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Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Ebastine and Phenylephrine Hydrochloride In Bulk And Pharmaceutical Dosage Form

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ABSTRACT

A simple UV-Visible spectrophotometric method is developed for the simultaneous determination of Ebastine and Phenylephrine hydrochloride in pharmaceutical dosage form using the first order derivative spectrophotometric method. The determination of both the drugs is based on the respective zero crossing point (ZCP) of their first order derivative spectra obtained in methanol. The first order derivative spectra were obtained using methanol as a solvent and the determinations were made at 241.0 nm (ZCP of Phenylephrine HCl) for Ebastine and 232.0 nm (ZCP 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 0.9994 and 0.9991 for Ebastine and Phenylephrine hydrochloride respectively. The percentage purity of drugs in combined tablet dosage form was found to be 100.02 % for Ebastine and 99.89 % for Phenylephrine hydrochloride. The % recoveries were found to be 99.88% for Ebastine and 99.24% 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.

Keywords: Ebastine (EBS), Phenylephrine hydrochloride (PHE), recovery, first order derivative spectrophotometric method, validation.

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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 spectroscopy[10], LC-MS[11] and RP- HPLC[12] 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 Pharmacopoeia[16], British Pharmacopoeia [17] 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] HPTLC[21] methods were reported for estimation Phenylephrine hydrochloride in single as well as combined dosage form.

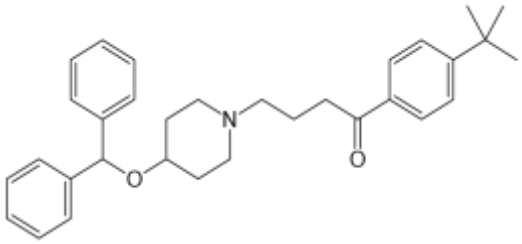

Figure 1: Structure of Ebastine

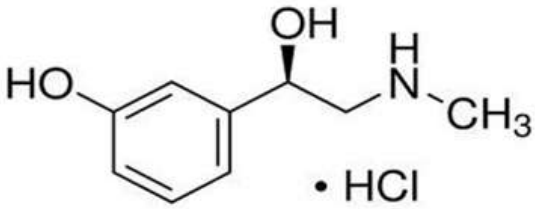

Figure 2: Structure of Phenylephrine HCl

The combination of Ebastine and Phenylephrine hydrochloride is marketed as tablet dosage form. This combination claims to produce pronounced relief of nasal congestion, cold and flu. Ofcourse, the patient compliance and convenience are benefits of combination compared with therapy using the same drugs singly. For the estimation of PHE and EBS in combination, some methods like UV-Visible spectroscopy by Q-Ratio method and Simultaneous equation method have been reported. Further, there existed the scope of exploring the possibility of developing other alternative methods that can offer good results and therefore an attempt has been made to develop a simple, sensitive and accurate method for simultaneous estimation of EBS and PHE by derivative spectroscopy and is presented herewith

MATERIALS AND METHOD:

Instrument:

A Shimadzu model UV1800 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 Pharma. 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 (1000 µg/ml) and PHE (1000 µg/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 (100 µg/ml) and PHE (100 µg/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 1000 µg/ml EBS and 1000 µg/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 (100 µg/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 to Selection of Wavelength

The spectrum of the standard solution was taken in the range of 200 nm to 400 nm. This spectrum of the drugs was converted to first derivative forms and ZCP'S (Zero Crossing Points) were determined and it were found to be 232.0 nm and 253.0 nm for Ebastine and 209.3 nm, 217.0 nm and 241.0 nm for Phenylephrine HCl.

The abs. of the Ebastine solution was measured at 241.0 nm (ZCP of Phenylephrine HCl) and for the Phenylephrine HCl, solution absorbance was measured at 232.0 nm (ZCP of Ebastine). The linearity was found in the range of 4-24 µg/ml for both Ebastine and Phenylephrine HCl. The calibration curves for derivative spectroscopy were constructed by plotting drug absorbance at ZCP Vs concentration and regression equations were computed.

