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Novel spectrophotometric methods for simultaneous determination of timolol and dorzolamide in their binary mixture

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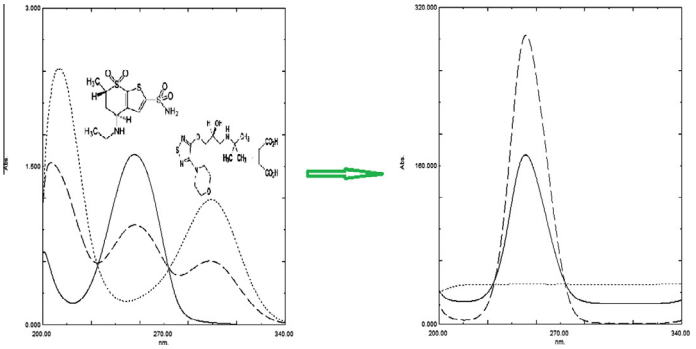
HIGHLIGHTS

- Two novel methods namely; absorbance subtraction (AS) and amplitude modulation (AM) methods were developed.
- Six recently well established spectrophotometric methods (SRS, RD, RS, EXRS, CM and MCR) were applied.
- The proposed methods are very simple, accurate, precise.
- They do not require any sophisticated apparatus or computer programs.

GRAPHICAL ABSTRACT

Zero-order spectra of 40 μg/mL of DOR (—) and TIM (. . .), separately in methanol, and binary of a mixture of DOR and TIM, 20 μg/mL of each (---) and their ratio spectra using the spectrum of TIM (1 μg/mL) as a divisor.



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ABSTRACT

Two smart and novel spectrophotometric methods namely; absorbance subtraction (AS) and amplitude modulation (AM) were developed and validated for the determination of a binary mixture of timolol maleate (TIM) and dorzolamide hydrochloride (DOR) in presence of benzalkonium chloride without prior separation, using unified regression equation. Additionally, simple, specific, accurate and precise spectrophotometric methods manipulating ratio spectra were developed and validated for simultaneous determination of the binary mixture namely; simultaneous ratio subtraction (SRS), ratio difference (RD), ratio subtraction (RS) coupled with extended ratio subtraction (EXRS), constant multiplication method (CM) and mean centering of ratio spectra (MCR). The proposed spectrophotometric procedures do not require any separation steps. Accuracy, precision and linearity ranges of the proposed methods were determined and the specificity was assessed by analyzing synthetic mixtures of both drugs. They were applied to their pharmaceutical formulation and the results obtained were statistically compared to that of a reported spectrophotometric method. The statistical comparison showed that there is no significant difference between the proposed methods and the reported one regarding both accuracy and precision.

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Introduction

Timolol maleate (TIM), (S)-1-[(1,1-dimethyl)amino]-3-[[4-(4-morpholinyl9-1,2,5-thiadiazol-3-yl]oxy]-2-propanol is a nonspecific-adrenergic blocker (Fig. 1a). It was the first-blocker to be used as an antiglaucoma agent [1]. None of the newer-blockers were found to be more effective than timolol [13]. Dorzolamide hydrochloride (DOR), (4S,6S)-4-ethylamino-5,6-dihydro-6-methyl-4H-thieno (2,3-b)thiopyran-2-sulfonamide-7,7-dioxide monohydrochloride (Fig. 1b), is a human carbonic anhydrase-II inhibitor used in eye drops to treat increased pressure in the eye caused by open-angle glaucoma and to treat a condition called ocular hypertension [1]. Both drugs have been co-formulated and widely used to reduce intraocular pressure in open-angle glaucoma and for treating ocular hypertension.

Only few methods have been reported for the determination of DOR based on HPLC assay with ultraviolet detection in human serum and urine [2–4] and capillary electrophoresis [5]. Several analytical procedures have been reported for determination of TIM, including GC [6], HPLC in plasma [7,8], in pharmaceuticals [9–11] and HPTLC [12].

To the best of my knowledge, very few spectrophotometric methods [13,14], capillary electrophoretic method [15] and few HPLC methods [16,17] were described for the simultaneous determination of both drugs in eye drops.

The main problem of spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. Several univariate spectrophotometric methods have been used for resolving mixtures with overlapping spectra such as derivative spectrophotometry [18], H-point standard addition method for binary [19] and ternary [20] mixtures, the ratio derivative spectrophotometry for binary [21] and derivative ratio spectra-zero crossing for ternary mixtures [22–27], the double divisor-ratio spectra derivative method for determination of ternary mixtures [18,26,28]. Multivariate spectrophotometric methods also were reported as partial least squares regression [29], principal component regression [28] and multiple linear regression analysis [30].

