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Research Article

STABILITY INDICATING HPLC METHOD FOR ANTIHISTAMINE DRUG EBASTINE ALONG WITH DEGRADATION KINETIC STUDY

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A B S T R A C T

A sensitive, precise, accurate and stability indicating HPLC method was developed and validated for analysis of ebastine. The separation of ebastine from its degradation product was achieved on a C_{18} column using acetonitrile: 0.5% phosphoric acid (68:32 %v/v) as a mobile phase and detection was performed at 254 nm. The degradation of ebastine was studied under different ICH recommended stress conditions. The method was linear over the range of 5-120 µg/ml with correlation co-efficient of 0.9993. Limit of Detection and Limit of Quantitation, calculated mathematically, were 0.30 µg/ml and 0.91µg/ml, respectively. Ebastine was found to degrade under acid and oxidation conditions with first order reaction and zero order reaction, respectively. Half time at room temperature in acidic and oxidative condition was calculated as 11,403.67 min. and 503.32 min., respectively. The Arrhenius plot was constructed and activation energy of degradation was calculated for both conditions.

INTRODUCTION

Ebastine (EBS) is a second generation antihistaminic drug, chemically known as 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tertbutylphenyl) butan-1-one. It blocks histamine H_1- receptor activity, and inhibits the release of anti-IgE-induced prostaglandin D2 (PGD2), leukotriene C4/D4 and cytokines which act as inflammatory mediators. Ebastine and its active metabolite, carebastine, are selective histamine H_1 peripheral receptor antagonists devoid of untoward CNS action and anticholinergic effects[1]. It is indicated mainly for allergic rhinitis and chronic idiopathic urticaria[2].

EBS is official drug in British Pharmacopoeia[3], which recommends a titrimetric method for determination of EBS in pure form using 0.1 M perchloric acid as a titrant. Scientific literature includes some analytical methods for determination of EBS in pharmaceutical formulations and/or biological fluids, such as spectrophotometry[4-9], voltametry[10], HPLC[11-14], HPTLC[15] and LC-MS/MS[16-17]. Few stability indicating methods have been reported, such as LC-DAD[18], LC method after pre column derivatization with Zn^{2+} ions[19] and spectrofluorimetric methods[20]. UHPLC method was reported that determined the drug in presence of impurities[21]. These studies gave useful information about ebastine degradation but it is not sufficient for a complete stability protocol of EBS.

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Stability studies and degradation kinetics are integral parts of the quality control of a drug on an industrial scale. Degradation kinetics is the study of the rate at which degradation occur. It is useful to predict shelf life period of the medicine and it gives an insight into the mechanisms of changes involved. International Conference on Harmonization (ICH) guideline[22] stipulates that the stability of active drug substances must be assessed. An ideal stability-indicating method is one which quantifies the drug and resolves it from its degradation products. These facts initiate the present study to establish an accurate, specific, repeatable, and stability-indicating HPLC method for analysis of EBS in the presence of its degradation products. The proposed method was validated in accordance with ICH guidelines[23-24]. The kinetics of degradation of the drug was studied by use of the method.

MATERIALS AND METHODS

Ebastine (certified purity of 99.49%, batch no. EBSN/150) was kindly gifted by Kiwi Laboratories, Vadodara (Gujarat). Ebast 10 tablets were purchased from local pharmacy. Acetonitrile and water (HPLC grade) were purchased from Merck, India. *o*-phosphoric acid (OPA), sodium hydroxide, hydrochloric acid, and hydrogen peroxide used were of analytical grade and purchased from S.D. Fine Chemicals, India. The quantitative analysis was performed on a LC-2010 C_{HT} by Shimadzu equipped with quaternary solvent manager, auto sampler, column compartment, and PDA detector controlled by LC solution software. Separation was achieved on Phenomenex C_{18} (250 x 4.6 mm, 5µm) column. The standards and samples

were separated using mobile phase consisting of acetonitrile: 0.5% phosphoric acid (68:32 %v/v). The flow rate was 1.0 ml/min. The column temperature was set at 40°C, and the injection volume was 20 µl. Samples were analyzed over a wavelength range of 210 - 410 nm and a λ_{max} of 254 nm was used for characterization.