VALIDATION OF THE PROPOSED METHOD: [22]

The proposed method was validated according to the 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 241.0 nm (ZCP of PHE) and 232.0 nm (ZCP of EBS) for EBS and PHE respectively. The calibration curves were constructed by plotting absorbances at ZCP 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 wee 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.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

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 absorbance was measured at 241.0 nm and 232.0 nm for quantification of EBS and PHE, respectively. 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 Phenylephrine HCl in mixture.

RESULTS AND DISCUSSION:

In this method, the absorbance of Ebastine was measured at 241.0 nm (ZCP of Phenylephrine HCl) and for the Phenylephrine HCl absorbance was measured at 232.0 nm (ZCP 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 0.9994 and 0.9991 for EBS and PHE respectively (Figure-4, 5, 6 & 7; Table-1). The limit of detection was found to be 0.529 µg/ml and 0.880 µg/ml for EBS and PHE respectively while limit of quantification was found to be 1.603 µg/ml and 2.933 µg/ml for EBS and PHE respectively. (Table-4) The percentage purity of drugs in combined dosage form was found to be 100.02 % for EBS and 99.89 % 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 99.88% for EBS and 99.24% 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)

Table-1: Regression Analysis Data for Ebastine and Phenylephrine HCl

Parameter	Ebastine (EBS)	Phenylephrine HCl (PHE)
Analytical wavelength(nm)	241.0 nm	232.0 nm
Linearity range (µg/ml)	4-24	4-24
Regression Equation:	y = 0.00156x - 0.00013	y = -0.00264x + 0.00007
Slope	0.00156	-0.00264
Intercept	-0.00013	0.00007
Correlation coefficient	0.9994	0.9991

Table 2: Repeatability of Ebastine and Phenylephrine HCl

Replicates	Ebastine(12 µg/ml)		Phenylephrine HCl(12 µg/ml)	
	Absorbance at 241.0 nm	Concentration found (µg/ml)	Absorbance at 232.0 nm	Concentration found (µg/ml)
1	0.018	11.81	-0.0312	11.84

2	0.0178	11.67	-0.0316	11.99
3	0.0177	11.61	-0.0319	12.10
4	0.0183	12.00	-0.0317	12.03
5	0.0182	11.94	-0.0311	11.80
6	0.0181	11.87	-0.0314	11.92
Mean ± SD	-	11.82 ± 0.151	-	11.95 ± 0.115
%RSD	-	1.280	-	0.970

Table-3: Inter-day and Intra-day precision of Ebastine and Phenylephrine HCl

Drug	Conc. Taken (µg/ml)	Intra-day precision Conc. Found* ± S.D	%R.S.D	Inter-day precision Conc. Found* ± S.D	%R.S.D
Ebastine	3	3.88 ± 0.218	0.561	3.84 ± 0.055	1.426
	12	11.82 ± 0.126	1.064	11.81 ±0.115	0.976
	20	19.87 ± 0.099	0.502	19.78 ±0.188	0.950
Phenylephrine HCl	3	3.92 ± 0.057	1.453	3.94 ±0.036	0.926
	12	11.89 ± 0.098	0.825	11.92±0.041	0.340
	20	19.78 ± 0.077	0.390	19.75±0.107	0.543

*Average of three determinations at three times

Table-4: LOD and LOQ parameters of Ebastine and Phenylephrine HCl

Parameters	Ebastine	Phenylephrine HCl
LOD(µg/ml)	0.529	0.880
LOQ(µg/ml)	1.603	2.933

Table-5: Recovery study of Ebastine and Phenylephrine HCl

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	7.98 ± 0.099	99.70 ± 0.099
	10	10 (100%)	20	10.02 ± 0.038	100.24 ± 0.038
	10	12 (120%)	22	11.96 ± 0.099	99.69 ± 0.099
	10	8 (80%)	18	7.99 ± 0.079	99.92 ± 0.079
Phenylephrine HCl	10	10 (100%)	20	9.88 ± 0.058	98.88 ± 0.058
	10	12 (120%)	22	11.87 ± 0.079	98.92 ± 0.079

* 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	10.024	100.02 ± 0.114	1.141
Phenylephrine HCl	10	9.969	99.69 ± 0.071	0.711

