

Research Article

MATHEMATICAL PROCESSING FOR SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF CYCLOPENTOLATE AND PHENYLEPHRINE IN THEIR BINARY MIXTURE

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ABSTRACT

Objective: Development and validation of three simple, accurate, selective and low cost spectrophotometric methods for simultaneous determination of cyclopentolate hydrochloride (CLO) and phenylephrine hydrochloride (PHE) in their binary mixture without need of any pre-separation step or sophisticated instruments. **Methods:** These methods are (I) area under the curve method using two ranges of wavelengths (255 - 260) and (271 - 275) nm. (II) Q-analysis method using two wavelengths 281.2 nm (iso-absorptive point) and 291 nm. (III) bivariate method using two wavelengths 255 and 270 nm chosen according to Kaiser method to calculate the sensitivity matrices of different wavelengths. **Results:** Calibration graphs were established in the range of 10-130 µg/ml for both drugs with good correlation coefficients. **Conclusion:** All the proposed methods were validated and successfully applied for simultaneous determination of cyclopentolate hydrochloride (CLO) and phenylephrine hydrochloride (PHE) in their bulk powder and pharmaceutical preparation Cyclophrine® eye drops. The obtained results were statistically compared with those of the reported method by applying student's t-test and F-test at 95 % confidence level and no significant difference was observed regarding accuracy and precision.

Keywords: cyclopentolate hydrochloride; phenylephrine hydrochloride; enriching technique; area under the curve; Q-analysis; bivariate; Kaiser method and sensitivity matrices.

INTRODUCTION

Cyclopentolate hydrochloride is 2-(dimethylamino)ethyl-(2*RS*)-(1-hydroxycyclopentyl)phenyl acetate hydrochloride, **figure 1**. It is official in British and United states pharmacopoeias [1,2] which is considered as antimuscarinic drug with actions similar to those of atropine and is used as eye drops to produce mydriasis and cycloplegia for ophthalmic diagnostic procedures and for the treatment of uveitis and iritis.[3] Phenylephrine hydrochloride is (1*R*)-1-(3-hydroxyphenyl)-2-(methylamino) ethanol hydrochloride, **figure (2)**. It is official in British and United states pharmacopoeias [1,2] which is considered as selective α_1 -adrenergic receptor agonist used primarily as a vasoconstrictor, decongestant and as mydriatic agent. Combination of cyclopentolate hydrochloride and phenylephrine hydrochloride is used as an ophthalmic solution to produce mydriasis and the amount of mydriasis produced by this combination is greater than that produced by either agent alone [3].

Literature survey reveals few methods for determination of cyclopentolate hydrochloride alone in pharmaceutical preparations including two colorimetric methods [4,5], spectrofluorimetric method [5], electrochemical method [6], gas chromatographic method [7] and HPLC method [8]. Also literature survey reveals few methods for determination of phenylephrine hydrochloride alone in pharmaceutical preparations including spectrophotometric methods [9-13], electrochemical methods [14-19], and HPLC methods [20-22].

Reviewing the literature on the simultaneous determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their binary mixture revealed only two reported separation techniques including thin layer chromatography and HPLC methods [23] and the lack of any spectrophotometric methods for the simultaneous determination of the two drugs in their binary mixture. The aim of this work is to develop and validate simple, sensitive, selective and cost effective spectrophotometric methods for the simultaneous determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their binary mixture without preliminary separation.

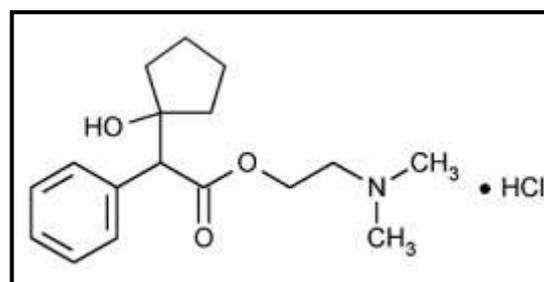


Fig. 1 Structural formula of cyclopentolate hydrochloride.

