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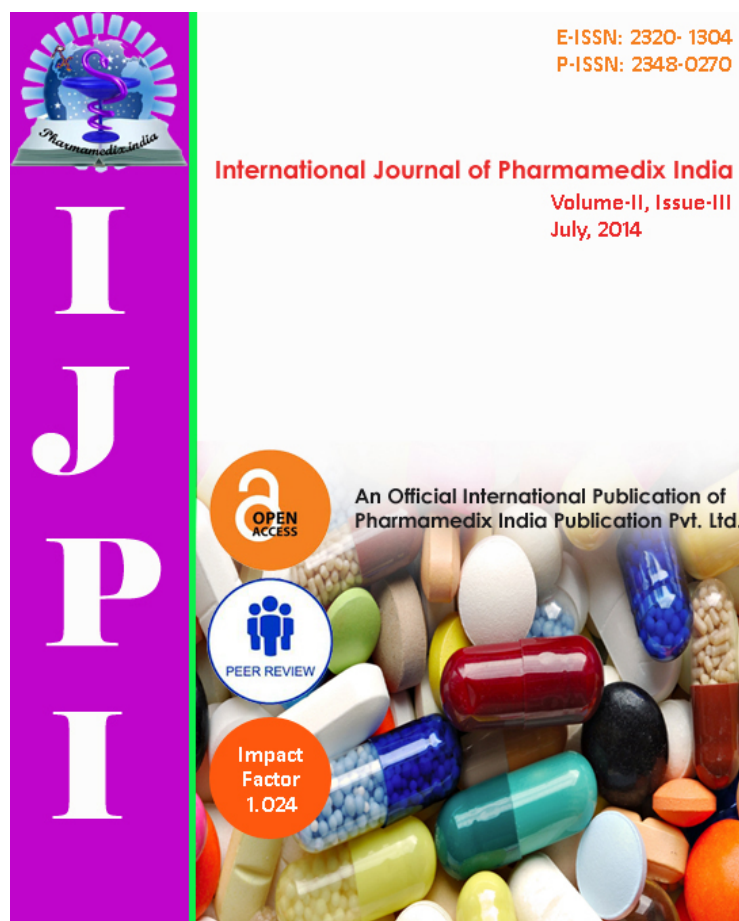
“Development and validation of dual wavelength method for Simultaneous Estimation of Ebastine and Phenylephrine hydrochloride in Bulk and Pharmaceutical Dosage Form”.

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Abstract:

A simple, rapid, accurate and precise Dualwavelength spectrophotometric method was developed for the simultaneous determination of Ebastine and Phenylephrine hydrochloride in pharmaceutical dosage form. The Dual wavelength spectrophotometric method was based on the determination of difference in absorbance of both the drugs at the respective Isoabsorptive point (IAP) of other drug. The Dualwavelength spectra were obtained using methanol as a solvent and the determinations were made at 209.2 nm & 221.6nm (IAP of Phenylephrine) for Ebastine and 253.0 nm & 216.0nm (IAP of Ebastine) for Phenylephrine hydrochloride. The linearity was obtained in the concentration range of 4-24 µg/ml for both drugs and correlation coefficient (r^2) were found to be 0.9998 and 0.9992 for Ebastine and Phenylephrine hydrochloride respectively. The percentage purity of drugs in combined tablet dosage form was found to be 99.33 % for Ebastine and 98.75 % for Phenylephrine hydrochloride. The % recoveries were found to be 99.84% for Ebastine and 99.18% for Phenylephrine hydrochloride. The method was found to be simple, accurate and precise and was applicable for the simultaneous determination of Ebastine and Phenylephrine in tablet dosage form.

Keyword: Ebastine (EBS), Phenylephrine hydrochloride (PHE), recovery, dualwavelength spectrophotometric method, validation.

Introduction

Ebastine is chemically 1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one (figure:1). Ebastine, a piperidine derivative, is a long-acting, non-sedating, second-generation histamine receptor antagonist that binds preferentially to peripheral H_1 receptors so devoid of untoward CNS action and anticholinergic effects.¹⁻⁷ Ebastine is official in European Pharmacopoeia and British

Pharmacopoeia.^{8,9} Liquid chromatography and potentiometric titration method is reported for the estimation. Many methods like UV-Visible spectroscopy¹⁰, LC-MS¹¹ and RP- HPLC¹² methods were reported for estimation of Ebastine.

