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STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF EBASTINE AS BULK DRUG AND IN TABLET DOSAGE FORM

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ABSTRACT

The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Ebastine as bulk drug and in tablet dosage form. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Ebastine was found susceptible to all the analyzed stress conditions except photolysis. Chromatographic resolution of Ebastine and its degradation products was achieved by use of silica gel 60 F_{254} precoated aluminium plates as stationary phase and solvent mixture comprising of Ethyl acetate: Triethylamine (9.5: 0.5, v/v) as optimum mobile phase. Densitometric detection was carried out at 253 nm. The retention factor was found to be 0.59 ± 0.05. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The developed method was found to be linear in the concentration range of 100-600 ng band$^{-1.}$ The developed method has been effectively applied for the drug estimation in tablet dosage form.

KEYWORDS: Ebastine, HPTLC, Forced degradation, Tablet dosage form, ICH

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 stability-indicating high performance thin layer chromatographic (HPTLC) method. This paper describes development and validation of simple, precise, accurate and selective stability indicating HPTLC 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

Analytically pure 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. Ethyl acetate, Triethylamine, (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic resolution of drug was achieved by use of precoated silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) Merck TLC plates as stationary phase with the help of Camag Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz,

Switzerland) by using mixture of ethyl acetate: triethyl amine (9.5: 0.5, v/v) as mobile phase. The chamber was saturated with mobile phase for period of 15 min. TLC plates were dried in a current of air with the help of a hair drier after development. Densitometric scanning was completed on Camag thin layer chromatography scanner III at 253 nm for all developments operated by win CATS software version 1.4.2. Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as radiation source.

Preparation of standard stock solution

Accurately weighed 10 mg of drug was transferred to 10 mL volumetric flask and dissolved in methanol to acquire solution of concentration 1000 ng μL^{-1} which was diluted further to 10 mL using methanol to get working standard solution of 100 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 253 nm. So, 253 nm was selected as the wavelength for detection.

Analysis of tablet formulation

Commercial brand of tablet namely Ebanit 10 was selected to estimate the amount of ebastine in Tablet formulation. Twenty tablets were weighed and powdered. Tablet powder equivalent to 10 mg was transferred to 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 100 ng $band^{-1}$. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 200 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 253 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation studies

Forced degradation studies were carried out to check the stability by exposing the bulk drug to the physical stress conditions. The study was carried out at concentration of 1000 ng μL^{-1}. The hydrolytic studies were carried out by treatment of stock drug solution separately with 0.05 N HCl and 0.05 N NaOH at room temperature for 30 min. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 400 ng $band^{-1}$. The drug was treated with water at room temperature for 30 min for neutral hydrolysis. Standard drug solution was treated with 3 % H_2O_2 at room temperature for 30 min to perform the oxidative degradation and was diluted with methanol to obtain 400 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 5 h. The solid drug powder was exposed UV light up to 200 watt h square $meter^{-1}$ for 3 d to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of 400 ng $band^{-1}$.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The goal of present research work was to develop stability indicating HPLTC method which would be proficient to give the satisfactory resolution between ebastine and its degradation products. Varied solvent systems comprising different ratios of benzene, chloroform, toluene, methanol, ethyl acetate, triethylamine were examined (data not shown) to separate and resolve spot of ebastine from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of ethyl acetate: triethyl amine (9.5: 0.5 v/v) was chosen as optimum for attaining well defined and resolved peak. Densitometric detection was carried out at 253 nm. The retention factor was found to be 0.59 ± 0.05. Representative densitogram of standard solution of Ebastine is represented in Fig. 1.