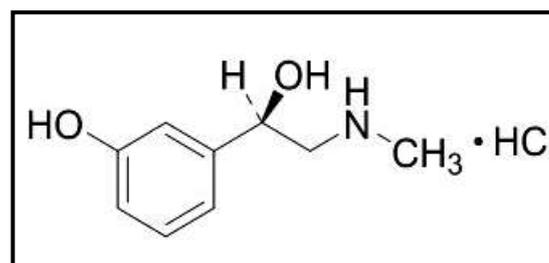


Fig. 2: Structural formula of phenylephrine hydrochloride.

Theory

Theory of area under the curve method:

Area under curve method utilizes two wavelength ranges. From the overlain spectra of both drugs the area under curve is determined at both the selected analytical wavelength ranges.

Within the above selected wavelength ranges, the area under curve was determined for both the drugs and analysis was performed using "Cramer's Rule" and "Matrix Method" [24].

Consider a binary mixture consisting of two components **X** and **Y**, from the spectra of two components, following information is obtained:

- $AUC_{\lambda_1-\lambda_2}^X$: area under curve for component **X** at the wavelength range $\lambda_1 - \lambda_2$.
- $AUC_{\lambda_3-\lambda_4}^X$: area under curve for component **X** at the wavelength range $\lambda_3 - \lambda_4$.
- $AUC_{\lambda_1-\lambda_2}^Y$: area under curve for component **Y** at the wavelength range $\lambda_1 - \lambda_2$.
- $AUC_{\lambda_3-\lambda_4}^Y$: area under curve for component **Y** at the wavelength range $\lambda_3 - \lambda_4$.

The total area under the curve of a mixture at a particular wavelength range is equal to the sum of area under curve of the individual components at same wavelength range. The area under curve of the mixture containing component **X** and **Y** can be given as follows:

$$AUC_{\lambda_1-\lambda_2} = AUC_{\lambda_1-\lambda_2}^X + AUC_{\lambda_1-\lambda_2}^Y \tag{1}$$

$$AUC_{\lambda_3-\lambda_4} = AUC_{\lambda_3-\lambda_4}^X + AUC_{\lambda_3-\lambda_4}^Y \tag{2}$$

Now the above equations can also be written as follows:

$$AUC_{\lambda_1-\lambda_2} = a_{\lambda_1-\lambda_2}^X bC^X + a_{\lambda_1-\lambda_2}^Y bC^Y \tag{3}$$

$$AUC_{\lambda_3-\lambda_4} = a_{\lambda_3-\lambda_4}^X bC^X + a_{\lambda_3-\lambda_4}^Y bC^Y \tag{4}$$

Where,

$$a_{\lambda_1-\lambda_2} = \text{absorptivity at } (\lambda_1 - \lambda_2)$$

$$a_{\lambda_3-\lambda_4} = \text{absorptivity at } (\lambda_3 - \lambda_4)$$

By applying "Cramer's Rule" and "Matrix Method", the concentration of component **X** and component **Y** can be determined as follows:

$$C^X = \frac{(a_{\lambda_1-\lambda_2}^Y AUC_{\lambda_3-\lambda_4}) - (a_{\lambda_3-\lambda_4}^Y AUC_{\lambda_1-\lambda_2})}{(a_{\lambda_1-\lambda_2}^X a_{\lambda_3-\lambda_4}^Y) - (a_{\lambda_3-\lambda_4}^X a_{\lambda_1-\lambda_2}^Y)} \tag{5}$$

$$C^Y = \frac{(a_{\lambda_1-\lambda_2}^X AUC_{\lambda_3-\lambda_4}) - (a_{\lambda_3-\lambda_4}^X AUC_{\lambda_1-\lambda_2})}{(a_{\lambda_1-\lambda_2}^Y a_{\lambda_3-\lambda_4}^X) - (a_{\lambda_3-\lambda_4}^Y a_{\lambda_1-\lambda_2}^X)} \tag{6}$$

Theory of Q-analysis method:

Suppose the first drug is **X** and the second is **Y**. According to Q-absorbance ratio method, uses the ratio of absorption at two selected wavelengths, one is at λ_{iso} and other being the λ_{max} of one of the two components [25,26].