Phenylephrine hydrochloride is chemically (1R)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol hydrochloride (figure 2). Phenylephrine is a selective α_1 -adrenergic receptor agonist. It acts predominantly on α -

adrenergic receptors. It causes powerful vasoconstriction by stimulating the post-synaptic alpha receptors. It causes increased systemic vascular resistance, decreased cardiac output, increased stroke volume and bradycardia. It is mainly used to treat nasal congestion, but may also be useful in treating hypotension and shock.^{13,14,15} Phenylephrine hydrochloride is official drug in Indian Pharmacopoeia¹⁶, British Pharmacopoeia¹⁷ and United state Pharmacopoeia.¹⁸ End point potentiometry and iodometric titration method is reported for the estimation. Many methods like UV-Visible spectroscopy,¹⁹ HPLC,²⁰ HPTLC²¹ methods were reported for estimation Phenylephrine hydrochloride in single as well as combined dosage form.

The combination of Ebastine and Phenylephrine hydrochloride is available in tablet dosage form. Earlier Ebastine and Phenylephrine HCl used as single dosage form to treat allergy and nasal congestion. These two drugs combine to give relief of nasal congestion, cold and flu. Very few analytical methods were reported for estimation of combination of Ebastine and Phenylephrine hydrochloride in pharmaceutical dosage form.

MATERIALS AND METHODS:

Instrument

A Shimadzu model UV-1800 double beam UV-visible Spectrophotometer, attached to a

computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched quartz cells was used. Shimadzu analytical balance (Sartorius, Gottingen, Germany), and Ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used throughout the practical. Class 'A' volumetric glassware were used.

Reagents and materials

Methanol (AR grade) was used as solvent. Ebastine bulk powder was kindly gifted by Kiwi pharmaceuticals Pvt. Ltd. Phenylephrine hydrochloride was gifted by Kaptab pharmaceuticals Pvt. Ltd.

Preparation of Standard Stock Solutions:

Accurately weighed portions of EBS (50mg) and PHE (50mg) were transferred to a separate 50ml volumetric flask and dissolved and diluted to the mark with Methanol to obtain standard solution having concentrations of EBS (1000µg/ml) and PHE (1000µg/ml).

From the above stock solution 5 ml was transferred to 50 ml of volumetric flask and diluted to the mark with Methanol to obtain standard solution having concentrations of EBS (100µg/ml) and PHE (100µg/ml).

Preparation of Sample Solution

Equivalent amount 50 mg of EBS and 50 mg of PHE was taken and transferred in to a 50 ml volumetric flask and dissolved in

methanol. The solution was sonicated for 20 min and filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with Methanol. This solution is expected to contain 1000µg/ml EBS and 1000µg/ml PHE. From this solution(5 ml) was taken in to a 50 ml volumetric flask and the volume was adjusted up to mark with Methanol to get concentration of EBS (100µg/ml) and PHE (100 µg/ml). From this solution(5 ml) was taken in to a 50 ml volumetric flask and the volume was adjusted up to mark with Methanol to get a final concentration of EBS (10 µg/ml) and PHE (10 µg/ml).

Development of method

The solutions of PHE (10 µg/ml) and EBS (10 µg/ml) were prepared and scanned over the range of 200- 400 nm and the overlain spectra were observed for development of suitable method for analysis.

From the overlay spectra two wavelengths 209.2 nm and 221.6 nm were selected as λ_1 and λ_2 for the estimation of EBS. (i.e. Iso-absorptive wavelengths for PHE) Similarly, 253.0 nm and 216.0 nm were selected as λ_3 and λ_4 for estimation of PHE.(i.e. Iso-absorptive wavelengths for EBS)

The calibration curve was plotted using difference in absorbance value at selected wavelength vs Concentration.

VALIDATION OF THE PROPOSED METHOD:²²

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity

Calibration curves were plotted over a concentration range of 4-24µg/ml for both EBS and PHE.

Accurately measured standard working solutions of EBS (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4ml) and PHE (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with Methanol, and absorbance were measured at 209.2 nm& 221.6 nm(IAP of PHE) and 253 nm&216 nm(IAP of EBS) for EBS and PHE respectively.

The calibration curves were constructed by plotting difference absorbances at IAP Vs concentrations.

Method precision (repeatability):

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of EBS and PHE (12 µg/ml for both drugs) without changing the parameters for the method.