Standard stock solution preparation

A standard solution of EBS containing 1000 µg/ml was prepared in acetonitrile and diluted as appropriate with the same solvent to obtain the working concentration range. Solutions were found to be stable for at least 5 days when kept at room temperature.

Sample solution preparation

20 tablets (label claim 10 mg) were accurately weighed and powdered, and powder equivalent to 20 mg EBS was mixed with acetonitrile (10 ml) and sonicated for 20 min. The solution was filtered through Whatman no. 41 filter paper and the residue was washed thoroughly with acetonitrile. The filtrate and washings were combined in a 20 ml volumetric flask and diluted to volume with acetonitrile. One ml of this solution was transferred to a 10 ml volumetric flask and diluted to volume with acetonitrile.

Validation of the method

The method was validated for system suitability, specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness as per ICH guideline

The system suitability ensures suitability of HPLC testing system for the intended application. The system suitability was assessed by seven replicate analyses of 100 µg/ml solution of EBS and chromatographic parameters were evaluated

To evaluate the linearity of the method, accurately measured volumes of the EBS standard solution were successively transferred into of a series of 10 ml volumetric flasks to obtain final concentrations of 5-120 µg/ml and diluted to the mark with acetonitrile and solutions were mixed properly. 20 µl aliquots of each solution were injected three times and were chromatographed. The average peak area of EBS was plotted against the drug concentration and the regression equation was derived.

System repeatability of the method was checked by analysis of seven injections of 100 µg/ml EBS solution. Percentage relative standard deviation for retention time and peak area of drug was calculated. The intraday and interday precision for analysis of EBS was performed with three concentrations (20, 40 and 60 µg/ml) for three times on same day and for three different days, respectively.

For accuracy of the method evaluation, 20 tablets (brand name - Ebast 10) were crushed and powder equivalent to 20 mg of EBS was taken in 3 different 10 ml volumetric flasks. 10 mg, 20 mg and 30 mg of EBS standard was added to volumetric flasks containing tablet powder and diluted up to mark with acetonitrile. Further they were diluted to have 40 µg/ml of sample in each flask. The experiment was performed triplicate. % recovery was calculated for each level.

For robustness evaluation of HPLC method a few parameters like flow rate, percentage of acetonitrile in the mobile phase and column oven temperature were deliberately changed. One factor was changed at one time to estimate the effect. To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase (1.0±0.1 ml/min), column oven temperature (40±1°C) and acetonitrile composition (68±2 %) were varied. In each case, the %RSD values were calculated for the obtained peak area. The number of theoretical plates and tailing factors was compared with that obtained under the optimized conditions.

The limits of detection (LOD) and quantification (LOQ) were estimated mathematically. The mathematical formulas used were:

LOD = 3.3(standard deviation of y-intercept/slope of the calibration plot)
LOQ = 10(standard deviation of y-intercept/slope of the calibration plot)

Specificity- Forced degradation studies

The forced degradation studies were executed to demonstrate whether the analytical method was stability indicating and could unequivocally assess the EBS in the presence of impurities and degradation products. EBS standard solution was stressed under thermal, photolytic, acid hydrolytic, base hydrolytic and oxidative stress conditions to result in partial degradation of the drug. 2 ml of the standard solution of EBS (1000 µg/ml) was transferred to three 20 ml volumetric flask followed by 2 ml of 1.0 M HCl, 2 ml of 1.0 M NaOH and 2 ml of 3% H_2O_2 to different flask and then the mixture were heated in a thermostatically water bath at 80°C for 1 hr, 2 hr and 30 min. to induce degradation by acid, base and oxidation, respectively. Then the mixtures were diluted with acetonitrile up to the mark. 50 mg EBS powder was placed in convection oven and exposed to heat at 70°C for 8 h and further preceded as per sample preparation for thermal degradation. To check photolytic degradation EBS drug (50 mg) was taken in two petridishes. One petridish was wrapped with aluminum foil and other was not wrapped. Both the petridishes were kept in UV chamber (365 nm) for 24 hr at room temperature and further preceded as per sample preparation.