Two equations were constructed as described below, using the relationship $a_{x1}=a_{y1}$ and $L=1$. Equations are;

$$\text{At } \lambda_1 : A_1 = a_{x1}C_x + a_{y1}C_y \quad (\text{because } a_{x1} = a_{y1}) \tag{1}$$

$$\text{At } \lambda_2 : A_2 = a_{x2}C_x + a_{y2}C_y \tag{2}$$

Dividing equation (2) by (1), we get;

$$A_2/A_1 = (a_{x2}C_x + a_{y2}C_y)/(a_{x1}C_x + a_{y1}C_y) \tag{3}$$

$$C_x/(C_x + C_y) = F_x \quad C_y/(C_x + C_y) = F_y$$

Let &

Dividing equation (3) by C_x+C_y , we get;

$$A_2/A_1 = (a_{x2}F_x + a_{y2}F_y)/(a_{x1}F_x + a_{y1}F_y) \tag{4}$$

$$F_y - 1 = F_x$$

,But

$$A_2/A_1 = (a_{x2}F_x + a_{y2} - a_{y2}F_x)/a_{x1} \tag{4}$$

$$a_{x1} - a_{y1}$$

, Because

$$A_2/A_1 = (a_{x2}F_x/a_{x1}) - (a_{y2}F_x/a_{y1}) + (a_{y2}/a_{y1})$$

$$\text{Let } \frac{a_{x2}/a_{x1} - Q_x}{\&} \frac{a_{y2}/a_{y1} - Q_y}{\&} \frac{A_2/A_1 - Q_M}{\&}$$

$$Q_M = F_x Q_x - F_x Q_y + Q_y$$

So,

$$F_x = (Q_M - Q_y)/(Q_x - Q_y) \tag{5}$$

This equation gives the fraction of mixture that determine the absolute concentration of **X** and **Y**.

$$C_x/(C_x + C_y) = (A_2/A_1) - (a_{y2}/a_{y1})/(a_{x2}/a_{x1}) - (a_{y2}/a_{y1}) \tag{6}$$

Both equation (5) & (6) gives the fraction, rather than the concentration of **X** and consequently of **Y** in the mixture in the term of absolute ratio. As, these are independent of concentration only approximate rather than accurate.

If the absolute concentration of **X** & **Y** than rearrange equation (1), we get;

$$C_x + C_y = A_1/a_{x1} \tag{7}$$

From equations (6) & (7), we get;

$$C_x/(A_1/a_{x1}) - (Q_M - Q_y)/(Q_x - Q_y) \tag{8}$$

$$C_y - [(Q_M - Q_x)/(Q_y - Q_x)] \times (A_1/a_{y1}) \tag{9}$$

Finally equations (8) & (9) gives the absolute concentration value of drug **X** & **Y**.

Theory of bivariate method:

- The principle of bivariate calibration is the measurement of two components (**X** and **Y**) at two selected wavelengths (λ_1 and λ_2) to obtain two equations:

$$A_{XY1} = m_{X1}C_x + m_{Y1}C_y + e_{XY1} \tag{1}$$

$$A_{XY2} = m_{X2}C_x + m_{Y2}C_y + e_{XY2} \tag{2}$$

- The resolution of such equations set allows the evaluation of C_x and C_y values:

$$C_x = (A_{XY1} - e_{XY1} - m_{Y1}C_y) / m_{X1} \tag{3}$$

$$C_y = [m_{X2} (A_{XY1} - e_{XY1}) + m_{X1} (e_{XY2} - A_{XY2})] / m_{X2}m_{Y1} - m_{X1}m_{Y2} \tag{4}$$

Where:

- C_x, C_y are the concentration of component **X**, component **Y**.
- m_{X1}, m_{X2} are the slope values of component **X** at λ_1, λ_2 .
- m_{Y1}, m_{Y2} are the slope values of component **Y** at λ_1, λ_2 .
- A_{XY1}, A_{XY2} are the absorbance values of the binary mixture at λ_1, λ_2 .
- e_{XY1}, e_{XY2} are the sum of the intercepts of components **X, Y** at λ_1, λ_2 .