Intermediate precision (reproducibility):

The intraday and interday precisions of the proposed methods were determined by analyzing the corresponding responses 3 times on the same day and on 3 different days with 3 different concentrations of

standard solutions of EBS and PHE (8, 12 and 20 µg/ml for both the drugs). The results were reported in terms of relative standard deviation (RSD).

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Accuracy (recovery study)

The accuracy of the methods was determined by calculating recoveries of EBS and PHE by the standard addition method. Known amounts of standard solutions of EBS and PHE were added at 80, 100 and 120% levels to prequantified sample solutions of EBS and PHE (10 µg/ml for both drugs). The amounts of EBS and PHE were estimated by applying the obtained values to the equation. The results were reported in terms of % Recovery.

ANALYSIS OF EBS AND PHE IN COMBINED DOSAGE FORM

Binary mixture was prepared for combination of both drug in the ratio of 1:1 (EBS and PHE). The difference in absorbance was measured at 209.2 nm and 221.6 nm for quantification of EBS and 253 nm & 216 nm for quantification of PHE. The amounts of EBS and PHE present in sample solutions were determined by fitting the response into the equation for EBS and PHE. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Ebastine and PhenylephrineHCl in mixture.

RESULTS AND DISCUSSION

In this method, the difference in absorbance of Ebastine was measured at 209.2 nm & 221.6 nm (IAP of PhenylephrineHCl) and for the PhenylephrineHCl difference in absorbance was measured at 253.0 nm & 216.0 nm (IAP of Ebastine) (Figure-3). Both the drugs obeyed Beer's law in the range of 4-24 µg/ml and correlation coefficient (r^2) were found to be 0.9998 and 0.9992 for EBS and PHE respectively (Figure-4, 5, 6 & 7; Table-1). The limit of detection was found to be 0.207 µg/ml and 0.219 µg/ml for EBS and PHE respectively while limit of quantification was found to be 0.629 µg/ml and 0.66 µg/ml for EBS and PHE respectively. (Table-4) The

percentage purity of drugs in combined dosage form was found to be 99.33 %for EBS and 98.75 % for PHE (Table-6). The accuracy of the method was determined by performing recovery study by standard addition method. The % recoveries were

found near to 99.84% for EBS and 99.18% for PHE (Table-5). The experiment was repeated six times in a day for precision. The method was found to be precise as % RSD for precision were < 2. (Table-2 & 3)