The aim of this work is to develop and conduct two novel methods namely; absorbance subtraction (AS) and amplitude modulation (AM) methods for resolving those binary mixtures with spectral interfering problems, either drugs in mixture or drug and its degradation product without preliminary separation using unified regression equation, in addition to a comparative study was done between the two novel methods (AS and AM) and six recently well established spectrophotometric methods (SRS, RD, RS, EXRS, CM and MCR) in terms of specificity and validation. These two novel methods are considered as new approach of the isosbestic point method either in zero order absorption spectrum i.e. isoabsorptive point [27,31–37] or in the ratio spectrum [38] at which the total concentration of both components in the mixture was calculated, by using smart mathematical techniques utilizing the absorption factor [39,40] in zero order absorption spectra or constants in the ratio spectra which could be adapted to isosbestic point analysis for separate quantitative estimation of each drug in their mixture using unified regression equation. The proposed methods are very simple, accurate, precise and do not require any sophisticated apparatus or computer programs.

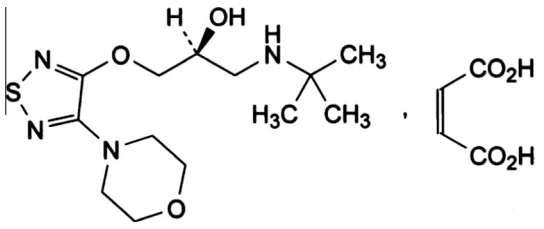

Fig. 1a. Chemical structure of timolol maleate.

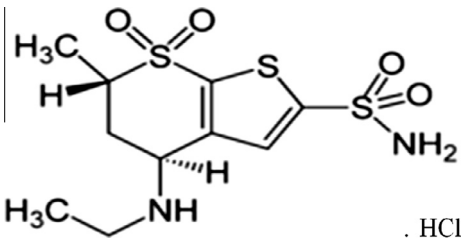

Fig. 1b. Chemical structure of dorzolamide hydrochloride.

Theoretical background

Absorbance subtraction method (AS)

This method is based on the same principles as the absorption factor method [39,40]. The method could be applied for the analysis of a mixture of two drugs X and Y having overlapped spectra intersect at isoabsorptive point and Y is extended over X, while X does not show any contribution at another wavelength (λ_2). In this method the isoabsorptive point λ_{iso} could be used for separate quantitative estimation of each X and Y in their mixture ($X+Y$). The determination can be done using mathematically calculated factor of one of these components. By simple manipulation step, we can get the absorbance value corresponding to X and Y, separately. So, the concentration of each component could be obtained via the isoabsorptive point regression equation without any need for a complementary method.

The absorbance values corresponding to X and Y at λ_{iso} were calculated by using absorbance factor which is a constant for pure Y representing the average of the ratio between the absorbance values of different concentrations of pure Y at λ_1 (A_{iso}) to those at λ_2 (A_2) i.e $\{A_{iso}/A_2\}$

$$\text{Absorbance of } Y \text{ in the mixture at } \lambda_{iso} = \frac{\text{abs1}}{\text{abs2}} \times \text{abs}\lambda_2(X+Y)$$

$$\text{Absorbance of } X \text{ in the mixture at } \lambda_{iso} = \text{abs}\lambda_{iso}(X+Y) - \frac{\text{abs1}}{\text{abs2}} \times \text{abs}\lambda_2(X+Y)$$

where $\text{abs}_{\lambda iso}$, $\text{abs}_{\lambda 2}$ is the absorbance of Y at λ_{iso} and λ_2; $\frac{\text{abs1}}{\text{abs2}}$ is the absorbance factor and $\text{abs}_{\lambda iso}(X+Y)$ and $\text{abs}_{\lambda 2}(X+Y)$ are the absorbance of the mixture at these wavelengths .

The concentration of each X and Y, separately, is calculated using the isobsorptive point unified regression equation (obtained by plotting the absorbance values of the zero order spectra of either X or Y at isobsorptive point (λ_{iso}) against the corresponding concentrations X or Y respectively).

Amplitude modulation method (AM)

The amplitude modulation method is a novel ratio spectrum manipulating method using normalized spectrum of the divisor obtained by dividing certain spectrum of Y' component by its concentration. For a mixture of X and Y, where Y is more extended than X; X and Y shows isoabsorptive point at the zero spectrum and consequently retained as an isosbestic point at the ratio spectrum.

At isoabsorptive point λ_{iso}

$$[A_m] = [A_X] + [A_Y]$$