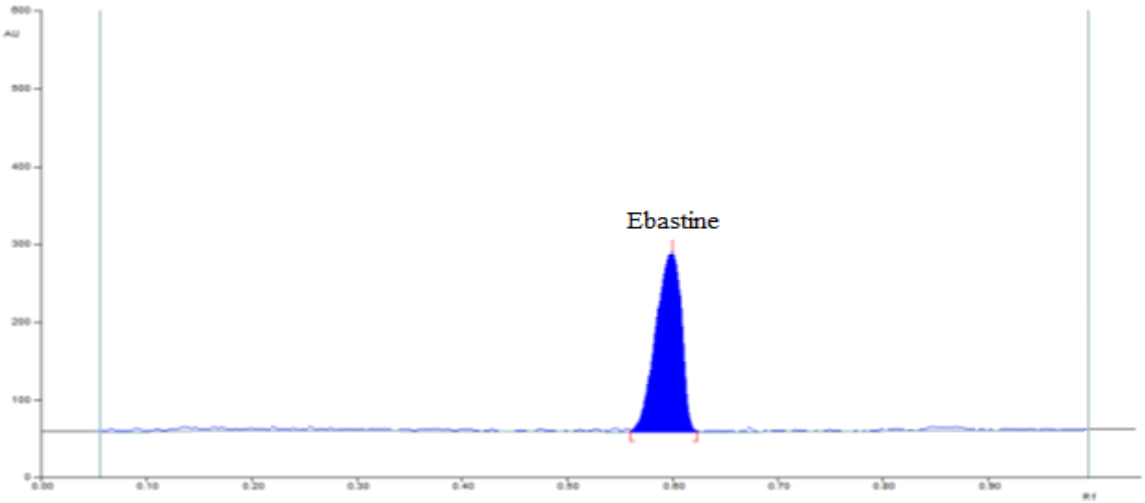

Fig 1: Representative densitogram of standard solution of Ebastine. (400 ng $band^{-1}$, Rf= 0.59 ± 0.05)

The forced degradation results demonstrated susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and its stability under photolytic stress conditions. The degradation products formed under the stress conditions were not interfering with active drug indicating the specificity of developed procedure. Fig. 2-4 denotes the densitograms of acid, alkali and neutral hydrolytic degradation, while Fig. 5 and 6 illustrates the densitograms of oxidative and thermal degradation, respectively. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 1.

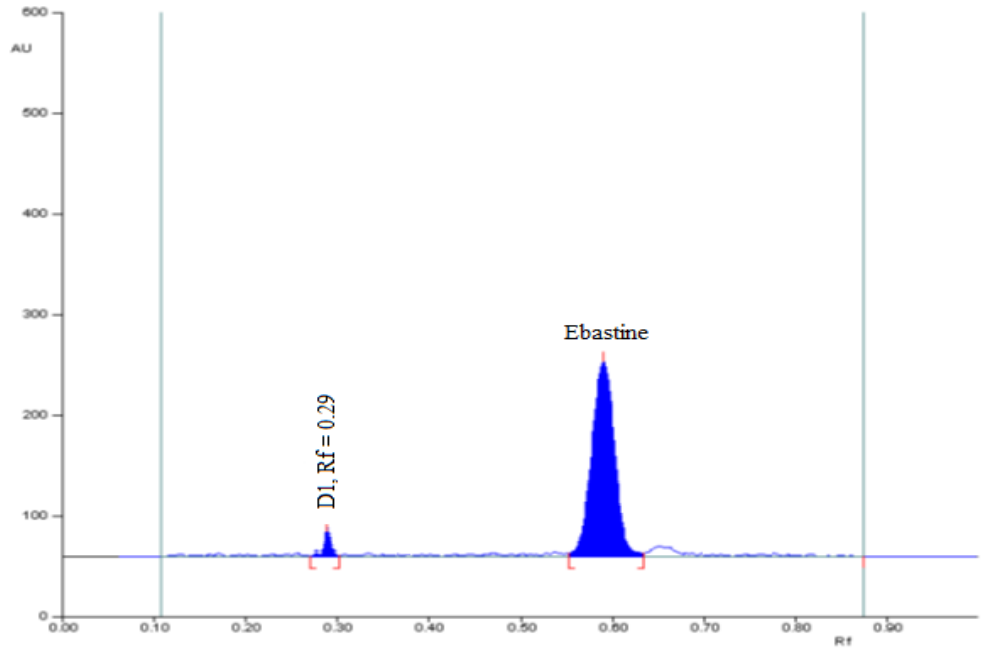

Fig 2: Densitogram obtained after treatment with 0.05 N HCl with degradation product (D1, Rf = 0.29).