Analysis of the Marketed Formulation

To determine the EBS content of a tablet (label claim 10 mg), 20 tablets were accurately weighed and powdered, and powder equivalent to 20 mg EBS was mixed with acetonitrile (10 ml) and sonicated for 20 min. The solution was filtered through Whatman no. 41 filter paper and the residue was washed thoroughly with acetonitrile. The filtrate and washings were combined in a 20 ml volumetric flask and diluted to volume with acetonitrile. One mL of this solution was transferred to a 10 ml volumetric flask and diluted to volume with acetonitrile. The analysis was repeated in triplicate. The possibility of interference of excipients with the analysis was studied.

Study of degradation kinetics

The EBS drug showed degradation under acidic and oxidative conditions. For this reason, a study was proposed to evaluate the degradation kinetics under these conditions. 2ml of standard EBS solution (1000 µg/ml) was placed in 20 ml volumetric flasks. To each flask 2 ml of 0.1 M HCl was added and the mixture was heated under reflux at 50, 60, 70, or 80 °C. After specific time interval volumetric flask was taken out from water bath, cooled it and diluted to the volume with acetonitrile. They were analyzed by the developed HPLC

method. Similar procedure was followed using 1% H_2O_2 to study degradation kinetics under oxidative condition.

RESULTS AND DISCUSSION

Development of Method

To achieve good separation of EBS from degradation products within reasonable time, mobile phases of various compositions were investigated and system suitability test was performed for method optimization. The mobile phase ratio of acetonitrile and 0.5% phosphoric acid 68:32% (v/v) was found to furnish sharp and well defined peak with very good symmetry(1.32), theoretical plates(>3000) and low retention time (4.3 min.)(Fig.1). Other tried mobile phases gave too broad peak or split peak or asymmetric peak, so were not considered. A wavelength maximum of the EBS, 254 nm was chosen for detection of eluents. The effect of flow rate was also optimized and 1.0 ml/min. was considered optimum for the separation method. After this experimental study, good separation of EBS from acidic and oxidative degradation product was achieved.

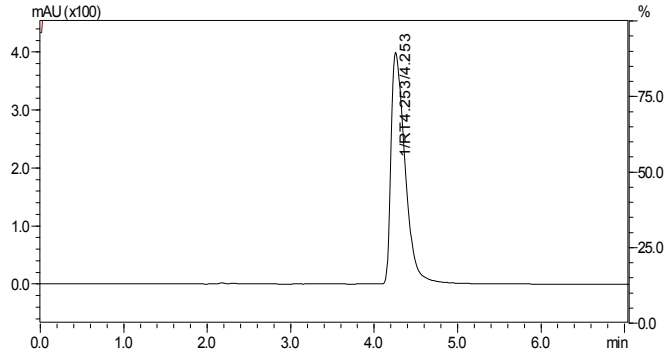

Fig 1 Chromatogram of EBS standard (100 µg/ml)

Validation of Method

The proposed method was validated according to the ICH guideline to prove its suitability for the intended purpose. The calibration graph was linear over the concentration range 5-120 µg/ml (r^2 ± SD = 0.9981 ± 0.0002). In chromatograms of drug samples extracted from tablets, no interference was observed from excipients commonly present in the tablets. The results of forced degradation studies showed the ability of the proposed method to separate EBS from possible degradation products. The method was found to be robust regarding any small variation in the column temperature (40 ± 1°c) and detection wavelength (254 ± 1 nm), as revealed by the constancy of the resolution and the peak area.

