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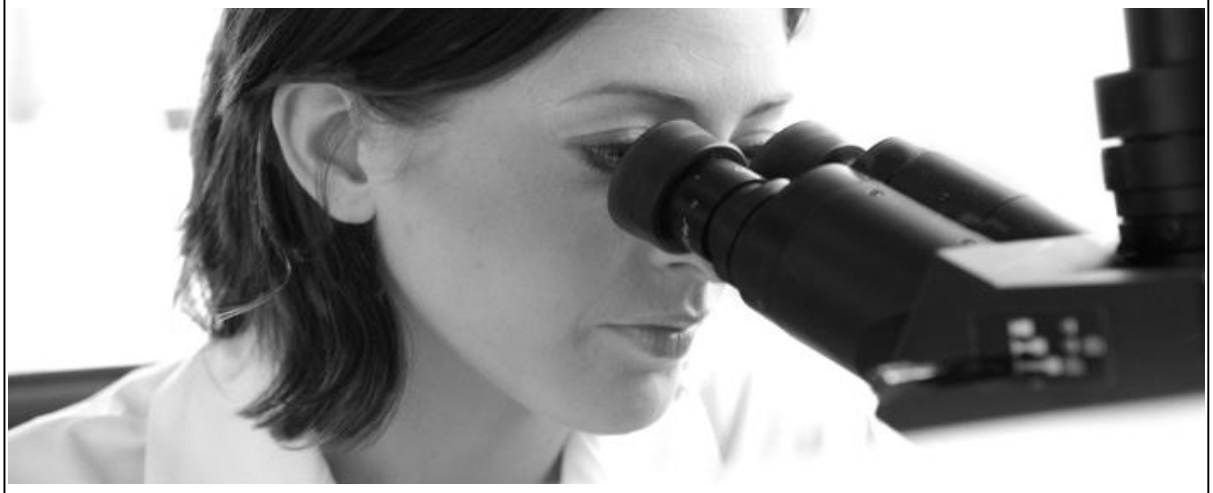

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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF Q-ABSORBANCE RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF PHENYLEPHRINE HCL AND BROMHEXINE HCL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

New spectrophotometric Q-Absorbance Ratio method has been developed for the simultaneous determination of Phenylephrine HCl and Bromhexine HCl in tablet dosage form. The various parameters, such as linearity, precision, accuracy, limit of detection and limit of quantitation were studied according to International Conference on Harmonization (ICH) guidelines. The Iso-absorptive point was found to be 218 nm. Calibration curves were linear over a concentration range of 5-15 µg/ml for Phenylephrine HCl and 4-12 µg/ml for Bromhexine HCl respectively. Accuracy of method was determined through recovery studies which were found 98.10-100.24 % for Phenylephrine HCl and 98.11 – 99.01 % for Bromhexine HCl. Method was found to be reproducible with relative standard deviation (RSD) for intra and interday precision to be < 1.5% over the said concentration rang.LOD and LOQ were found to be 0.958-2.904 to 240nm for PLE and 0.467-1.415 to 240nm for BHX. The proposed method is found to be highly simple, sensitive, precise and accurate.

INTRODUCTION

Phenylephrine HCl (PLE), chemically hydrochloride salt known as (R)-1-(3-hydroxyphenyl)-2methyl amino ethanol, is a sympathomimetic drug, used in the treatment of decongestant, it acting on α1- adrenergic receptors in the arterioles of the nasal mucosa to produce constriction; this leads to decreased edema and increased drainage of the sinus cavities(Horak, 2009). It is Official in IP and BP.

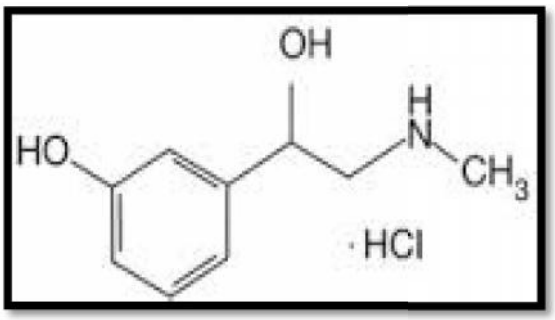

Figure 1. Chemical Structure of Phenylephrine HCl

Bromhexine HCl (BHX) is the hydrochloride salt of Bromhexine chemically known as 2,4-dibromo-6-{[cyclohexyl(methyl)amino]methyl}aniline, it is act as a mucolytic used in the treatment of respiratory disorders associated with productive cough & abnormal mucus secretion. It is official in IP, BP.

