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VALIDATED STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR DETERMINATION OF EBASTINE IN TABLET DOSAGE FORM

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ABSTRACT

A new simple, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method for determination of Ebastine as bulk drug and in tablet dosage form has been developed and validated. The drug was exposed to hydrolytic, oxidative, thermal and photolytic stress conditions to check the stability nature of drug. Chromatographic resolution of Ebastine and its degradation products was accomplished by use of Jasco HPLC system equipped with HiQSil C_{18} column (150 x 4.6 mm i.d.) using Potassium dihydrogen o-phosphate buffer: Acetonitrile (30: 70, v/v) as mobile phase. Densitometric detection was carried out at 253 nm. The retention time was found to be 3.12 ± 0.02 min. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results were linear in the range of 5-30 μg mL^{-1}. The developed method has been successfully applied for the estimation of drug in tablet dosage form.

KEYWORDS: Ebastine, RP-HPLC, Forced degradation, Tablet dosage form, Validation.

INTRODUCTION

Ebastine, chemically, 4-tert-butyl-4[4-(diphenylmethoxy) piperidino] butyrophenone, is a second-generation H1 receptor antagonist with long duration of action and is used mainly for allergic rhinitis and chronic idiopathic urticaria.[1]

Extensive literature review with respect to analytical methods revealed that Spectrophotometric methods[2-3] has been reported for the estimation of ebastine in tablet dosage form either as single drug or in combination with other drugs. Analytical reports also revealed availability of High Performance Liquid Chromatographic (HPLC)[4-16] methods for estimation of ebastine either as single drug or in combination with other drugs in pharmaceutical dosage form. Analysis of ebastine in pharmaceutical preparations by High Performance Thin Layer Chromatography (HPTLC) was also found in the literature.[17]

To best of our information, no reports were available in the literature for determination of ebastine in tablet dosage form by reverse phase stability-indicating high performance liquid chromatography (RP-HPLC) method. This paper describes development and validation of simple, precise, accurate and selective stability indicating RP-HPLC method for determination of ebastine in tablet dosage form in accordance with International Conference on Harmonisation Guidelines.[18,19]

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard ebastine was received as gift sample from Wallance Pharmaceuticals Pvt. Ltd., (Goa, India). The pharmaceutical tablet dosage form used in this study was Ebanit 10 labeled to contain 10 mg of ebastine was procured from the local pharmacy. Acetonitrile (HPLC grade), Potassium dihydrogen o-phosphate (AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

JASCO HPLC system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column HiQSil C_{18} (150 x 4.6 mm i.d. 3µm) was used. Separation was carried out at flow rate of 1 mL min^{-1} using Potassium dihydrogen o-phosphate buffer: Acetonitrile (30: 70, v/v) as mobile phase and detection was carried out at 253 nm.

Preparation of standard stock solution

Standard stock solution of Ebastine was prepared by dissolving 10 mg drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} which was further diluted with mobile phase to get final concentration 100 µg mL^{-1}.

Estimation of drug in tablet dosage form

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of Ebastine was weighed and transferred to 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and filtered through Whatman paper No. 41 and volume was made upto the mark with the mobile phase. One mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the methanol to get solution of concentration 10 µg mL^{-1} for Ebastine After setting the chromatographic conditions, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of drug present per tablet was estimated from the calibration curve.

System suitability

The system suitability was assessed by six replicate injections of the standard Ebastine having concentration 15 µg mL^{-1}. The resolution, peak asymmetry, number of theoretical plates and height equivalent to theoretical plate (HETP) were calculated. The values obtained demonstrated the suitability of the system for the analysis of drug. The results obtained are represented in Table 1.

Table 1: System suitability parameters for proposed RP-HPLC method.

Sr. No.	Parameters	Ebastine
1	Theoretical plates	3896.27
2	HETP (cm)	0.0082
3	Resolution	4.20
4	Asymmetry factor	1.37

Forced degradation study

The stability studies were accomplished by subjecting the bulk drug to the physical stress and stability was accessed. The study was carried out at concentration of 100 µg μL^{-1}. The hydrolytic studies were performed by treating the stock solution of drug with 0.05 N HCl and 0.05 N NaOH at room temperature for 30 min. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 20 µg μL^{-1} The oxidative degradation was carried out in 3 % H_2O_2 at room temperature for 30 min and sample was diluted with mobile phase to obtain 20 µg μL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 5 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square meter^{-1} for 3 d. Thermal and photolytic samples were diluted with mobile phase to obtain 20 µg µL^{-1} concentration.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The prime objective of developing this stability indicating RP-HPLC method is to accomplish the resolution between Ebastine and its degradation products. In order to attain the separation, we have used HiQSil C_{18} column as stationary phase and mobile phase comprising of mixture of Potassium dihydrogen o-phosphate buffer: Acetonitrile (30: 70, v/v) at flow rate of 1 mL min^{-1}. The retention time for ebastine was found to be 3.12 ±0.02 min. The representative chromatogram of the standard drug solution is shown in Fig. 1.

