SIMULTANEOUS DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE AND TROPICAMIDE IN OPHTHALMIC DOSAGE FORM WITH THREE RAPID DERIVATIVE SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Three spectrophotometric methods have been developed for simultaneous estimation of Phenylephrine hydrochloride (PHE) and Tropicamide (TPC) in their combined dosage form. Method-I: zero-crossing derivative spectrophotometry involves amplitudes measurement of the first derivative spectra of the binary mixture at 284.0nm for PHE and 241.2nm for TPC. Method-II: dual wavelength, which uses the difference in absorbance at 260.8 nm and 268.2 nm for estimation of PHE and 245.4nm and 271.8nm for TPC. Method III: Ratio spectra derivative spectrophotometry, in which division of the absorption spectrum of the binary mixture by a standard spectrum of one of the components and then calculating first derivative amplitudes at 270.8nm for PHE and 240.8nm for TPC. The calibration graph follows Beer's law in the range of 25-125 mg/ml for PHE and 4-20 mg/ml for TPC for all the three methods. The accuracy and precision of the methods were validated statistically.

Keywords: Phenylephrine Hydrochloride(PHE), Tropicamide(TPC), zero-crossing derivative spectrophotometry, dual wavelength, Ratio spectra derivative spectrophotometry.

INTRODUCTION

Chemically, Phenylephrine hydrochloride (PHE), is (*R*)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride^[1-3]. It is an alpha-adrenergic (sympathomimetic) agent which stimulates alpha-adrenergic receptors, producing pronounced vasoconstriction. PHE alone or in combination with other drugs is reported to be estimated by spectrophotometric method^[4-7], HPLC^[8-13] and voltametry^[14].

Similarly, Tropicamide (TPC), is (*R,S*)-*N*-ethyl-3-hydroxy-2-phenyl-*N*-(pyrid-4-ylmethyl) propionamide^[1-3]. It is a tropic acid derivative endowed with short duration of antimuscarinic activity. Tropicamide alone is reported to be estimated by spectrophotometric method^[15] and HPLC^[16-17].

Since no spectrophotometric method is reported for simultaneous estimation of phenylephrine hydrochloride and tropicamide in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by three simple UV spectrophotometric methods (zero crossing derivative spectrophotometry, Dual wavelength method, Ratio spectra derivative spectrophotometry).

EXPERIMENTAL

Apparatus

A Shimadzu 1700PC double beam spectrophotometer with a fixed slit width (1nm) used coupled with shimadzu UVPC software was used for all the absorbance measurements and treatment of all data.

Chemicals used

PHE and TPC were kindly supplied as gift samples by Promed pharmaceuticals (Gurgaon, India) and were used as received. Analytical grade HCl and distilled water were used as a solvent.

Commercial Ophthalmic formulation

The quantitative assay of the commercial pharmaceutical formulations TROPICACYL PLUS eye drops (INTAS) containing 8mg TPC and 50mg PHE (1:6.25) respectively were carried out using the developed methods.

Standard solutions and synthetic mixtures

Stock solution of 1mg/1ml PHE and TPC were prepared separately in 0.1N HCl. The standard series of the solutions containing 4-20mg/ml TPC and 25-125 mg/ml were prepared from the stock solutions in distilled water for obtaining calibration graphs and spectra. Similarly, accurate volumes of each of PHE and TPC stock solutions were transferred in 10ml volumetric flasks and diluted to volume with distilled water to obtain binary mixtures as well as synthetic mixtures. All the solutions were prepared freshly. The zero order overlay spectra of PHE, TPC and synthetic mixture is shown in figure 1.

Figure1: Zero Order Overlay Spectra Of PHE(50µg/ml), TPC(8µg/ml) and Synthetic Mixture.

Zero Crossing Derivative Spectrophotometry (Method I):

The absorption spectra of the binary mixture solutions of PHE and TPC were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with $\Delta\lambda = 8\text{nm}$ and scaling factor 10. The amplitudes at 284.0nm were plotted against the respective concentrations of PHE. Similarly, the amplitudes at 241.2 nm were plotted against the respective concentrations of TPC [Figure 2(a)].

