3}$$

$$D_{1(312.5)} = 1.016 \times 10^{-3}C - 2 \times 10^{-4}$$

The slopes and intercepts do not differ significantly from those obtained from the analysis of mixed standard solutions and, therefore, we can conclude that no interference occur in the determination of each substance in the presence of the other.

4.3. Ratio spectra first derivative spectrophotometry

Fig. 5 shows the ratio spectra of different DORZ standards (spectra divided by the spectrum of a 17.5 μg ml^{-1} TIM) and their first derivatives. The first derivative values at 244 nm corresponding to a maximum wavelength are proportional to the DORZ concentration.

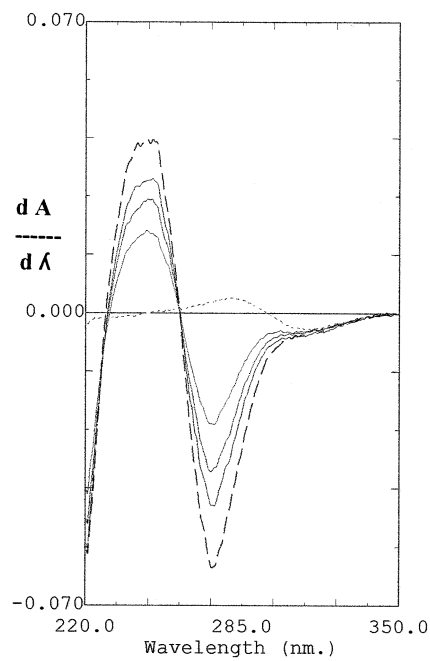


Fig. 3. First-order derivative spectra of mixtures containing 5.0 μg ml^{-1} timolol plus increasing amounts of dorzolamide ranging from 10.0 to 64.0 μg ml^{-1} (—), and first-order derivative spectrum of 5.0 μg ml^{-1} timolol (- - -) in 0.1 M sodium hydroxide.

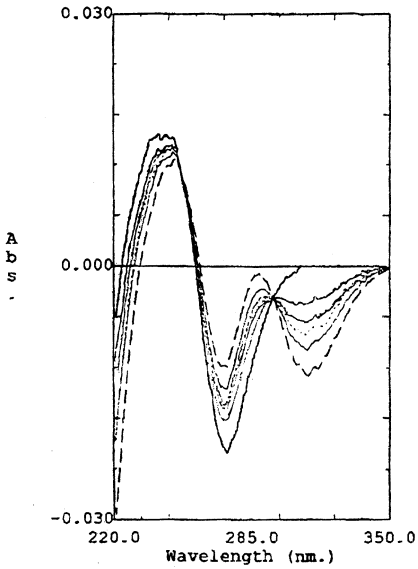


Fig. 4. First-order derivative spectra of mixtures containing 20.0 μg ml^{-1} dorzolamide plus increasing amounts of timolol ranging from 2.5 to 16.0 μg ml^{-1} (—), and first-order derivative spectrum of 20.0 μg ml^{-1} dorzolamide (- - -), in 0.1 M sodium hydroxide.

For determining the other component, TIM, an analogous procedure was followed. Fig. 6 shows the divided spectra of different standards of TIM and their first derivatives. Calibration graphs gave straight lines by measuring at 306.2 nm, corresponding to a maximum wavelength. Under the experimental conditions described, standard calibration curves for DORZ and TIM were constructed by plotting the absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in (Table 1). The correlation coefficient were 0.9989 and 0.9998 indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations (R.S.D.) were found to be less than 1% indicating reasonable repeatability of the proposed method.

In order to assess the precision (R.S.D.%) the accuracy (E_r%) of the proposed methods for assay each drug in the presence of the other, synthetic mixtures of DORZ and TIM were carried. The results obtained for the recovery of both drugs,