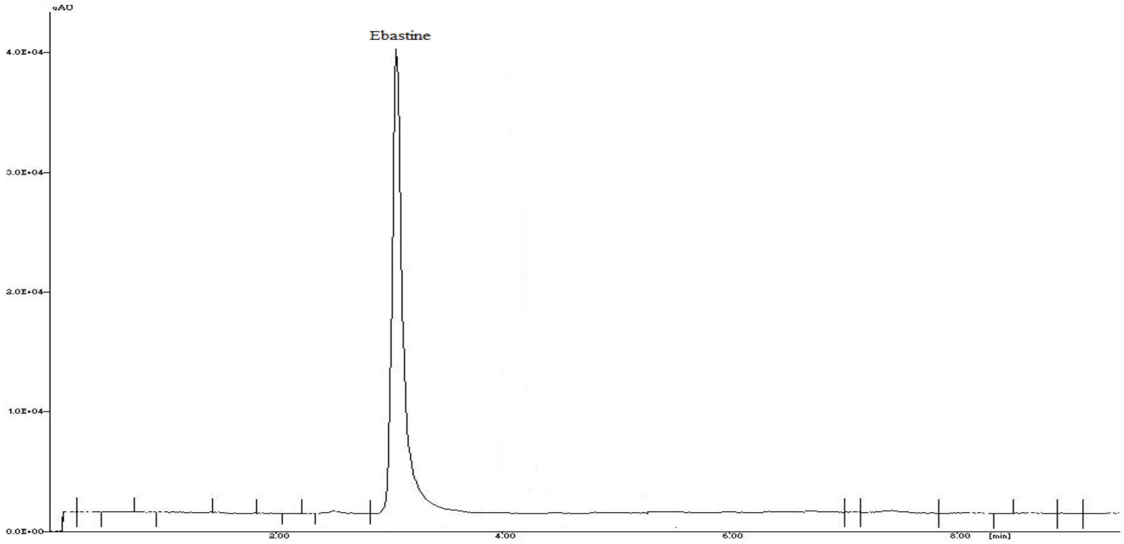

Fig. 1: Representative chromatogram of standard drug solution (15 µg mL^{-1}, 3.12min).

Result of forced degradation studies

The forced degradation results indicated susceptibility of the drug to hydrolytic, oxidative, thermal stress conditions and stability under photolytic stress conditions. Marked degradation product peaks were observed in acidic and oxidative conditions. The drug was degraded in alkaline and thermal stress conditions without appearance of any peak for degradation product. Forced degradation study revealed the specificity of developed method as no degradation products were eluted at retention time of drug. Fig. 2 and 3 denotes the chromatograms of acid and alkali degradation while Fig. 4-6 show the chromatograms of oxidative, thermal and photolytic degradation. The data obtained for forced degradation studies data is represented in Table 2.

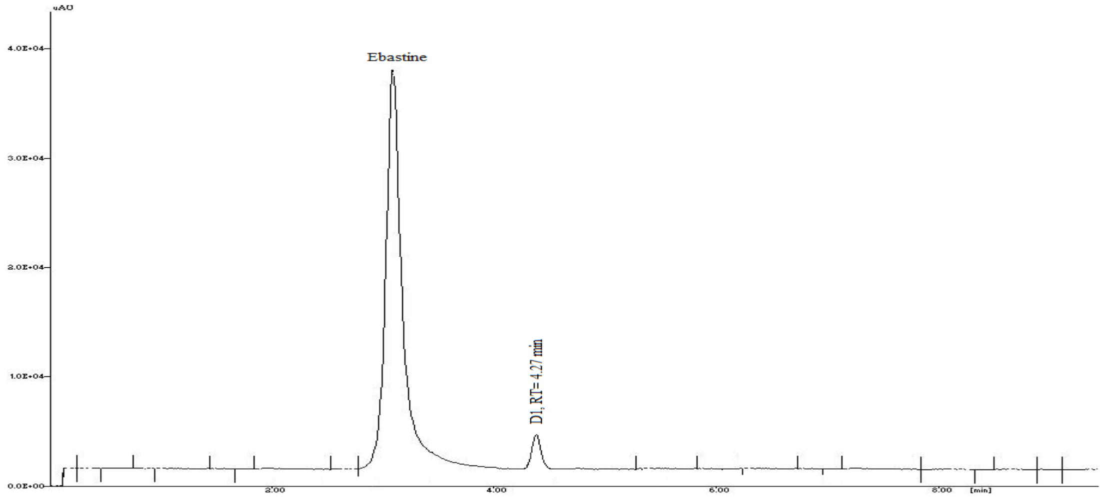

Fig. 2: Chromatogram obtained for ebastine after treatment with 0.05 N HCl with degradation product (D1, RT= 4.27 min).