- This simple mathematic algorithm allows the resolution of the two components by measuring the absorbance of their mixture at the two selected wavelengths and using the parameters of the linear regression functions evaluated individually for each component at the same wavelengths [27].

- According to Kaiser method,[28] the slope values of the linear regression equations for both components at different wavelengths were used to calculate the sensitivity matrices (K)

to find out the optimum pair of wavelengths (highest matrix value) at which the binary mixture was determined.

$$K = \begin{vmatrix} m_{X1} & m_{Y1} \\ m_{X2} & m_{Y2} \end{vmatrix} \quad (5)$$

EXPERIMENTAL

Instruments

Shimadzu UV-Visible 1800 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. The bundled software, UV-Probe personal spectroscopy software version 2.21 (Shimadzu).

Materials and solvents

- Pure cyclopentolate hydrochloride (99.45%) and phenylephrine hydrochloride (99.70%) were kindly supplied by Kahira pharmaceutical and chemical industrial company – Cairo – Egypt.
- Cyclophrine® eye drops: each (1ml) claimed to contain 10 mg of cyclopentolate hydrochloride and 100 mg of phenylephrine hydrochloride (batch number: 1660119), manufactured by Kahira pharmaceutical and chemical industrial company – Cairo – Egypt, purchased from local market.
- Methanol, analytical grade (Sigma-Aldrich, Germany).

Standard solutions

Cyclopentolate hydrochloride and phenylephrine hydrochloride standard solutions (each, 1 mg/ml), were prepared by dissolving 100 mg of the drugs powder separately in 50 ml of methanol into two 100-ml volumetric flasks and then completing to volume with the same solvent.

Procedures

General procedure

Different aliquots equivalent to (0.1-1.3 mg) of cyclopentolate hydrochloride and phenylephrine hydrochloride were accurately transferred from their standard solutions (1 mg/ml) into two separate series of 10-ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 300 nm) of these solutions were recorded using methanol as a blank.

For area under the curve method: Area under the curves obtained from the scanned spectra over the ranges of wavelengths (255 - 260) and (271 - 275) nm were recorded and then the corresponding regression equations were computed for both cyclopentolate hydrochloride and phenylephrine hydrochloride. Areas under the curve and the absorptivity values at the selected wavelength ranges were used for calculating the concentration of cyclopentolate hydrochloride using equation (5) and phenylephrine hydrochloride using equation (6) mentioned under "2.1".

For Q-analysis method: The absorbance values of cyclopentolate hydrochloride and phenylephrine hydrochloride were measured at 260 nm (λ_{iso}) and 273 nm (λ_{max} of phenylephrine hydrochloride) and the calibration graphs were constructed. The absorptivity values for each component at the selected wavelengths were used to calculate the concentration of cyclopentolate hydrochloride using equation (8) and phenylephrine hydrochloride using equation (9) mentioned under "2.2".

For bivariate method: The absorbance was measured at 255 and 270 nm and then the corresponding regression equations were computed at the selected wavelengths for both drugs. The obtained slopes and intercepts values were used for calculating the concentration of cyclopentolate hydrochloride using equations (3) and phenylephrine hydrochloride (4) mentioned under "2.3".

Application to laboratory prepared mixtures

The general procedure was repeated for each method using aliquots of cyclopentolate hydrochloride standard solution (1 mg/ml)

containing (0.1–0.6 mg) with aliquots of phenylephrine hydrochloride standard solution (1 mg/ml) containing (0.2–1.2 mg) and each drug concentrations were calculated.