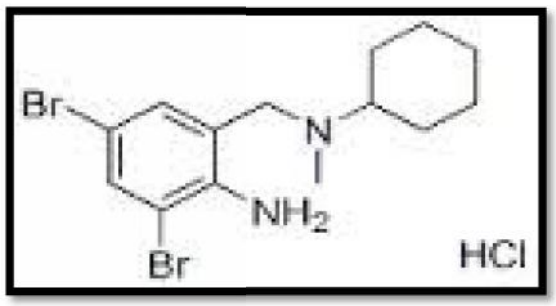

Figure 2 Chemical Structure of Bromhexine HCl

Combination of PLE and BHX is available in tablet dosage form in the ratio of 10:8 mg. PLE and BHX tablet is used for treatment of Lower respiratory track infection (Backett and Stenlake, 2001). Literature reveals that there is no Q-Absorbance Ratio UV spectroscopic method for PLE and BHX in pharmaceutical dosage form has not been reported yet. So, the present study was carried out with the aim of development of a simple, accurate, precise method for simultaneous

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estimation of PLE and BHX in pharmaceutical dosage form by Q- Absorbance Ratio method.

MATERIALS AND METHODS

Apparatus and Instrument

Double beam UV- visible spectrophotometer (Shimadzu, model 1800) having two matched quarts cells with 1 cm light path, Electronic analytical balance- BL220H, pH meter- Chemiline, India. All instruments and glass wares were calibrated.

Reagents and Materials

PLE (Umedica laboratory) BHX (Umedica laboratory) Methanol AR (Merck Pvt. Ltd) combined tablet formulation (Solvin tablet) was procured from local market.

Preparation of standard stock solution of PLE (100µg/ml)

100 mg of PLE was weighed and transferred to a 100ml volumetric flask and diluted with methanol. Take 10 ml and transfer in 100 ml volumetric flask and make up with methanol.

Preparation of standard stock solution of BHX (80µg/ml)

100 mg of BHX was weighed and transferred to a 100 ml volumetric flask and diluted with methanol. Take 8 ml and transfer in 100 ml volumetric flask and make up with methanol

Preparation of working standard solution

Suitable aliquots of above solution were diluted up to the mark with methanol to get the concentration range of 5-15 ppm for PLE and 4-12 ppm for BHX.

Selection of Detection Wavelength

PLE (15 µg /ml) and BHX (12µg/ml) were scanned over range of 390-190nm against Methanol as blank, using medium scan speed. It is revealed from overlay spectra that absorption maxima of PLE and BHX was found to be 240nm & 213nm respectively. Isobestic point was found at 218nm.

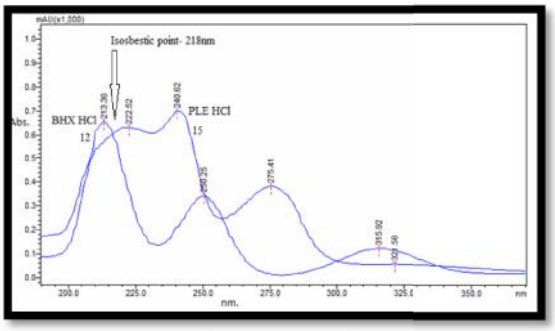

Figure 3 Overlay Spectrums of PLE and BHX

Calibration curve for PLE and BHX

To check linearity of the method, working standard solution having concentration in range of 5-15µg/ml for PLE and 4-12 µg/ml for BHX were prepared from the standard stock solutions of both drugs. The absorbance was measured at 240 nm (λ_{max} of PLE) and at 218 nm (iso-absorptive point).

Calibration curves were constructed by plotting concentration vs absorbance.