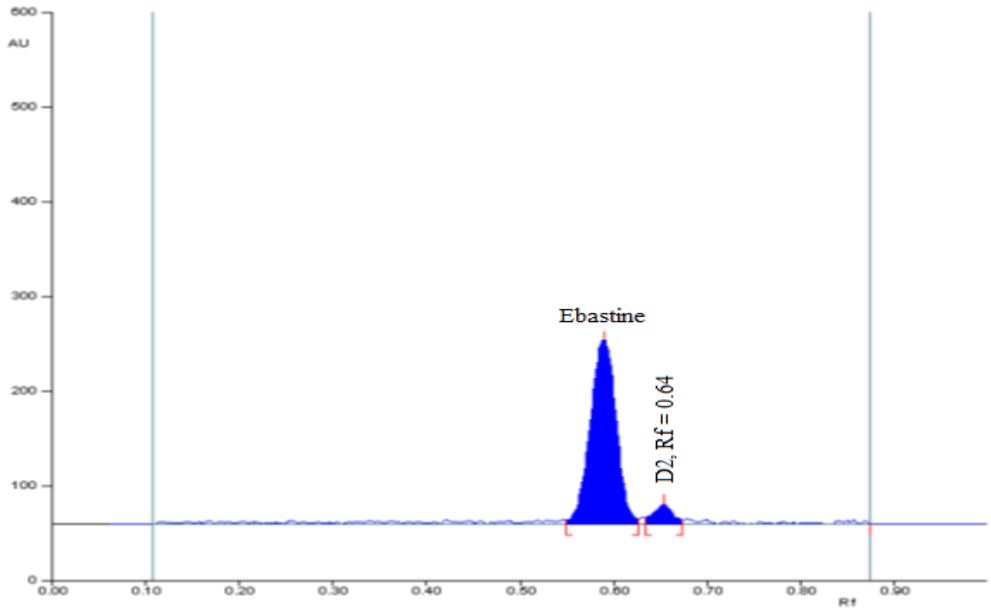

Fig 3: Densitogram obtained after treatment with 0.05 NaOH with degradation product (D2, Rf = 0.64).

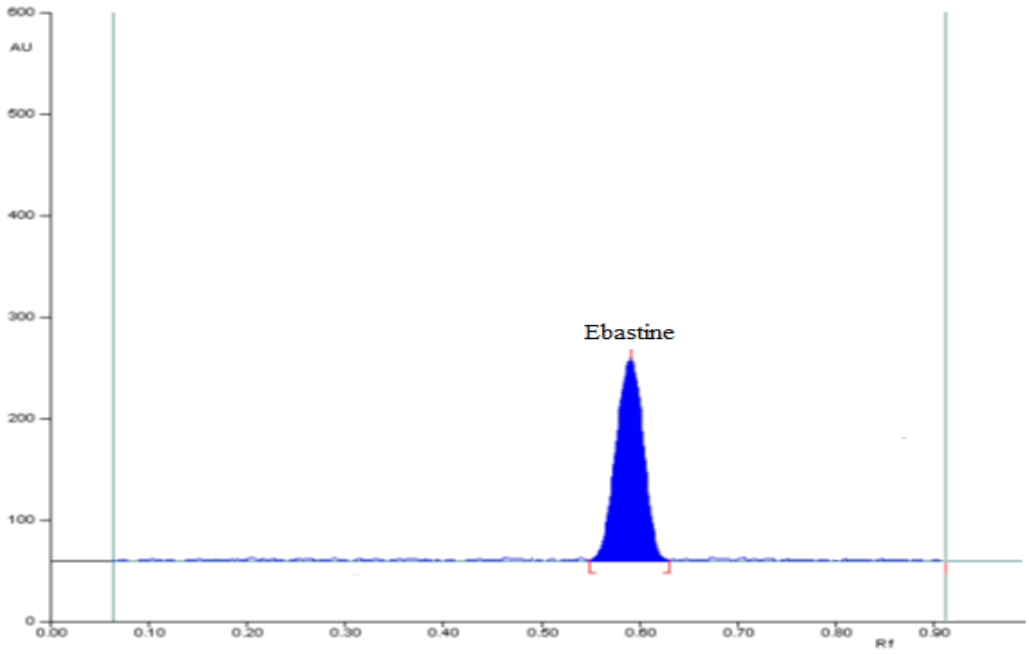

Fig 4: Densitogram after neutral hydrolysis with water.