*average of six determinations

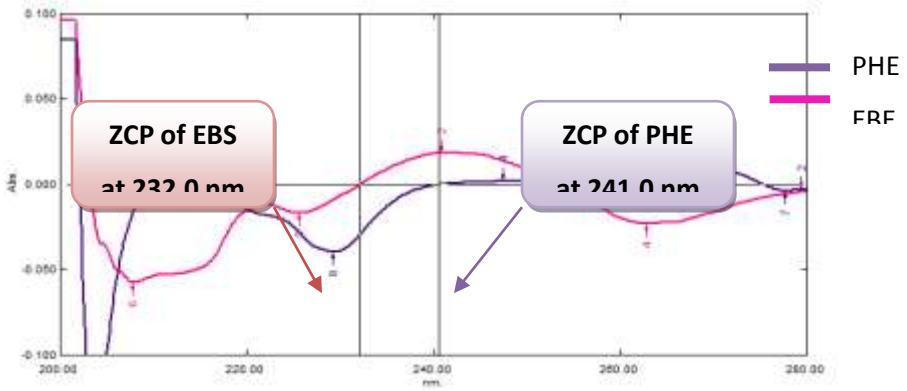

Figure 3: Overlain spectra of Ebastine and Phenylephrine HCl

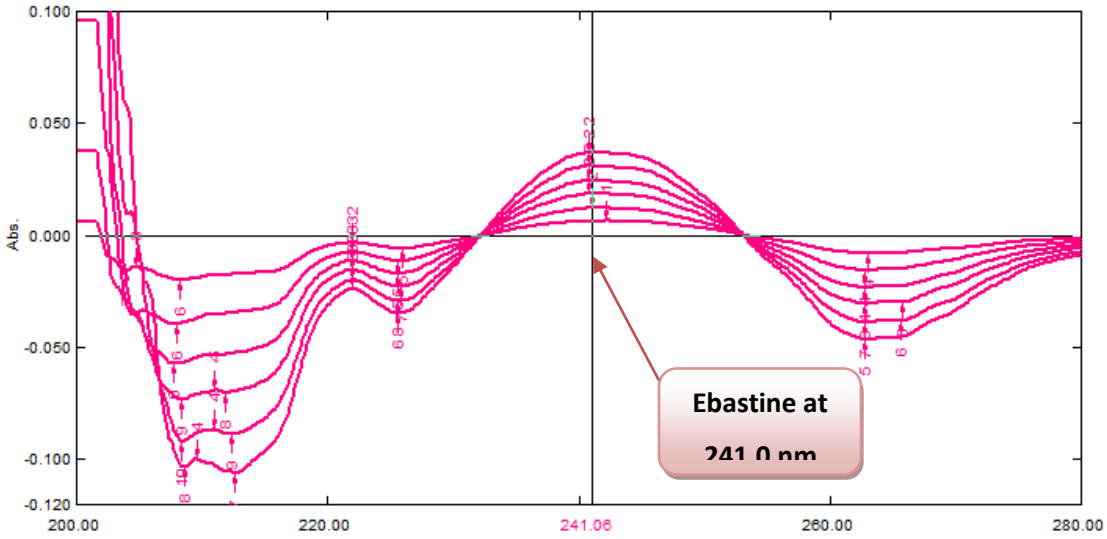

Figure 4: Overlain spectra of Calibration Range of Ebastine

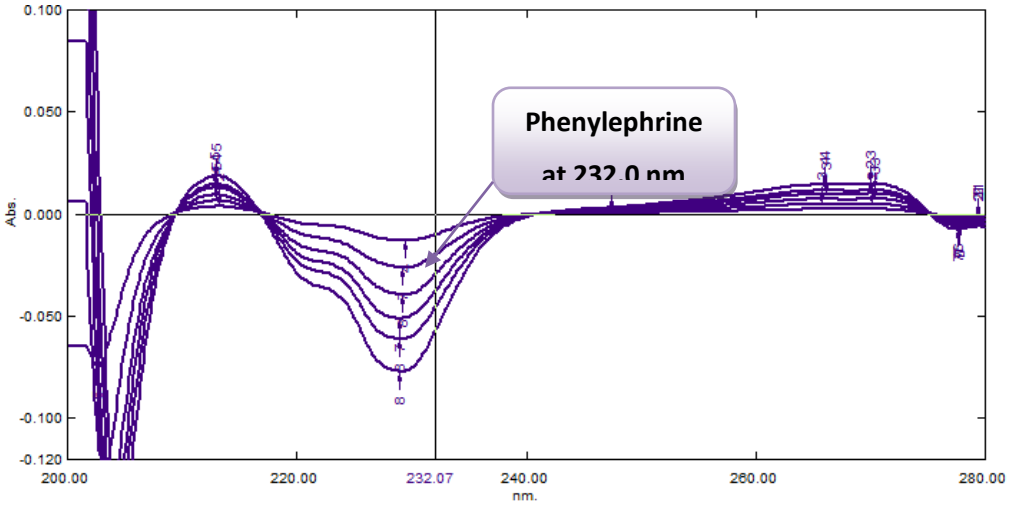

Figure 5: Overlain spectra of Calibration Range of Phenylephrine HCl