METHODOLOGY

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and other being the $\lambda_{max}$of one of the two components. From the overlay spectra of two drugs, it is evident that PLE and BHX show an iso-absorptive point at 218 nm. The second wavelength was selected 240 nm, which is the λ_{max} of PLE. Working standard solutions having concentration 5, 7.5, 10, 12.5, 15 µg/ml for PLE and 4, 6, 8,10,12 µg/ml BHX were prepared in methanol and the absorbance at 218 nm (iso-absorptive point) and 240 nm (λ_{max} of PLE) were measured and absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

For BHX,

$$CX = \frac{Q0-Q2}{Q1-Q2} \times \frac{A}{a1}$$

For PLE,

$$CY = \frac{Q0-Q1}{Q2-Q1} \times \frac{A}{a2}$$

$$Q0 = \frac{\text{Absorbance of Sample at 240nm}}{\text{Absorbance of Sample at218nm}}$$

$$Q1 = \frac{\text{Absorptivity of BHX at240nm}}{\text{Absorptivity of BHX 218nm}}$$

$$Q2 = \frac{\text{Absorptivity of PLE at240nm}}{\text{Absorptivity of PLEat 218nm}}$$

Where, CX and CY are concentrations of BHX and PLE respectively.

A1 is the absorbance of sample at iso-absorptive wavelength
a1 and a2 are absorptivity ofBHX andPLE at iso-absorptive wavelength

Quantitative estimation of PLE and BHX in marketed Tablet Formulation

Twenty tablets were finely powered. A quantity of powder equivalent was weighed and transferred to 100 ml volumetric flask. 60 ml methanol was added to the same flask and sonicated for 15 min. The volume was made up to 100 ml with

methanol. The solution was first filtered using Whitman filter paper No.41 and then through 0.45 µ filters paper in order to remove the excipient. After filtration, aliquots solutions were prepared by taking 10 ml sample stock solution. Volume was made up to 100 ml with methanol to produce of 10 µg/ml of PLE and 8 µg/ml of BHX.

Validation of Developed Method

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between concentration vs absorbance of PLE and BHX. The Linearity spectra and calibration curves of these two drugs at 240 nm and 213 nm are shown inFigure (4, 5, 6, 7) respectively and in Table 1 and 2.**Accuracy**

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 tablets were taken and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80 %, 100 % and 120 %) taking into consideration percentage purity of added bulk drug samples. (Table 3 and 4).

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous sample. It provides an indication of random error in results and was expressed as % RSD.

Intermediate precision (Reproducibility)

Variations of results within same day and amongst days are called as reproducibility. It includes following parameter,

Intra-day reproducibility: A variation of results within same day is called intraday variation. It was determined by repeating calibration curve 3 times on same day. Results are shown in table no. 5, 6.

Inter-day reproducibility: Variation of results amongst day is called interday variation. It was determined by repeating calibration curve daily for 3 different days. Results are shown in table no. 7,8.

Limit of Detection and Limit of Quantitation

Calibration curve was repeated for 6 times and the standard deviation (SD) of the intercepts was calculated then LOD and LOQ was calculated as follow from the formula.

LOD= (3.3*SD)/Slope

LOQ= (10*SD)/Slope

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

RESULTS AND DISCUSSION

Linearity

Table 1 linearity data for PLE at 240 nm and 218 nm in methanol

PLE (240nm)		PLE (218nm)	
Concentration (µg/ml)	**Absorbance (240nm)**	**Concentration (µg/ml)**	**Absorbance (218nm)**
5	0.212	5	0.214
7.5	0.363	7.5	0.339
10	0.485	10	0.438
12.5	0.677	12.5	0.548
15	0.815	15	0.724
Correlation coefficient: 0.998		Correlation coefficient: 0.994	
Intercept: 0.106		Intercept: 0.106	
Slope: 0.060		Slope: 0.049	
Regression Equation: y= 0.060x+0.106		Regression Equation: y=0.049x+0.106	
LOD: 0.958 µg/ml		LOD: 1.632 µg/ml	
LOQ: 2.904 µg/ml		LOQ: 4.947 µg/ml	