Figure2: Overlay first derivative spectra of PHE and TPC, for estimation of PHE and TPC.

a. Method I: Zero Crossing Derivative Spectrophotometry, showing zero crossing points at 240.4nm for determination of TPC and 280.2 for PHE.

Dual wavelength method (Method II):

The zero-order spectra of pure drugs were derivatized in first order with $\Delta\lambda = 8\text{nm}$ and scaling factor 20 for both drugs. In this method, the difference between absorbance at 260.8nm and 268.2 nm (Difference is zero for TPC) of the 1st derivative spectra of the binary mixture containing PHE and TPC were measured for the determination of PHE. Similarly the difference between absorbance at 245.4 nm and 270.8nm (Difference is zero for PHE) were measured for the determination of TPC [Figure 2(b)]. Standard laboratory mixtures of PHE and TPC in 1:6.25 ratio were prepared and successfully analyzed using the developed method.

b. Method II: Dual Wavelength method, showing difference in 246.2nm and 271.2nm for determination of TPC and difference in 260.8nm and 268.2nm for PHE determination.

c. Method III : i) Ratio spectra and first derivative spectra of PHE (25-125 $\mu\text{g/ml}$) using 28 $\mu\text{g/ml}$ TPC as a divisor, showing amplitude measurement at 270.8nm.

ii) Ratio spectra and first derivative spectra of TPC (4-20 µg/ml) using 125 µg/ml PHE as a divisor, showing amplitude measurement at 240.4 nm.

Ratio spectra derivative spectrophotometry (Method III):

For selecting the standard solution as a divisor, appropriate concentration of PHE and TPC were tested respectively. Thus, a concentration of 125 mg/ml of PHE and 28 mg/ml of TPC as divisor gave best results in terms of signal to noise ratio and highest correlation coefficient values, being an indication of the quality of fitting of the data to the straight line. Therefore, the absorption spectra of PHE for the solution in distilled water were divided by the spectrum of the standard solution of 28 mg/ml of TPC in the same solvent and the first derivative was plotted with the interval of $DI=8$ nm. It was found that the first derivative amplitude at 270.8 nm was suitable for the determination of PHE in binary mixture with TPC. In similar way, the stored spectra of TPC were divided by 125 mg/ml of PHE and the first derivative was plotted with the interval of $DI=8$ nm. It was found that the first derivative amplitude at 240.8 nm was suitable for the determination of TPC in binary mixture with PHE [Figure 2(c)]. Once the optimum working condition was established, calibration graphs were obtained at 270.8 nm for Phenylephrine Hydrochloride and 240.8 nm for Tropicamide in the standard binary mixture and showed that the proposed method is applicable over the ranges 25-125 mg/ml for PHE and 4-20 mg/ml for TPC.

Assay of ophthalmic formulation by Method I, II & III:

An accurate volume of ophthalmic solution equivalent to about 50 mg of PHE, and 8.00 mg of TPC was transferred to 100 ml volumetric flask, dissolved in 20 ml 0.1N HCl and sonicated for 10 minutes. The volume was then made up to the mark using the same solvent. The resulting solution was filtered through Whatmann filter paper grade I. Appropriate dilutions were prepared in distilled water taking suitable aliquots of the clear filtrates and was subjected to analysis using all the three methods described above. The result of analysis of ophthalmic formulation is reported in Table 1.

Table 1: Results of simultaneous estimation of marketed formulation (TROPICALYL PLUS eye drops), for Method I, II and III.

Formulation:-TROPICALYL PLUS EYE DROPS		
Labelled claim:-TPC:PHE(8mg:50mg)		
Method	TPC (% $\pm$ S.D.)	PHE (% $\pm$ S.D.)
I	98.10 $\pm$ 01.56	100.09 $\pm$ 0.68
II	100.27 $\pm$ 1.18	99.98 $\pm$ 1.15
III	100.87 $\pm$ 1.46	98.52 $\pm$ 0.87

* Mean value of five determinations.

RESULTS AND DISCUSSION

The proposed methods were validated as per ICH guideline (Table 2):

(i) **Linearity:** The linearity range was optimised with 25-125 mg/ml and 4-20 mg/ml for PHE and TPC for all three developed methods. The linear regression analysis shows good linearity having regression coefficient greater than 0.999 for all three developed methods.