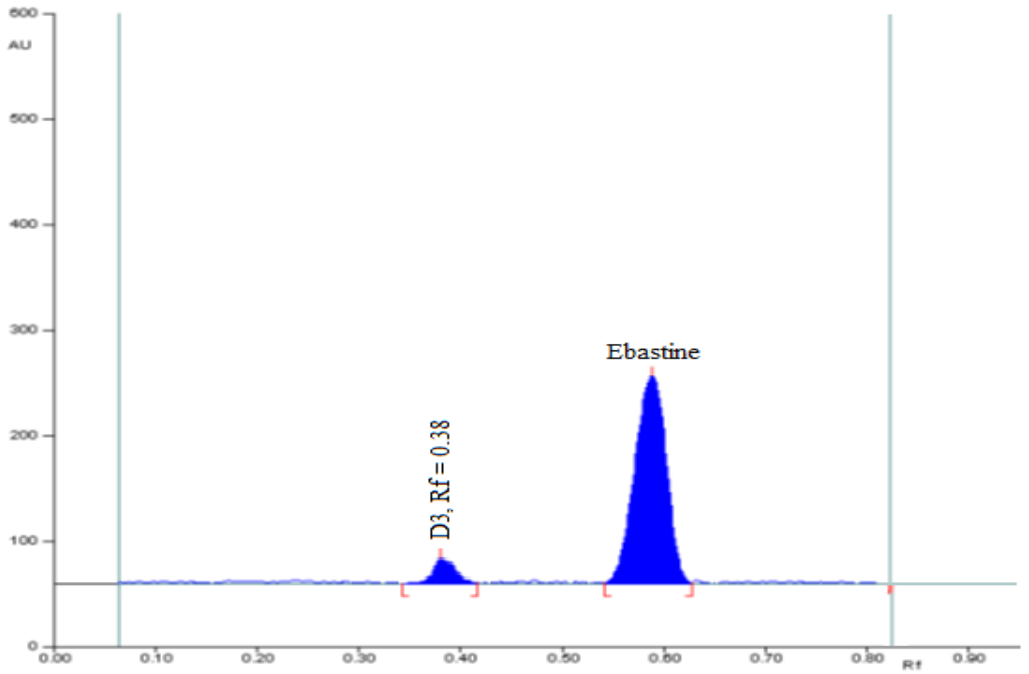

Fig 5: Densitogram after treatment with 3% H_2O_2 with degradation product. (D3, Rf = 0.38)

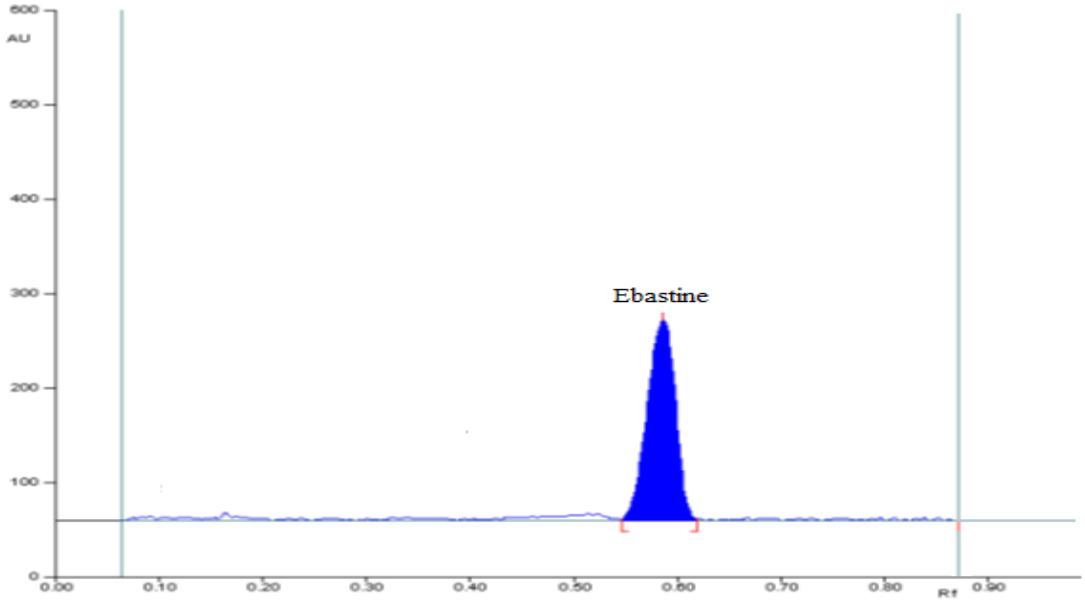

Fig 6: Densitogram obtained after exposure to 60ºC for 5 h.