Table 1 Summary of Validation Data

Data	Value
Linear range	5-120 µg/ml
Correlation coefficient	0.9993 ± 0.0001
Limit of detection	0.30 µg/ml
Limit of Quantitation	0.91 µg/ml
Accuracy	101.15 ± 0.86
Precision (%RSD)	
Repeatability	1.644
Interday (n=3)	0.885
Intraday (n=3)	1.297
Robustness	Robust in column temperature and detection wavelength
Specificity	Specific

%RSD is Percentage relative standard deviation for n=3 observations.

In case of flow rate of mobile phase (1 ± 0.1 ml/min.), where minor variation resulted in a significant change in the retention of the EBS and; in case of ratio of acetonitrile, it resulted in a significant change in the retention of the EBS and symmetry of the peak. Method validation data are summarized in Table 1.

Stability indicating aspects

Under acidic hydrolysis, EBS degraded yielding the degradation product at 2.317 minute with good resolution (Fig. 2 and 3) and EBS having peak purity of 0.999 (Fig.4). EBS degraded with 1% hydrogen peroxide yielding the degradation product at 3.943 with resolution of 1.56 (Fig.5 and 6) and EBS having peak purity of 0.999 (Fig.7).

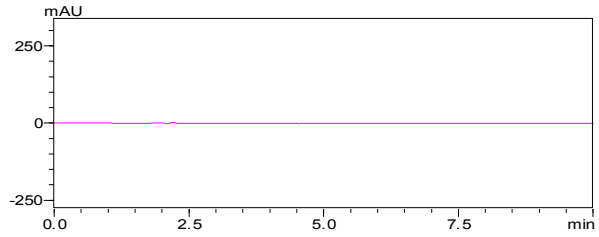

Fig 2 Chromatogram of 0.1 N HCl

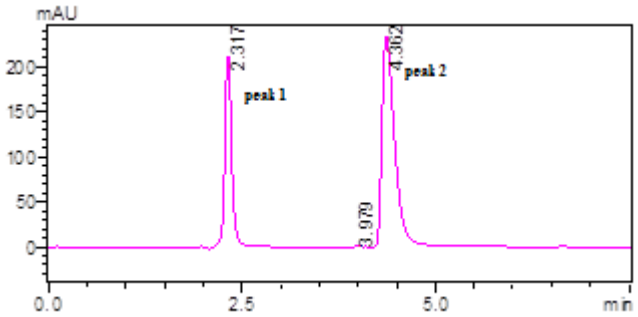

Fig 3 Chromatogram after degradation with 0.1 N HCl at 80° C for 1 hr Peak 1 is degradation product and peak 2 is Ebastine peak

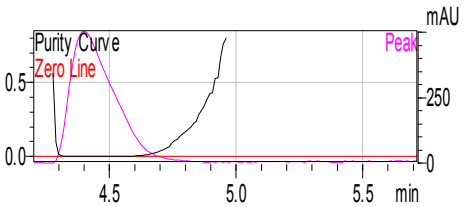

Fig 4 Peak purity curve of EBS in presence of acidic degradaton product

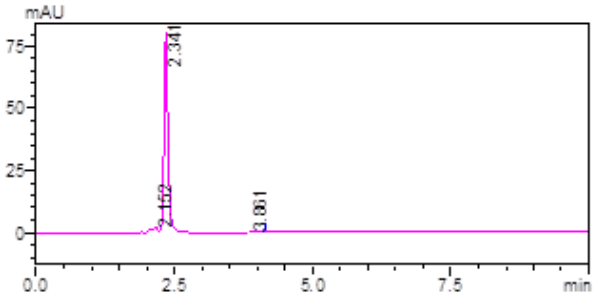

Fig 5 Chromatogram of 1% H_2O_2

The concentration of the EBS was found to decrease from the initial concentration, indicating EBS undergoes degradation under acidic condition (Fig.8) and oxidative condition (Fig.9). No degradation was observed for sample of EBS with 1 N NaOH for 5 hr at 80° C and EBS with distilled water for 5 hr at 80° C. No additional peak was observed and no significant change in initial concentration of EBS when solid EBS drug

kept at 365nm for 2 days. EBS samples kept under dry heat conditions furnished no additional peaks. So EBS is stable under alkali, dry heat, wet heat and photolytic condition.