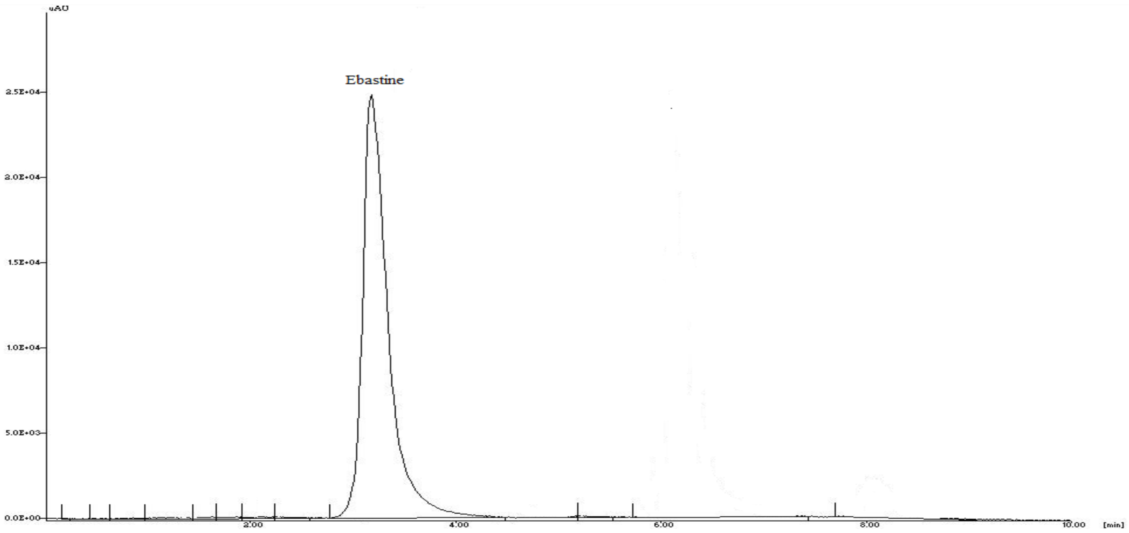

Fig. 3: Chromatogram obtained for ebastine after treatment with 0.05 N NaOH

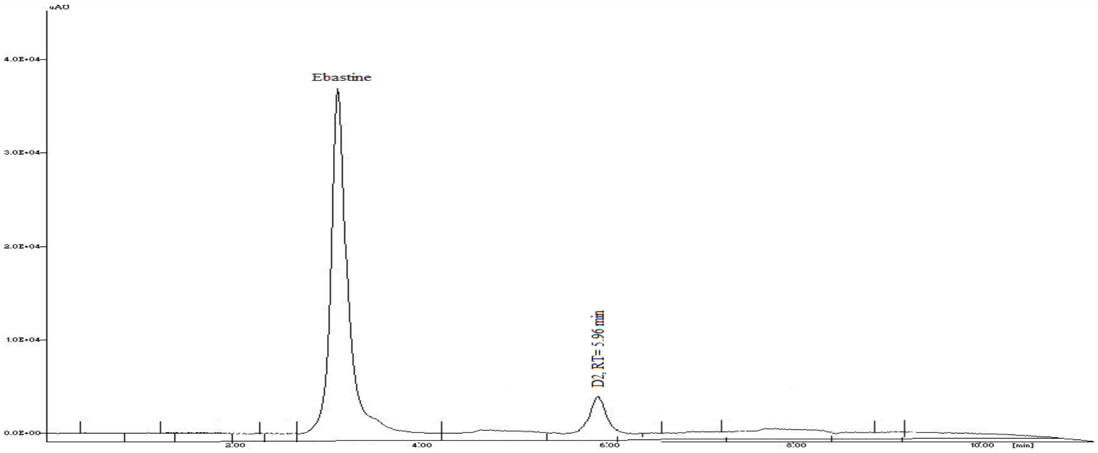

Fig. 4: Chromatogram obtained after treatment with 3% H_2O_2 with degradation product (D2, RT = 5.96 min).