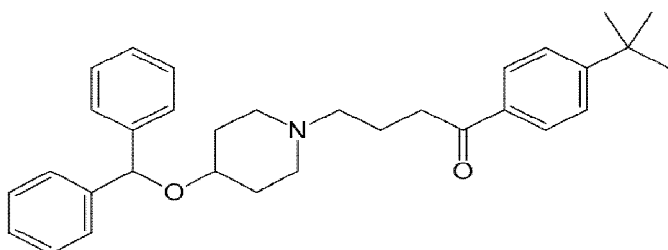


Figure 1: Structure of Ebastine

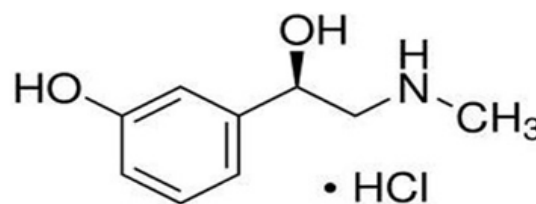


Figure 2: Structure of PhenylephrineHCl

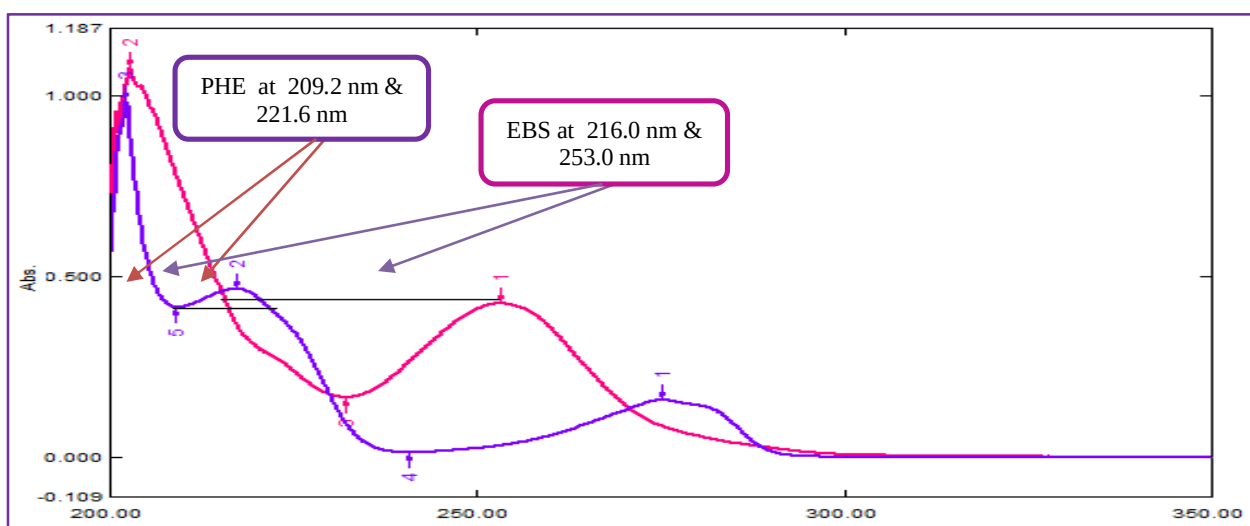


Figure 3: Overlay spectra of Ebastine and PhenylephrineHCl

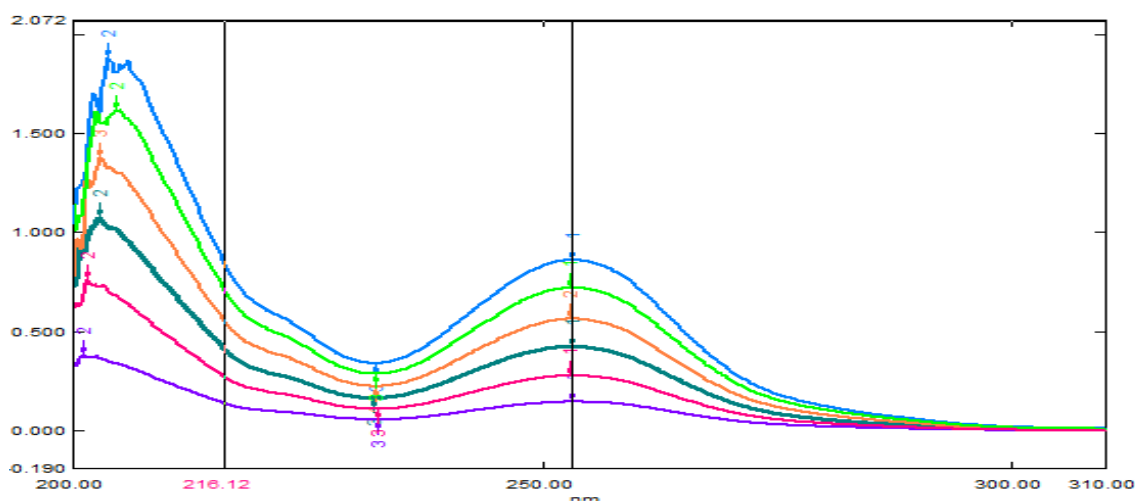


Figure 4: Overlay spectra of Calibration Range of Ebastine

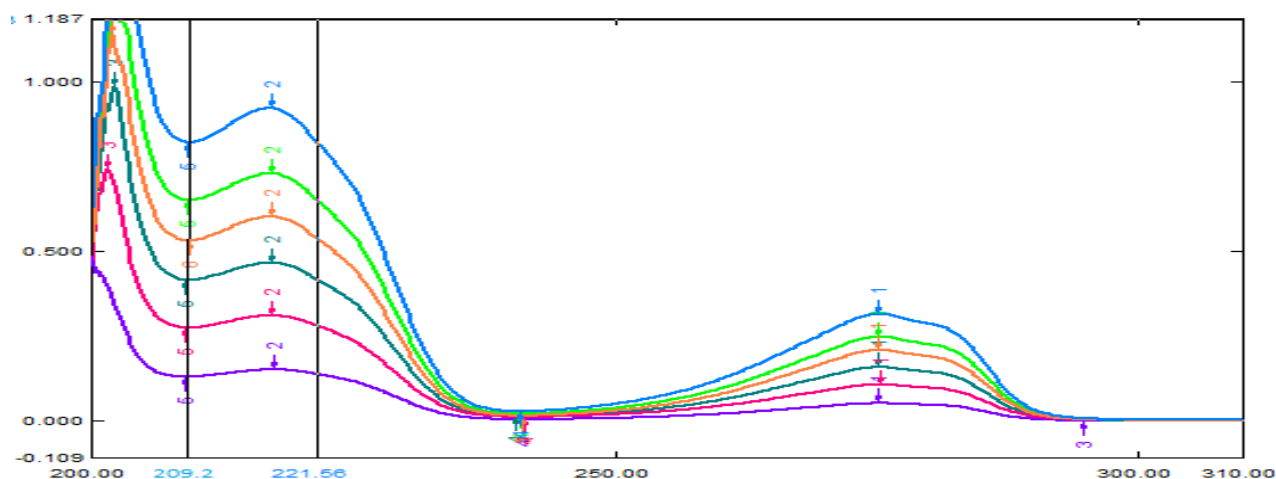


Figure 5: Overlay spectra of Calibration Range of PhenylephrineHCl

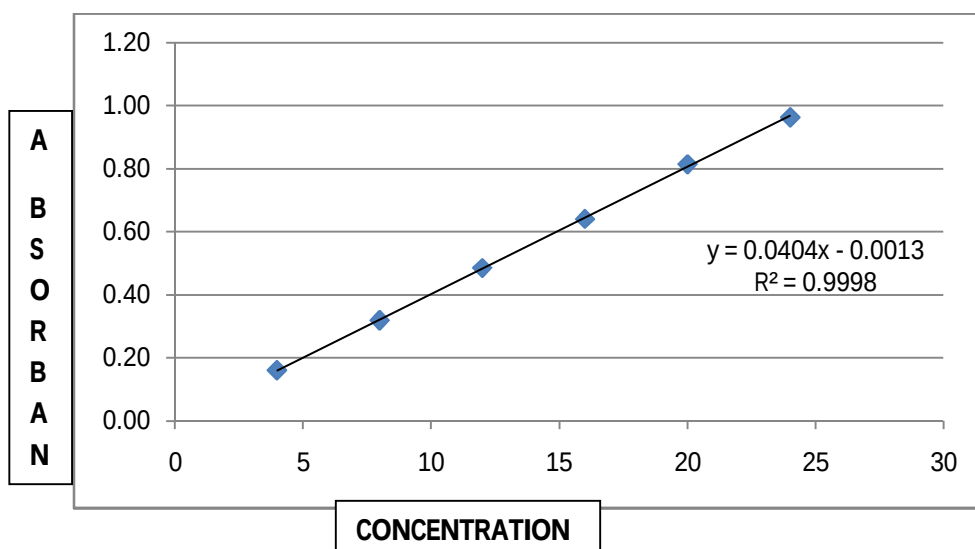


Figure 6: Calibration curve of Ebastine

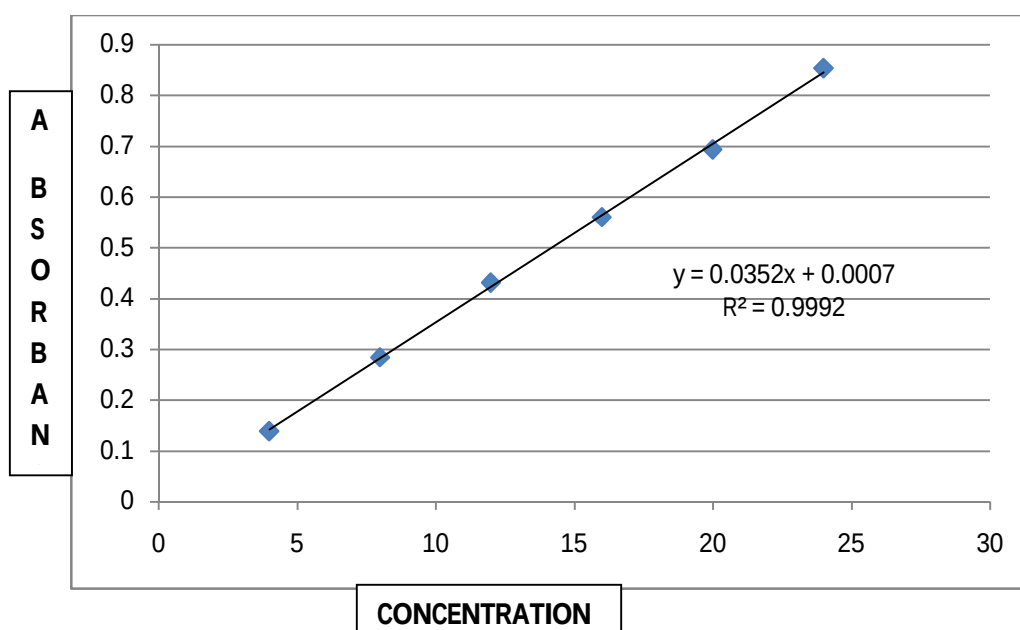


Figure 7: Calibration curve of PhenylephrineHCl

Table-1: Regression Analysis Data for Ebastine and PhenylephrineHCl

Parameter	Ebastine (EBS)	Phenylephrine HCl(PHE)
Analytical wavelength(nm)	209.2 nm& 221.6	253.0 nm& 216.0
Linearity range (µg/ml)	4-24	4-24
Regression Equation:	$Y = 0.0404 X - 0.0013$	$Y = 0.0352 X + 0.0007$
Slope	0.0404	0.0352
Intercept	-0.0013	0.0007
Correlation coefficient	0.9998	0.9992

Table 2: Repeatability of Ebastine and PhenylephrineHCl

Replicates	Ebastine (12 µg/ml)		PhenylephrineHCl (12 µg/ml)	