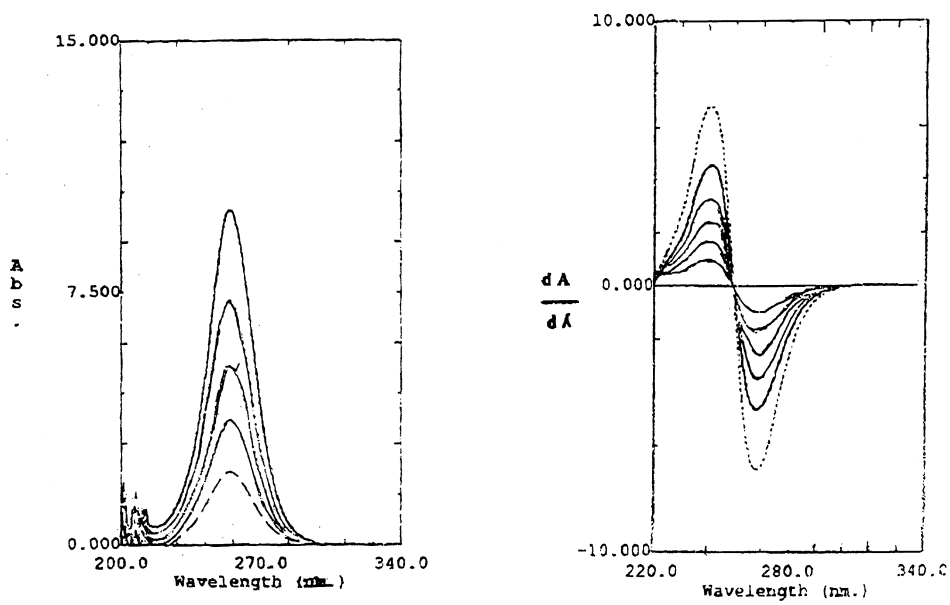


Fig. 5. (a) Ratio spectra of dorzolamide (a) 5.0 μg ml^{-1}; (b) 15.0 μg ml^{-1}; (c) 20.0 μg ml^{-1}; (d) 30.0 μg ml^{-1}; (e) 40.0 μg ml^{-1}; when 17.5 μg ml^{-1} timolol used as a divisor in 0.1 M sodium hydroxide ($\Delta\lambda = 8$ nm). (b) First derivative of the ratio spectra of dorzolamide of (a) 5.0 μg ml^{-1}; (b) 15.0 μg ml^{-1}; (c) 20.0 μg ml^{-1}; (d) 30.0 μg ml^{-1}; (e) 40.0 μg ml^{-1}; when 17.5 μg ml^{-1} timolol used as divisor in 0.1 M sodium hydroxide ($\Delta\lambda = 8$ nm).

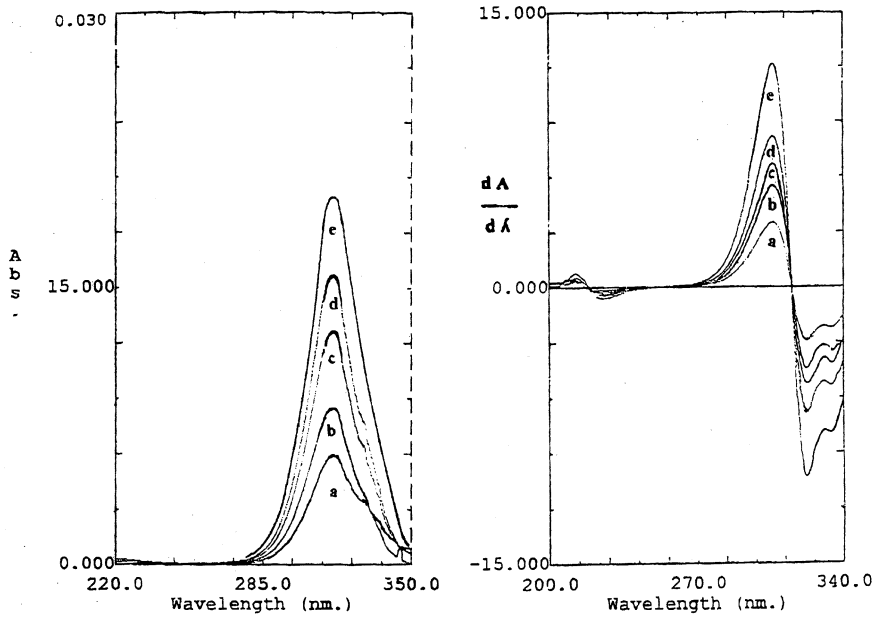


Fig. 6. (a) Ratio spectra of timolol (a) 5.0 μg ml^{-1}; (b) 7.5 μg ml^{-1}; (c) 10.0 μg ml^{-1}; (d) 12.5 μg ml^{-1}; (e) 17.5 μg ml^{-1}, when 40.0 μg ml^{-1} dorzolamide used as divisor in 0.1 M sodium hydroxide ($\Delta\lambda = 4$ nm). (b) First derivative of the ratio spectra of a dorzolamide of (a) 5.0 μg ml^{-1}; (b) 7.5 μg ml^{-1}; (c) 10.0 μg ml^{-1}; (d) 12.5 μg ml^{-1}; (e) 17.5 μg ml^{-1}, when 40.0 μg ml^{-1} dorzolamide used as divisor in 0.1 M sodium hydroxide ($\Delta\lambda = 4$ nm).