Application to pharmaceutical formulation:

Contents of 5 **Cyclophrine®** eye drops were mixed well. 1 ml of the mixture containing 10 mg of cyclopentolate hydrochloride and 100 mg of phenylephrine hydrochloride was transferred into 100-ml volumetric flask then enriching technique was applied by addition of 40 mg of authentic cyclopentolate hydrochloride into the 100-ml volumetric flask and the volume was completed to 100 ml with methanol to obtain a stock solution labeled to contain 0.5 mg/ml of cyclopentolate hydrochloride and 1 mg/ml of phenylephrine hydrochloride [29]. Repeat the general procedure for each method using aliquots covering the working concentration range and determine the content of the eye drops.

RESULTS AND DISCUSSION

In the present study, three different spectrophotometric methods were applied for the for simultaneous determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their binary mixture without previous separation. These methods were applied to pharmaceutical preparation using enriching technique [29] by addition of authentic cyclopentolate hydrochloride to the pharmaceutical preparation, because the concentration of cyclopentolate hydrochloride is very low and practically does not reach the lower limit of concentration range in Beer's law.

Spectral characteristics and optimization of experimental conditions

The zero-order absorption spectra of cyclopentolate hydrochloride and phenylephrine hydrochloride, as shown in **figure (3)**, show severe overlap, which does not permit direct determination of the drugs in their binary mixture.

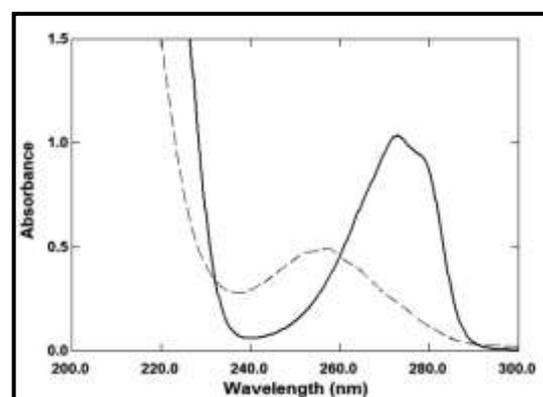


Fig. 3: Zero order absorption spectra of cyclopentolate hydrochloride, 100 µg/ml (---) and phenylephrine hydrochloride, 100 µg/ml (—) in methanol.

For area under the curve method, the area under the curves for cyclopentolate hydrochloride and phenylephrine hydrochloride were recorded over the ranges of (255-260) and (271-275) nm as shown in figure (4) for cyclopentolate hydrochloride and figure (5) for phenylephrine hydrochloride. The calibration graphs that relate the measured areas under the curve to the concentration of each component in µg/ml were constructed and the regression equations were computed. The absorptivity values and areas under the curve for each component at the selected wavelength ranges were used to calculate the concentration of cyclopentolate hydrochloride using equation (5) and phenylephrine hydrochloride using equation (6) mentioned under "2.1". To choose the optimum wavelength ranges at which calculations are done, different pairs of wavelength ranges were selected using the general procedure under "2.1".

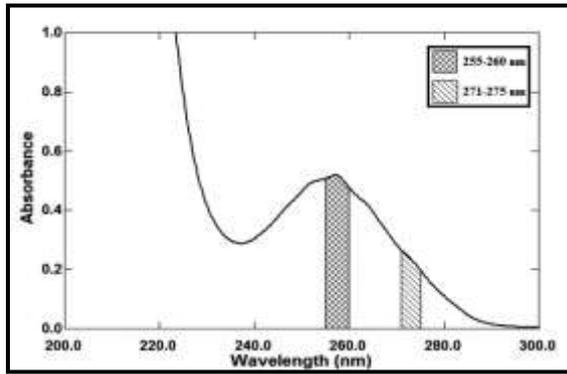


Fig. 4 : Zero order absorption spectrum of cyclopentolate hydrochloride (100 µg/ml) showing area under the curve over the ranges (255-260) and (271-275) nm.