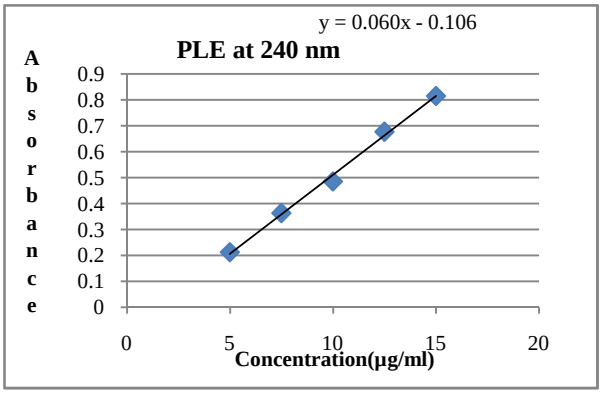

Figure 4 Calibration curve of PLE at 240 nm

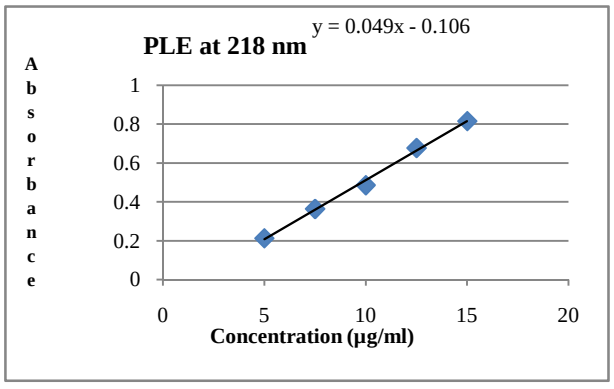

Figure 5 Calibration curve of PLE at 218 nm

Table 2linearity data for BHX at 240 nm and 218 nm in methanol

BHX (240nm)		BHX (218nm)	
Concentration (µg/ml)	**Absorbance (242nm)**	**Concentration (µg/ml)**	**Absorbance (276nm)**
4	0.126	4	0.252
6	0.198	6	0.428
8	0.278	8	0.574
10	0.347	10	0.728
12	0.411	12	0.882
Correlation coefficient: 0.999		Correlation coefficient: 0.999	
Intercept: 0.010		Intercept: 0.043	
Slope: 0.036		Slope: 0.078	
Regression Equation: y= 0.036x+0.010		Regression Equation: y=0.078x+0.043	
LOD: 0.467		LOD: 0.356	
LOQ: 1.415		LOQ: 1.079	

DISCUSSION

PLE and BHX were given linear response from 5-15 µg/ml and 4-12 µg/ml in Q- Absorbance Ratio method.

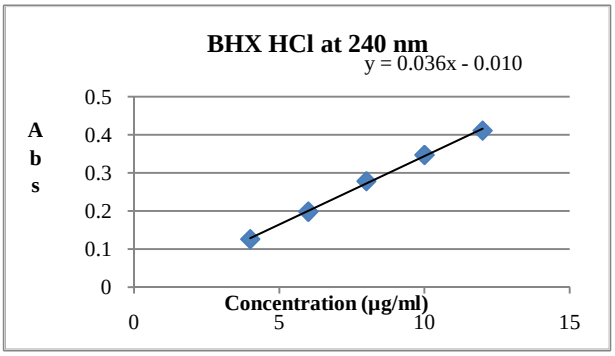

Figure 6 Calibration curve of BHX at 240 nm

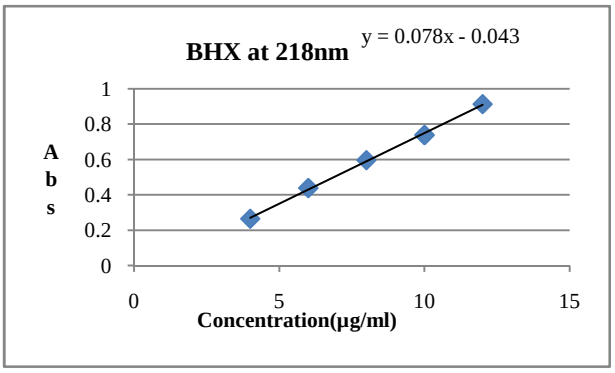

Figure 7 Calibration curve of BHX at 218 nm

Table 3 Recovery data of PLE

% level of recovery	Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Mean Total Concentration Found* (µg/ml)	%Recovery Mean*	%RSD
80	5	4	3.924	98.10	
100	5	5	5.012	100.24	
120	5	6	5.916	98.61	