Table 1: Forced degradation studies.

Stress conditions/ duration	% of active drug	% Degradation
Acid / 0.05 N HCl/ Kept at RT for 30 min	88.94	11.05
Alkal /0.05 N NaOH/ Kept at RT for 30 min	84.81	15.18
Neutral/H_2O/ Kept at RT for 30 min	89.09	10.90
Oxidative /3 % H_2O_2 / Kept at RT for 30 min	87.40	12.59
Dry heat/ 60ºC/ 5 h	88.26	11.73
Photolysis: UV light 200 watt h square meter^{-1} 3 d	99.73	---

Method Validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines.[18, 19]

Linearity

Volumes 1, 2, 3, 4, 5 and 6 µL from standard solution of Ebastine (100 ng µL^{-1}) were spotted onto the TLC plates, developed and scanned as described earlier. The established method was found to be linear in the concentration range 100-600 ng band^{-1} with high correlation coefficient. The linear regression equation was found to be y =7.0074x +556.47 with correlation coefficient value of 0.993. The calibration curve achieved by plot of concentration vs peak area is depicted in Fig. 7.

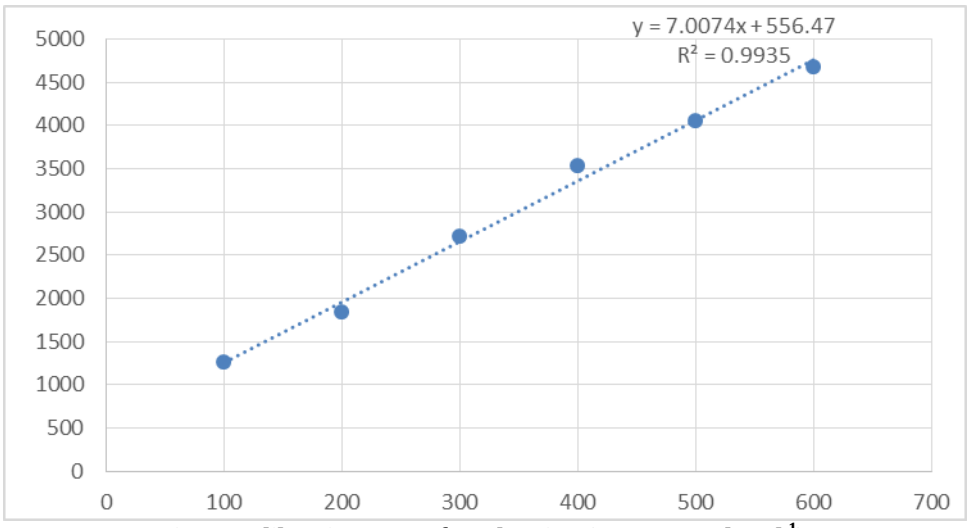

Fig 7: Calibration curve for Ebastine (100-600 ng band^{-1}).

Precision

Set of three different concentrations in three replicates of standard solution of Ebastine (100, 200 and 300 ng band^{-1}) were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as R.S.D. (%), was found to be in the range of 0.92 to 1.49. Three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days for inter day variation study. Interday variation, as RSD (%) was found to be in the range of 0.50 to 1.64. The smaller values of % R.S.D. (< 2) indicated that method was found to be precise.

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 12.65 ng band^{-1} and 38.35 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band^{-1} from tablet solution. The drug concentrations were calculated from linear regression equation. The results of the recovery studies indicated accurateness of developed method for estimation of drug in tablet formulation.

Table 2: Recovery studies.

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Amount found (ng band^{-1})	% Recovery±R.S.D.*
Ebastine	200	160	355.44	98.73±1.23
	200	200	396.31	99.07±0.60
	200	240	438.52	99.66±0.98

*Average of three determinations

Robustness

The deliberate variation in the method parameters were made to check the robustness of the method. The parameters varied were mobile phase composition (± 2 % ethyl acetate), wavelength (± 1 nm) and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated robustness of the method.

CONCLUSIONS

Stability-indicating HPTLC-densitometric method without interference from the excipients and/or from degradation products has been developed and validated for the estimation of Ebastine as bulk drug and in tablet dosage form. The developed method is simple, precise, accurate, reproducible and selective. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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