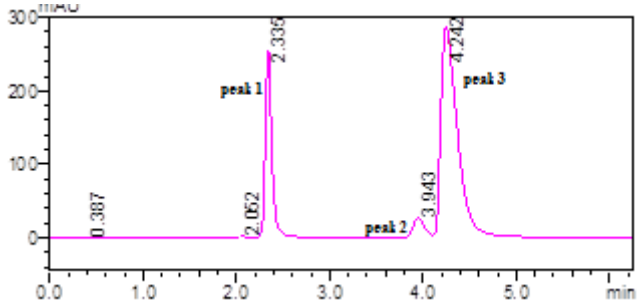

Fig 6 Chromatogram after degradation with 1% H_2O_2 at 80° C for 1 hr Peak 1 is H_2O_2 peak, peak 2 is degradation product and peak 3 is Ebastine peak

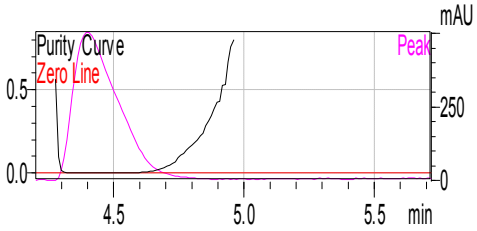

Fig 7 Peak purity curve of EBS in presence of oxidative degradation product

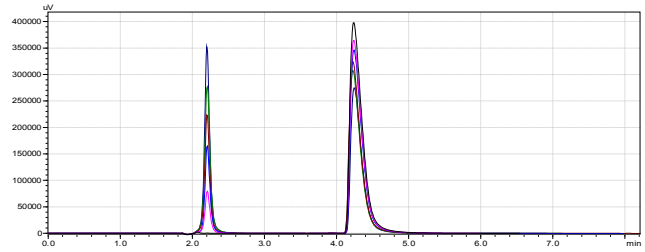

Fig 8 Overlay of chromatograms of HCl degradation at 70° C

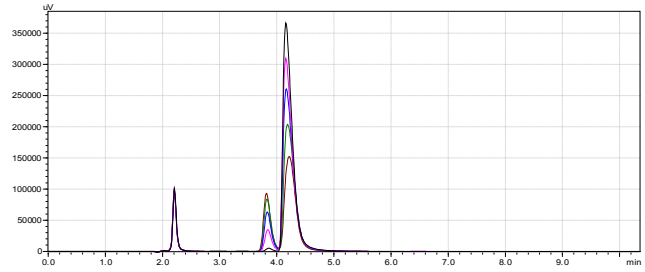

Fig 9 Overlay of chromatograms of 1% H_2O_2 degradation at 70° C

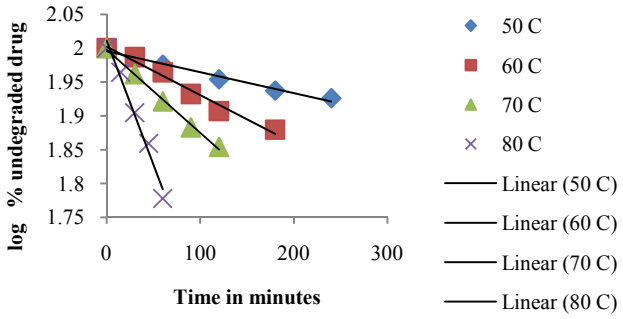

Fig 10 Temperature effect on acidic degradation of EBS

Degradation Kinetics

In acidic and oxidative condition, the drug concentration decreased with time. The effect of temperature (50, 60, 70 and 80° C) on the degradation process is shown in fig. 10 and 11 for acidic and oxidative condition, respectively.