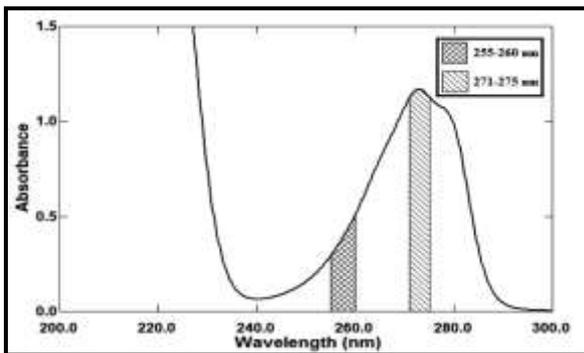


Fig. 5: Zero order absorption spectrum of phenylephrine hydrochloride (100 µg/ml) showing area under the curve over the ranges (255-260) and (271-275) nm.

For Q-analysis method, the absorption spectra of 100 µg/ml of cyclopentolate hydrochloride, 100 µg/ml of phenylephrine hydrochloride, and a mixture containing equal concentration of them (50 µg/ml of each) showed isoabsorptive point at 260 nm, as shown in figure (6). The spectra show also isoabsorptive point at 232.4 nm which was not involved in the method due to the low sensitivity at this wavelength. The absorbance values were measured at 273 nm (λ_{max} for phenylephrine hydrochloride) and 260 nm (λ_{iso}) in the range of 10-130 µg/ml for both drugs. Absorptivity values were determined for both cyclopentolate hydrochloride and phenylephrine hydrochloride. The values and the absorbance ratio were used to calculate the concentration of cyclopentolate hydrochloride and phenylephrine hydrochloride in their binary mixture using equations (8) and (9) mentioned under "2.2", respectively.

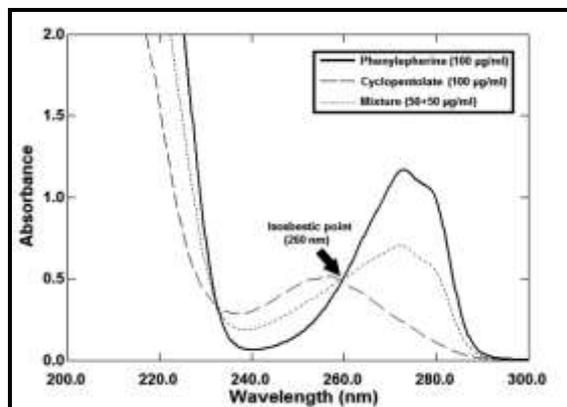


Fig. 6: Zero-order absorption spectra of cyclopentolate hydrochloride 100 µg/ml (—), phenylephrine hydrochloride 100 µg/ml (—) and their mixture 50 µg/ml of each (....).

For bivariate method, In order to apply the method in the resolution of cyclopentolate hydrochloride and phenylephrine hydrochloride in their mixture, the absorbance of the two components individually at seven different wavelengths was recorded in the region of overlap; 245, 250, 255, 260, 265, 270 and 275 nm. The calibration curves equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that; there was a linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient ($r > 0.9987$). According to Kaiser method, the slope values of the linear regression equations for both drugs at the selected wavelengths were used to calculate the sensitivity matrices (K) to find out the optimum pair of wavelengths at which the binary mixture was recorded. It was found that; the slopes at 255 and 270 nm gave the maximum value of K table (1) and thus chosen for the analysis.

Table 1: The absolute values of the sensitivity matrix determinates calculated according to Kaiser's method ($k \times 10^5$) for the mixture of cyclopentolate hydrochloride and phenylephrine hydrochloride:

λ/λ	245	250	255	260	265	270	275
24	5	12.5	246.3	903.4	1538.5	1982.6	1923.8
5	0	7	5	6	9	6	7
25	0	0	262.0	926.9	1570.8	1705.5	1652.9
0			5	8	1	1	1
25			0	665.9	1289.1	2021.9	1962.0
5				4	5	3	8
26				0	562.42	887.14	853.63
0							
26					0	276.56	256.72
5							
27						0	15.83
0							
27							0
5							

Methods validation

Validations of the proposed methods were assessed as per the ICH guidelines [30].