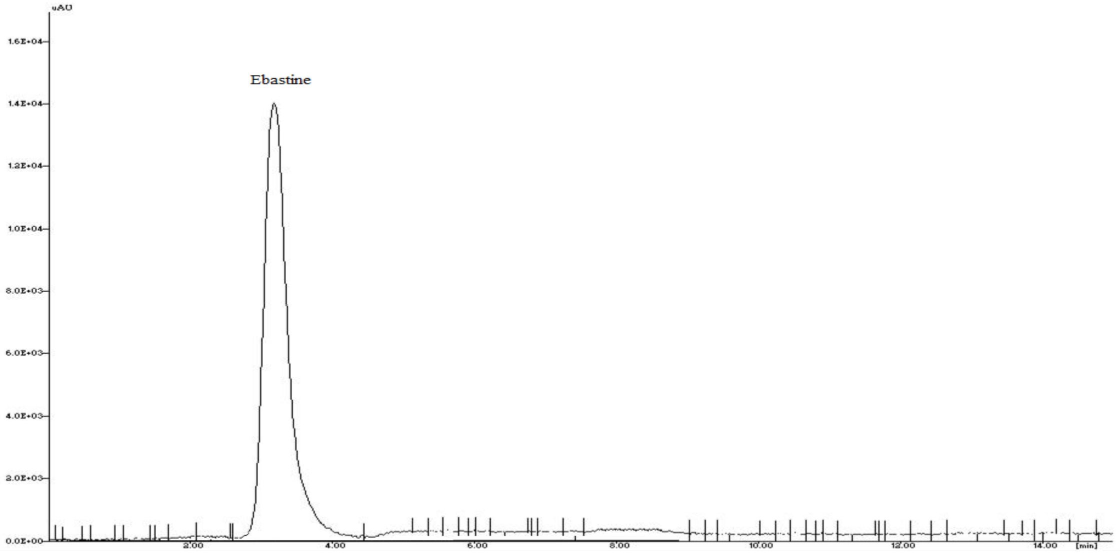

Fig. 5: Chromatogram obtained after thermal degradation.

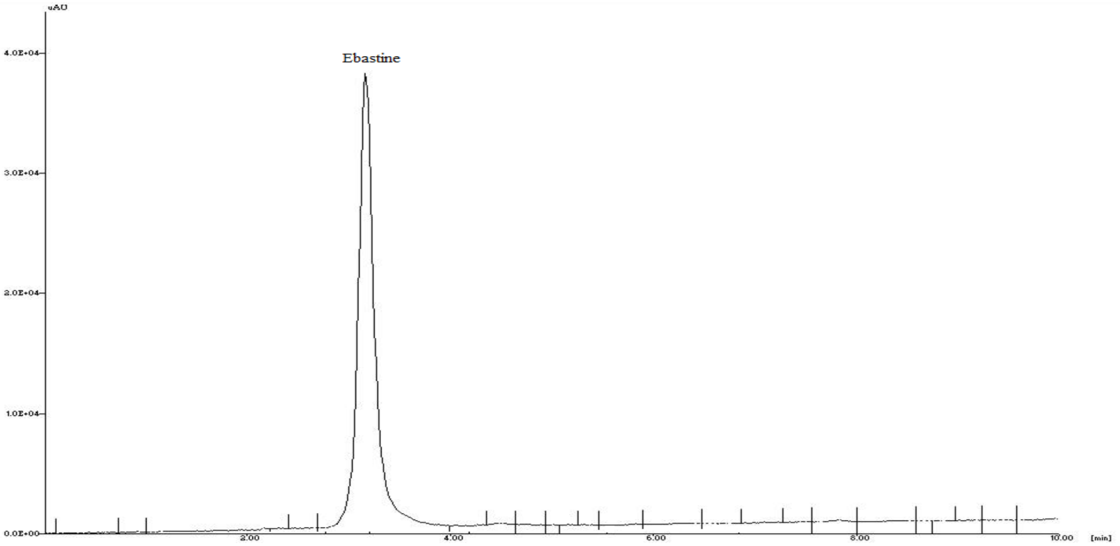

Fig. 6: Chromatogram obtained after exposure to UV light.

Table 2: Forced degradation studies data.

