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Research Article

Stability and Degradation Kinetic study of Bilastine in Solution State by RP-HPLC Method

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Abstract

Aim: The current study dealt with the degradation behavior of Bilastine and degradation kinetics of a drug in solution state. **Background:** Very limited information on the effect of pH on maximum stability has been published. In order to understand the degradation kinetics of bilastine, aqueous stability studies were carried out, because such studies on bilastine have not been reported in the literature, further no methods have reported about shelf-life determination of bilastine. The study design involves selection of stability indicating RP-HPLC method for estimation of drug then evaluation of degradation kinetics, shelf-life determination and validation of proposed method. **Results:** The Shimadzu HPLC series 1100 was used for stress degradation analysis of bilastine in tablet dosage form. The analysis was performed using Agilent ZORBAX SB-C8 (4.6×150×5µm) column and Phosphate Buffer: Acetonitrile (pH-5.0) in the ratio of 60:40 as mobile phase; wavelength selected for analysis was 254nm with the flow rate of 1mL/min at which drug showed sharp peak. The analysis was performed on the isocratic pump mode with the injection volume of 20µl. The mobile phase is used as diluent. The proposed method was found to be linear over the range 10 to 50 µg/mL. The analysis was performed by placing standard and samples with 7 different pH buffer, oxidative and neutral hydrolytic solutions in oven at 40ºC, 60^{0}C and room temperature for an interval of 30, 60, 90, 120, 150, 180 mints for standard and samples. The results indicated that the pH, temperature, ionic strength and oxidation greatly influence the stability of Bilastine and the degradation behavior of Bilastine followed pseudo-first-order kinetics. Bilastine was most stable in neutral, alkaline, lower temperature conditions and lower ionic strength. **Conclusion:** The proposed method was found to be specific, selective and robust and successfully applied for its assay, degradation (stress testing) of drug and degradation kinetics in solution state.

Keywords: Degradation, Stability, Bilastine, RP-HPLC, Kinetics

1. INTRODUCTION

Stability studies of drug products helps in establishing the shelf life of the pharmaceuticals. Stability testing of pharmaceuticals requires significant cost, time and scientific proficiency to generate safety, in quality and efficacy in a drug formulation as it involves a complex set of procedures. Scientific as well as commercial success of any pharmaceutical product the understanding of the drug development process and the infinite tasks and milestones is essential to abroad development plan [1]. Drug stability refers to the extent to which a drug substance or product retains, the same properties and characteristics that it possessed at the time of its manufacture within specified limits and throughout its period of storage and use [2]. The main objective of stability studies is to determine the shelf-life of the drug product. During stability it is expected that any degradation product in dosage form not to achieve a sufficient level that represent a risk to the patient before storage time allowed. Based on this time, the product shelf life or expiration date is determined [3]. The FDA and pharmaceutical Conference on Harmonization (ICH) guidance provides very little information about strategies and principles for conducting forced degradation studies, including problems of poorly soluble drugs and exceptionally stable compounds. In particular, the issue of how much stress is sufficient to carry out the stress testing has not addressed specifically. Degradation profiles generated using overstresses of a molecule are not representative of real storage conditions and perhaps not relevant to method development. Therefore, in stress-testing it is the amount of stress that is important and not necessarily the extent of degradation and therefore the conditions should be realistic and not excessive. It has been observed that some compounds may not degrade significantly after considerable exposure to stress conditions. From both a regulatory and a scientific perspective it is unclear about what should be done at each development phase. Although FDA does not require degradation studies for an investigational new drug (IND) application, preliminary degradation studies are useful for the development of the stability-indicating methods that will be used during the clinical trials [4].

Bilastine[2-[4-[2-[4-[1-(2-ethoxyethyl)benzimidazol-2-yl] piperidin-1-yl]ethyl]phenyl]-2-methylpropanoic acid] is a second generation antihistamine medication which is used in the treatment of allergic rhino-conjunctivitis and urticaria (hives). It exerts its effect as a selective histamine H1 receptor antagonist, and has effectiveness similar to cetirizine, fexofenadine, and desloratadine. Its molecular formula is $C_{28}H_{37}N_{3}O_{3}$ having molecular weight of 463.622 g/mol (Fig. 1). It is white to off-white solid slightly soluble in Chloroform and Methanol [5,6,7].