Linearity and range

Calibration graphs were constructed by repeating the general procedure for each method. The regression plots were found to be linear over the range of 10-130 µg/ml for both drugs. The yielded statistical results are summarized in table (2).

Limits of detection and quantitation

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$$LOD = 3.3 \sigma / S \qquad LOQ = 10 \sigma / S$$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. LOD and LOQ values were mentioned in table (2) and indicate good sensitivity of the methods.

Accuracy and precision

Accuracy and precision of the method were determined by applying the proposed procedure for determination of three different concentrations of each drug (30, 70 and 110 µg/ml), each in triplicate, in pure form in the same day (intra-day) and in three successive days (inter-day), then the accuracy as percent recovery (%R) and precision as percent relative standard deviation (%RSD) were calculated and results are listed in table (2).

Accuracy of the method was also determined by applying the standard addition technique where the general procedure of each method was repeated using aliquots of standard solutions (1 mg/ml) of each drug containing (100:200 µg), (110:220 µg), (120:240 µg) and (130:260 µg) of cyclopentolate hydrochloride and

phenylephrine hydrochloride respectively with already analyzed aliquot of Cyclophrine® eye drops containing (0.5:1 mg), then the percent recovery (%R) of pure added concentrations were calculated and results are listed in table (3). From these data the methods show high accuracy and precision.

Specificity

The specificity of the proposed methods was assured by applying it to laboratory prepared mixtures of cyclopentolate hydrochloride and phenylephrine hydrochloride. The results were listed in table (4).

Table 2: Regression and validation data for the determination of cyclopentolate hydrochloride and phenylephrine hydrochloride by the proposed methods:

Parameters	Area under the curve		Q-analysis		Bivariate	
	CLO	PHE	CLO	PHE	CLO	PHE
Wavelength (nm)	(255-260) & (271-275)		260 & 273		255 & 270	
Linearity range (µg/ml)	10-130		10-130		10-130	
LOD (µg/ml)	2.685	2.581	2.483	2.333	2.048	2.236
LOQ (µg/ml)	8.137	7.821	7.525	7.069	6.205	6.776
Regression Equation	$y = 0.0256 x + 0.0130$	$y = 0.0193 x + 0.0257$	$y = 0.0049 x + 0.0071$	$y = 0.0051 x + 0.0014$	$y = 0.0053 x + 0.0040$	$y = 0.0029 x + 0.0053$
	$y = 0.0097 x + 0.0083$	$y = 0.0454 x + 0.0617$	$y = 0.0025 x + 0.0019$	$y = 0.0115 x + 0.0087$	$y = 0.0030 x + 0.0054$	$y = 0.0106 x + 0.0080$
Correlation coefficient (r)	0.9997 & 0.9996		0.9997 & 0.9997		0.9998 & 0.9998	
Accuracy (% R)	100.39		99.72		100.23	
Precision (% RSD)	100.17		99.96		99.35	
- Repeatability ^a	1.176	1.141	0.976	0.959	1.509	1.178
-Intermediate precision ^b	1.001	0.919	0.965	0.847	0.790	1.133

^a Average of three concentrations of each drug (30, 70 and 110 µg/ml) repeated three times within the day. ^b Average of three concentrations of each drug (30, 70 and 110 µg/ml) repeated three times in three days.