Table 2
Precision and accuracy for the determination of dorzolamide hydrochloride and timolol maleate by the proposed methods

Drug	Added[d]	Found		
		Mean ± S.D.[a]	R.S.D.%[b]	E_r%[c]
TLC method				
Dorzolamide hydrochloride	12.000	12.060 ± 0.050	0.41	−0.50
Timolol maleate	3.000	2.986 ± 0.011	0.37	0.47
First derivative				
Dorzolamide hydrochloride	40.000	40.50 ± 0.120	0.30	−0.13
Timolol maleate	10.000	9.990 ± 0.012	0.12	0.10
Ratio spectra derivative				
Dorzolamide hydrochloride	32.000	31.959 ± 0.100	0.31	0.13
Timolol maleate	8.000	7.987 ± 0.041	0.51	0.16

[a] Mean and S.D. for three determinations.
[b] Relative standard deviation.
[c] Percentage relative error.
[d] The concentration of the simulated mixtures was mentioned in μg for TLC and in μg ml^{-1} for both first derivative and ratio spectra derivative.

(Table 2), showed that the precision and accuracy were very satisfactory. The R.S.D. for both drugs were less than 1%.

The proposed methods were applied to the recovery of DORZ and TIM in commercial formulations which comprise the binary mixture (20

Table 3
Determination of dorzolamide hydrochloride and timolol maleate combination in synthetic mixtures and commercial ophthalmic solutions by the proposed methods

	Analytical methods mean found[a] % ± S.D.			
Synthetic mixtures	TLC	First derivative	Ratio spectra derivative	Reported method [21][c]
For DORZ	100.03 ± 0.57	99.67 ± 0.82	99.54 ± 0.34	
	$t = 1.87$	$t = 0.67$	$t = 0.57$	99.38 ± 0.53
	$F = 1.16$	$F = 2.39$	$F = 2.43$	
For TIM	99.57 ± 0.94	99.39 ± 0.95	100.71 ± 0.31	
	$t = 0.62$	$t = 0.97$	$t = 0.66$	99.89 ± 0.67
	$F = 1.97$	$F = 2.01$	$F = 4.67$	
Cosopt 2%	99.78 ± 1.03	99.77 ± 0.43	100.0 ± 0.64	(2.306)[b]
For DORZ	$t = 0.77$	$t = 1.28$	$t = 1.68$	(6.39)[b]
	$F = 3.78$	$F = 1.52$	$F = 1.46$	
For TIM	100.34 ± 0.75	99.55 ± 1.03	100.32 ± 0.54	
	$t = 1.00$	$t = 0.62$	$t = 1.13$	
	$F = 1.25$	$F = 2.36$	$F = 1.54$	
Recovery[c]				
For DORZ	100.64 ± 0.46	100.11 ± 0.72	100.16 ± 0.51	
For TIM	99.3 ± 0.79	100.50 ± 0.31	99.67 ± 0.72	

[a] Mean and S.D. for five determinations; percentage recovery for the labeled claim amount.
[b] Theoretical values for t- and F- at 95% confidence limit.
[c] For standard addition of 50% of nominal content ($n = 5$).

mg for DORZ and 5 mg for TIM in 1 ml). The results presented in (Table 3) are in good agreement with the labeled amount.

The recovery of the proposed methods was tested by adding known amount (standard addition) of DORZ and TIM to the ophthalmic solution.

The results obtained in (Table 3) were statistically compared with the reported HPLC [21] using Student t- and the F-tests. As shown from the table, the calculated t- and F- values were less than the theoretical ones, indicating no significant difference between the proposed and reported methods.

5. Conclusions

TLC, first derivative and ratio spectra derivative spectrophotometry are suitable technique for the reliable analysis of commercial formulations containing combinations of DORZ and TIM. The most striking features of the proposed methods are their simplicity, sensitivity, selectivity and rapidity, which renders suitable for routine analysis in control laboratories. TLC method is more sensitive than the first derivative and ratio derivative spectrophotometry and a large number of samples can be analyzed within a short time as from 6 to 8 samples can be applied to one plate. The proposed methods permit direct determination of binary mixtures without previous separations. Moreover, it has many advantages over other separation technique such as HPLC method. With these methods, one can gain the advantages of speed and reduced cost without scarifying accuracy. These methods can not be used as stability-indicating methods.

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