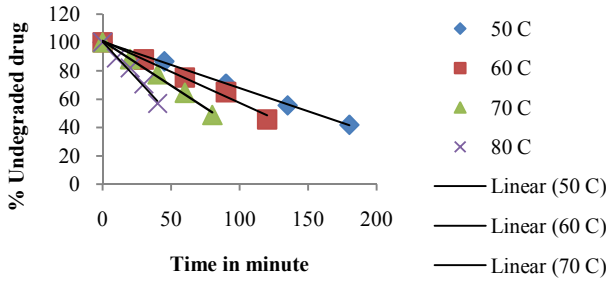

Fig11 Temperature effect on oxidative degradation of EBS

The degradation under acidic condition follows first order kinetics. The first order degradation rate constants (K) were calculated from the slopes of plots of log (a/a-x) versus time, t in accordance with equation (1).

$$Kt = 2.303 \log (a/a-x) \quad \ldots\ldots\ldots (1)$$

Here, a is the initial drug concentration and a-x is the remaining drug concentration.

The half life time ($t_{1/2}$) for the first order degradation was calculated according to equation (2).

$$t_{1/2} = 0.693/K \quad \ldots\ldots\ldots\ldots (2)$$

The degradation under oxidative condition follows zero order kinetics. The zero order degradation rate constant (K) were equal to the slopes of plots of undegraded drug versus time.

$$K = (a-x)/t \quad \ldots\ldots\ldots (3)$$

The half time ($t_{1/2}$) for the zero order degradation was calculated as following.

$$t_{1/2} = a/2K \quad \ldots\ldots\ldots (4)$$

Data obtained from order kinetics were further subjected to fitting to the Arrhenius equation:

$$\log K = \log A - Ea/2.303RT \quad \ldots\ldots\ldots\ldots (5)$$

Where K is the rate constant, A is the frequency factor, Ea is the activation energy (cal mol^{-1}), R is the gas constant (1.987 cal deg^{-1} mol^{-1}), and T is absolute temperature. A plot of K against 1000/T, Arrhenius plot, was linear in the range of 50-80 °C for acidic and oxidative degradation. The activation energy was calculated to be 18.272 and 8.685 Kcal/mol for acidic and oxidative condition, respectively.

Table 2 Degradation Kinetic Data for Ebs In The Presence of 0.1 M HCL

Temperature (°C)	Degradation rate constant K (min^{-1})	Half time $t_{1/2}$ (min.)	t_{10} (min.)
50 °C (323.15 K)	0.00069	1003.04	150.53
60 °C (333.15 K)	0.00161	429.87	64.51
70 °C (343.15 K)	0.00276	250.75	37.63
80 °C (353.15 K)	0.00852	81.33	12.21
25 °C (calculated)	0.0000608	11,403.67	1711.37

Table 3 Degradation Kinetic Data for Ebs In The Presence of 1% H_2O_2

Temperature (°C)	Degradation rate constant K (min^{-1})	Half time $t_{1/2}$ (min.)	t_{10} (min.)
50°C (323.15 K)	0.327	152.765	30.553
60°C (333.15 K)	0.439	113.895	22.779
70°C (343.15 K)	0.630	79.352	15.870
80°C (353.15 K)	1.045	47.847	9.569
25° C (calculated)	0.0993	503.32	100.66

The degradation rate constants at room temperature (25 °C) obtained by extrapolating to 25 °C (where 1000/T = 3.356) and half time were calculated (Table 2 and 3).

CONCLUSION

The present study represents precise, specific, accurate and stability-indicating HPLC method for EBS. Statistical analysis proves that the method is suitable for analysis of EBS in the pharmaceutical product without interference from excipients. A stress degradation study was conducted in order to investigate the degradation behaviour of EBS under ICH recommended condition; further kinetic investigation was preferred to check rate of reaction. All validation parameter is permitting the application of suggested method in quality control laboratory.

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