Table 3: Recovery study of cyclopentolate hydrochloride and phenylephrine hydrochloride by standard addition technique via the proposed methods in Cyclophrine® eye drops:

Drug	Pharmaceutical taken (µg/ml)	Pharmaceutical (µg/ml)	found	Pure (µg/ml)	added	Pure Recovery (%) Area under the curve	Q-analysis	Bivariate
Cyclopentolate hydrochloride	50	50.46 ^a	10	99.24	100.41	101.05		
		50.62 ^b	11	98.15	99.76	100.32		
		49.68 ^c	12	101.07	99.23	99.49		
			13	98.33	101.04	100.71		
	Mean % RSD			99.20	100.11	100.39		
Phenylephrine hydrochloride	100	100.63 ^a	20	98.33	100.35	100.68		
		100.14 ^b	22	100.21	99.69	101.89		
		99.22 ^c	24	98.96	101.51	99.75		
			26	99.78	100.58	100.19		
	Mean % RSD			99.32	100.53	100.63		
			0.845	0.749	0.918			

^a Average of five determinations using area under the curve method. ^b Average of five determinations using Q-analysis method. ^c Average of five determinations using bivariate method.

Table 4 :Determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in synthetic laboratory mixtures by the proposed methods:

Taken amount (µg/ml)	Found Recovery (%)	Area under the curve		Q-analysis		Bivariate	
		CLO	PHE	CLO	PHE	CLO	PHE
10	20	100.77	99.05	99.39	98.76	100.52	98.61
20	40	98.12	99.83	100.02	101.24	100.64	101.67
30	60	99.91	98.70	99.46	99.82	101.35	99.73
40	80	101.35	99.34	100.18	98.91	98.97	100.22
50	100	100.83	101.42	101.09	100.85	99.13	100.47
60	120	98.57	100.93	99.53	100.36	100.12	101.12
10	100	101.96	101.65	98.96	101.87	101.29	101.35
Mean		100.22	100.13	99.80	100.26	100.29	100.45
% RSD		1.423	1.192	0.699	1.164	0.947	1.052

Pharmaceutical applications

The proposed methods were applied for the simultaneous determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in Cyclophrine® eye drops using enriching technique

[29] by addition of authentic cyclopentolate hydrochloride to the pharmaceutical preparation, because the concentration of cyclopentolate hydrochloride is very low and practically does not reach the lower limit of concentration range in Beer's law. Satisfactory results were obtained in good agreement with the label

claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method^[23] indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their

pharmaceutical dosage form, as shown in **table (4)**. No significant differences were found by applying student's *t*-test and *F*-test at 95 % confidence level.

Table 5: Determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in Cyclophrine® eye drops by the proposed methods and the reported method:

Parameters	Area under the curve		Q-analysis		Bivariate		Reported method* [23]	
	CLO	PHE	CLO	PHE	CLO	PHE	CLO	PHE
Number of measurements	5	5	5	5	5	5	5	5
Mean % recovery	100.92	100.63	101.23	100.14	99.35	99.22	101.09	100.87
% RSD	0.984	0.676	0.614	1.568	1.487	1.444	0.823	1.069
Student's <i>t</i> -test**	0.300 (2.306)	0.425 (2.306)	0.297 (2.306)	0.855 (2.306)	2.294 (2.306)	2.066 (2.306)	—	—
<i>F</i> -value**	1.425 (6.388)	2.513 (6.388)	1.794 (6.388)	2.123 (6.388)	3.156 (6.388)	1.766 (6.388)	—	—

*Reported method is HPLC method using Waters Spherisorb ODS2 C₁₈ column and 0.1% heptane-1-sulphonic acid sodium salt in methanol : water (80:20, v/v) as a mobile phase with 1 ml/min flow rate and UV detection at 210 nm. ** The values in parenthesis are tabulated values of "*t*" and "*F*" at (P = 0.05)

CONCLUSION

This study described the first spectrophotometric methods developed and applied for simultaneous determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their binary mixture without need of any pre-separation step or sophisticated techniques and instruments. The proposed methods are simple, sensitive, rapid and inexpensive, so they are good alternative to the other few reported methods and to the high-cost HPLC and TLC methods. The methods were validated according to the ICH guidelines and can be easily applied for quality control and routine analysis of the studied drugs in bulk powder and in their pharmaceutical preparation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest..

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