(ii) **Precision:** The precision of the methods were established by carrying out the analysis of the analytes ($n=5$) using the proposed developed methods. The intra and inter day precision were found less than 2 for all the developed methods which shows that methods are precise.

(iii) **Sensitivity:** The LOD and LOQ were calculated using the following equations:

$$LOD = 3.3 \times \sigma / s \text{ and } LOQ = 10 \times \sigma / s$$

Where σ is standard deviation of 'y' intercept of calibration curve ($n=5$) and s is slope of regression equation.

(iv) **Accuracy:** To check the accuracy of all three developed methods, analytical recovery experiments were carried out by using standard addition method at 80 %, 100 % and 120 % levels. From the total amount of drug found, the % recovery was calculated. The results of recovery studies were summarised in Table 3.

Statistical Analysis

Statistical comparison of developed three methods was carried out using Analysis of variance (ANOVA) with Tukey's multiple comparison test (compares all pairs of columns with one another) with the help of software Graph Pad Prism 5. It was found that there is no significant difference between three methods for both PHE and TPC (Table 4).

Table 2: Results of validation parameters obtained by method I, II and III

Parameters	Regression and Validation parameters					
	Method I		Method II		Method III	
	PHE	TPC	PHE	TPC	PHE	TPC
$\lambda_{max}(nm)$	284.0	241.2	260.8-268.2	246.2-271.2	270.8	240.4
Beer's Law Limit, $\mu g/ml$	25-125	4-20	25-125	4-20	25-125	4-20
Correlation coefficient(r)	0.9999	0.9996	0.9995	0.9999	1.0000	0.9998
Intraday Precision (% RSD)	0.6902	1.250	1.512	1.129	1.265	1.231
Interday Precision (% RSD)	0.7880	1.486	1.513	1.252	1.458	1.313
LOD($\mu g/ml$)	1.138	1.283	1.272	1.037	1.173	0.416
LOQ($\mu g/ml$)	3.449	3.889	3.856	3.142	3.557	1.262

Table 3: Results of recovery study of PHE and TPC by method I,II and III.

Method	% Std addition	Amount added TPC : PHE (µg/ml)	Amount found* TPC (µg/ml)	Amount found* PHE (µg/ml)	Recovery of TPC (%)	Recovery of PHE (%)
I	80	7.2:45.0	7.24	44.72	100.55±1.68	99.37±0.88
	100	08:50.0	7.98	50.24	99.75±0.93	100.48±0.82
	120	8.8:55.0	8.76	55.75	99.75±1.37	101.36±0.90
II	80	7.2:45.0	7.26	45.54	100.83±1.25	101.20±1.74
	100	08:50.0	8.17	49.87	101.12±1.19	99.74±0.89
	120	8.8:55.0	8.96	54.21	101.81±1.15	98.56±0.84
III	80	7.2:45.0	7.41	44.80	101.9±1.14	99.55±1.00
	100	08:50.0	8.14	49.34	101.75±0.54	99.68±0.71
	120	8.8:55.0	8.87	54.86	100.79±1.19	99.56±0.84

*Mean of three determinations.

Table 4: Statistical comparison of the results of the developed three methods Using Tukey's multiple comparison test.

Methods	Mean difference		Q		Significant? P<0.05	
	PHE	TPC	PHE	TPC	PHE	TPC
I vs II	-0.595	1.152	1.203	3.562	NO	NO
I vs III	-1.441	0.363	2.911	1.121	NO	NO
II vs III	-0.845	-0.789	1.709	2.441	NO	NO

CONCLUSION

Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis of multicomponent mixtures. Derivative spectrophotometry is useful by means of resolving two overlapping spectra and eliminating matrix interferences in the assay of two or more component mixture. This paper demonstrates the potential of zero crossing derivative spectrophotometry, dual wavelength method and ratio spectra derivative spectrophotometry as an analytical technique and its usefulness to accurately, rapidly, simply and simultaneously quantitative active ingredients in multicomponent pharmaceuticals. The proposed three analytical UV spectrophotometric methods were developed and validated thoroughly for quantitatively determination of PHE and TPC in ophthalmic solution.

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