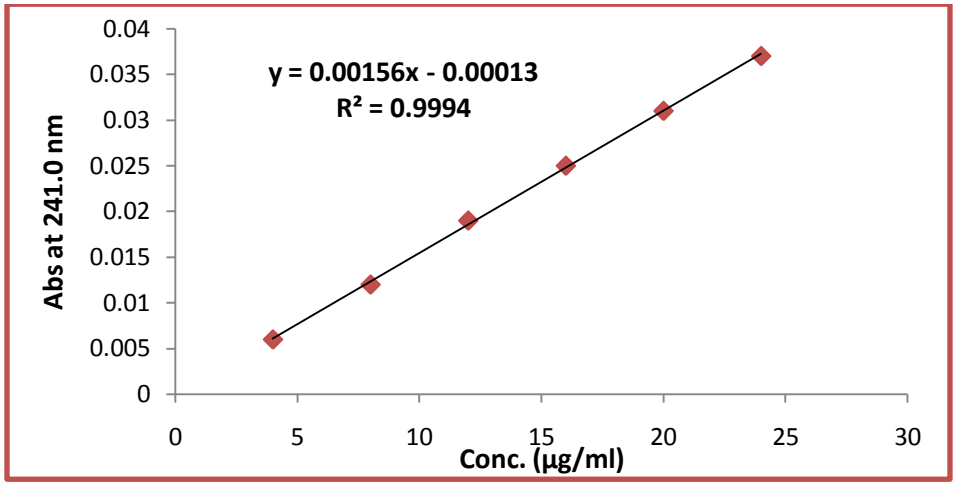

Figure 6: Calibration curve of Ebastine

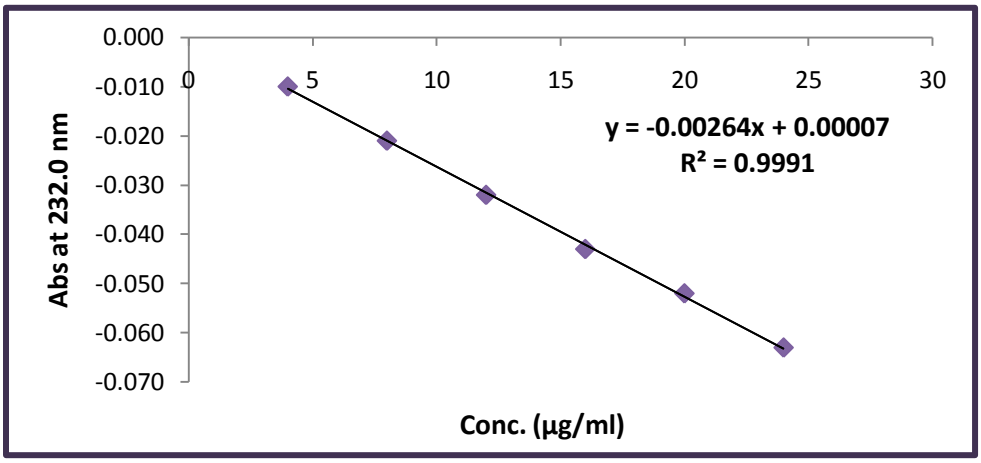

Figure 7: Calibration curve of Phenylephrine HCl

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) = 0.9994 and (r^2) = 0.9991 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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