Stress conditions/ duration	% Recovered	% Degradation
Acid / 0.05 N HCl/ Kept at RT for 30 min	86.00	14.00
Alkaline /0.05 N NaOH/ Kept at RT for 30 min	88.05	11.95
Oxidative /3 % H_2O_2 / Kept at RT for 30 min	86.40	13.60
Dry heat/ 60ºC/ 5 h	89.85	10.15
Photolysis: UV light 200 watt h square meter^{-1} 3 d	99.80	---

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision, specificity and robustness, in accordance with ICH guidelines.[18,19]

Linearity

Volumes 0.5, 1, 1.5, 2, 2.5 and 3 mL from standard stock solution (100 µg mL^{-1}) were diluted with mobile phase to obtain the solutions in concentration range of 5-30 µg mL^{-1}. The linearity of the response of the drug was verified at six concentration levels, ranging from 5-30 µg mL^{-1}. The calibration graph was obtained by plotting peak area versus the concentration and data was treated by least-squares linear regression analysis. The calibration graph was found to be linear in the plotted concentrations with coefficient of correlation 0.999. The calibration curve for ebastine is shown in Fig. 7.

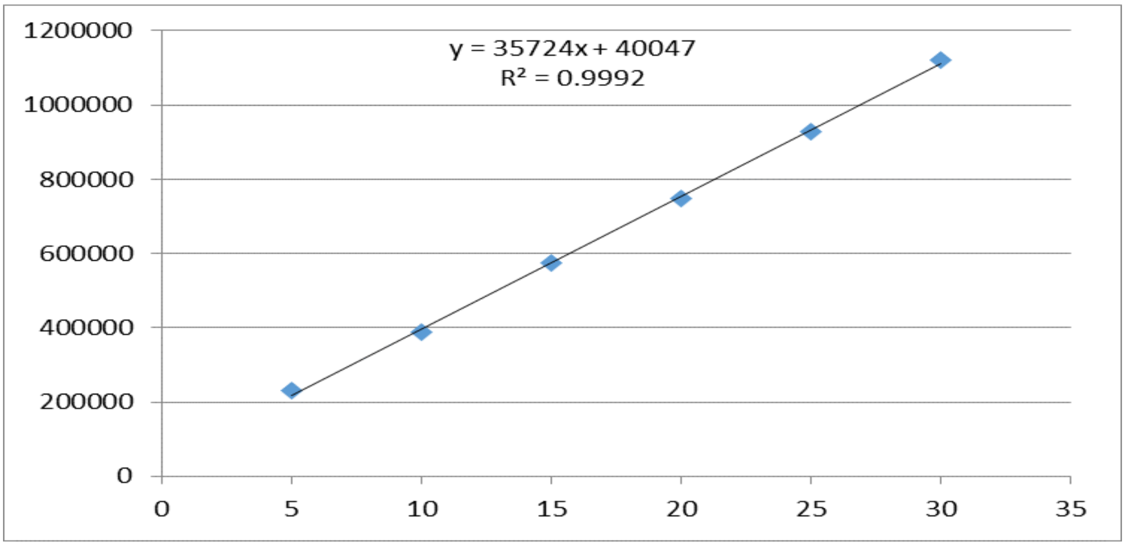

Fig. 7: Calibration curve for Ebastine.

Precision

One set of three different concentrations of standard solution of ebastine (10 µg mL^{-1}, 15 µg mL^{-1}, 20 µg mL^{-1}) were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.69 to 0.1.36. For Inter day variations study three different concentrations of the standard solution in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.80 to 1.76.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.72 µg mL^{-1} and 2.20 µg mL^{-1}, respectively.

Recovery studies

To check the accuracy of the method, recovery studies were carried out by standard addition method. It involved addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 10 μg mL^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results of recovery studies demonstrated that the proposed method is accurate for estimation of drug in tablet dosage form. The results obtained are represented in Table 3.

Table 3: Accuracy data.

Drug	Sample concentration taken (μg mL^{-1})	Concentration added (μg mL^{-1})	Total concentration found (μg mL^{-1})	% Recovery*	% R.S.D.^{a}
Ebastine	10	08	18.04	100.21	0.73
	10	10	20.03	100.16	0.92
	10	12	21.83	99.20	0.64

*Average of three determinations, R.S.D. is relative standard deviation

Robustness

The robustness of method was checked by making deliberate variations in method parameters. The parameters varied were wavelength (± 1 nm), mobile phase composition (± 2 % acetonitrile) and the effect on the area of drug was noted. No marked changes in the chromatograms and peak areas of drug demonstrated the robustness of the method.

CONCLUSION

A simple, accurate, precise and selective stability indicating RP-HPLC method without interference from excipients or degradation products has been developed and validated for determination of ebastine in tablet dosage form. All the validation parameters were within the acceptable limit as specified by ICH guidelines. As the developed method is stability indicating, it can be used for assessing the stability of ebastine in bulk drug and in pharmaceutical tablet dosage form.

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