	Difference in absorbance ($A_{209.2} - A_{221.6}$)	Concentration found (µg/ml)	Difference in absorbance ($A_{253.0} - A_{216.0}$)	Concentration found (µg/ml)
1	0.479	11.88	0.426	12.08
2	0.482	11.96	0.421	11.94
3	0.478	11.86	0.422	11.96
4	0.484	12.01	0.427	12.11
5	0.480	11.91	0.425	12.05
6	0.476	11.81	0.422	11.96
Mean ± SD	-	11.90 ± 0.070	-	12.02 ± 0.070
%RSD	-	0.593	-	0.586

Table-3: Inter-day and Intra-day precision of Ebastine and PhenylephrineHCl

Drug	Conc. Taken (µg/ml)	Intra-day precision		Inter-day precision	
		Conc. Found* ± S.D	%R.S.D	Conc. Found* ± S.D	%R.S.D
	4	3.95±0.028	0.732	3.93±0.049	1.264

Ebastine	12	11.94±0.049	0.416	11.93±0.056	0.470
	20	19.86±0.051	0.258	19.85±0.144	0.728
Phenylephrine HCl	4	3.94±0.034	0.864	3.91±0.053	1.374
	12	11.94±0.052	0.436	11.92±0.075	0.630
	20	19.86±0.055	0.279	19.80±0.118	0.599

*Average of three determinations at three times

Table-4: LOD and LOQ parameters of Ebastine and PhenylephrineHCl

Parameters	Ebastine	PhenylephrineHCl
LOD(µg/ml)	0.207	0.219
LOQ(µg/ml)	0.629	0.66

Table-5: Recovery study of Ebastine and PhenylephrineHCl

Drug	Amount Present (µg/ml)	Amount Added(µg/ml)	Total amount(µg/ml)	Amount recovered (µg/ml)	Recovery (%) *±S.D.
Ebastine	10	8 (80%)	18	7.95± 0.065	99.41± 0.81
	10	10 (100%)	20	10.01± 0.086	100.15± 0.869
	10	12 (120%)	22	11.99± 0.086	99.96±0.724
Phenylephrine HCl	10	8 (80%)	18	7.91± 0.099	98.94±1.247
	10	10 (100%)	20	9.95± 0.056	99.51± 0.568
	10	12 (120%)	22	11.89± 0.086	99.10± 0.729

*Average of three determinations

Table-6: Analysis of mixture by proposed method

Drug	Amount added(mg)	Amount Found (mg)* ±SD	%Purity* ±SD	% R.S.D.
Ebastine	10	9.93	99.33±0.92	0.93
PhenylephrineHCl	10	9.87	98.75±1.18	1.20

*average of six determinations

CONCLUSION

Based on the results, obtained from the analysis of using described method, it can be concluded that the method has linear response in the range of 4-24 µg/ml for both Ebastine and Phenylephrine with co-efficient of correlation, (r^2) = 0.9992 and (r^2) = 0.9998 for Ebastine and Phenylephrine HCl, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination Ebastine and Phenylephrine HCl. The method can be used for the routine analysis of Ebastine and Phenylephrine HCl in combined dosage form.

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