*denotes average of three determination

Table 4 Recovery data of BHX

% level of recovery	Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Mean Total Concentration Found* (µg/ml)	%Recovery Mean*	%RSD
80	4	3.2	3.139	98.11	
100	4	4	3.935	98.38	
120	4	4.8	4.752	99.01	

*denotes average of three determination

DISCUSSION

Result reveals that % recovery of PLE and BHX was within acceptance criteria given in ICH i.e. 98-102%

Method Precision

Intermediate precision (Reproducibility)

The intra-day and inter-day precisions of the developed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PLE (2.5, 5 and 8.75µg/ml) and BHX (12.5, 25 and 43.5 µg/ml). Results were reported in terms of % RSD.

Table 5 Intra-day precision data for PLE of 240 nm and 218 nm

Concentration (µg/ml)	Absorbance at 240 nm		Absorbance at 218 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
5	0.215 ± 0.002	1.168	0.213± 0.002	1.08
10	0.486 ± 0.001	0.205	0.443± 0.005	1.13
15	0.815 ± 0.003	0.368	0.729± 0.002	0.285

*denotes average of three determination

Table 6 Intra-day precision data for BHX of 240 nm and 218 nm

Concentration (µg/ml)	Absorbance at 240 nm		Absorbance at 218 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
4	0.124 ± 0.002	1.674	0.253± 0.002	0.821
8	0.274 ± 0.003	1.278	0.571± 0.002	0.440
12	0.413 ± 0.002	0.484	0.887± 0.005	0.567

*denotes average of three determination

Table 7 Inter-day precision for PLE of 240 nm and 218 nm

Concentration (µg/ml)	Absorbance at 240 nm		Absorbance at 218 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
5	0.216 ± 0.003	1.750	0.186± 0.001	0.819
10	0.484 ± 0.002	0.546	0.375± 0.003	0.935
15	0.814 ± 0.002	0.308	0.574± 0.003	0.611

*denotes average of three determination

Table 8 Inter-day precision for BHX of 240 nm and 218 nm

Concentration (µg/ml)	Absorbance at 240 nm		Absorbance at 218 nm	
	Mean* ± SD	% RSD	Mean* ± SD	% RSD
4	0.129± 0.002	1.605	0.221± 0.003	1.584
8	0.277 ± 0.002	0.722	0.445± 0.003	0.721
12	0.415 ± 0.001	0.277	0.775± 0.004	0.536

*denotes average of three determination

DISCUSSION

Result reveals that SD and % RSD of PLE and BHX was within acceptance criteria given in ICH i.e. less than 1 and less than 2 respectively. So, the proposed method for estimation of PLE and BHX in précised in nature.

Quantitation estimation of PLE and BHXin marketed formulation

The proposed method was evaluate in the assay of table formulation containing PLE and BHX. Three replicate determinations were carried out on tablets. % assay found was 101.7 % for PLE and that for BHX was 101.4%. Result is shown in table 9.

Table Quantitative estimation of PLE and BHX in marketed formulation

Parameters	SOLVIN TABLET	
	PLE	BHX
Actual Concentration (µg/ml)	10	8
Concentration Obtained* (µg/ml)	10.17	8.02
% Assay*	101.70	100.25
%RSD *	1.04	0.589
Limit	90-110%	90-110%

*denotes average of three determination

DISCUSSION

% assay of PLE and BHX was found in an acceptance limit so this method could be used for analysis of this combination.

CONCLUSION

The described method enables the quantification of PLE and BHX in combined tablet dosage form. The validation data demonstrates good precision and accuracy, which prove the reliability of proposed method. This method was based on the determination of graphical absorbance at two wavelengths, one being Iso-absorptive point for the two drugs (218 nm) and the other being the wavelength of PLE (240nm). Hence, this Q-Absorbance Ratio method can be used routinely for quantitative estimation of both components in solid dosage form.

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