Physicochemical properties, pharmacokinetics and pharmacodynamics of bilastine have been reported and described [8,9,10]. Yet very limited information on the effect of pH on maximum stability has been published. In order to understand the degradation kinetics of bilastine, aqueous stability studies were carried out, because such studies on bilastine have not been reported in the literature, further no methods have reported about shelf life determination of bilastine. The objectives of the present study were to investigate the stability of bilastine in aqueous solution as a function of pH and temperature; and to evaluate degradation kinetics at various hydrolytic conditions using stability indicating RP-HPLC method.

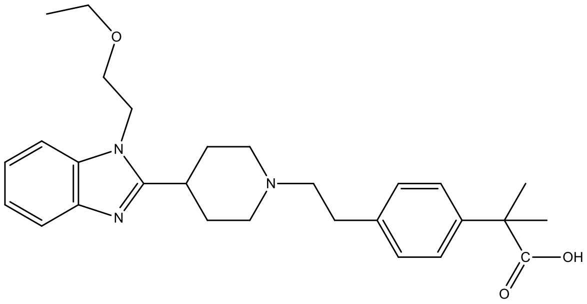

Figure 1: Structure of Bilastine

2. EXPERIMENTAL METHODS

2.1 Materials

Pure bilastine standard (Assigned purity 100.02%) was obtained as a gift sample from Glenmark Pharmaceuticals Ltd. Tablet formulation (Bilanix) was purchased from Ajanta Pharma Ltd. with labeled claim of 20mg is used in analysis. Acetonitrile (HPLC Grade), Ortho phosphoric acid (HPLC Grade), GR Grade Potassium Di-hydrogen Phosphate, Potassium Hydroxide, Potassium Chloride, Hydrochloric Acid, Potassium Bi-phthalate, Sodium Hydroxide, Monobasic Potassium Phosphate, Monosodium phosphate, Sodium Phosphate Dibasic, Boric acid and Hydrogen Peroxide etc. used during the analysis. In-house double distilled water was used during whole experiments.

2.2 Instrumentation and Chromatographic Conditions

The Shimadzu-HPLC series 1100 equipped with isocratic pump LC-10ADVP, PDA - SPD M20A detector was used for stress degradation analysis of Bilastine in tablet dosage form. The analysis was performed using Agilent ZORBAX SB-C8 (4.6×150×5µm) column and Phosphate Buffer: Acetonitrile (pH-5.0) in the ratio of 60:40 as mobile phase; wavelength selected for analysis was 254nm with the flow rate of 1mL/min at which drug showed sharp peak. The analysis was performed on the isocratic pump mode; the column temperature was set at 35˚C, with the injection volume of 20µl. The mobile phase is used as diluent.

2.3 System Suitability Procedures

The system suitability parameters were studied and after equilibration of column with mobile phase, six replicate injections of 20 µL solution were injected through the manual injection and chromatographed. For linearity study, aliquots of standard stock solution were in range 1.0 to 5.0 mL in 10 mL volumetric flask with Injection volume 20µl, Detection wavelength 254 nm, Column temperature 30-35^{0}C, Pump mode Isocratic, diluent (mobile phase) and volume was made up to mark with diluent to obtained concentration ranging from 10 – 50 µg/mL of Bilastine. The mobile phase was allowed to equilibrate with stationary phase till steady baseline was obtained. Each of the final solution was injected separated and recorded the chromatograms. The graph plotted between concentrations vs AUC is shown in figure 2.

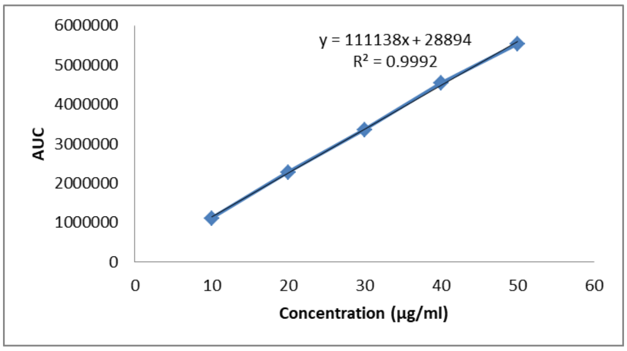

Figure 2: Calibration curve of Bilastine

2.4 Kinetics Procedures at Different pH and Temperatures

For kinetic studies of bilastine samples, 7 different pH buffer solutions were made for making different pH conditions from acidic to alkaline range. The pH ranged from low acidic range of 1.2 to most basic at 9.5. All of the pH buffers were prepared in 200 mL volumetric flasks with purified double distilled water and the final pH was adjusted. The pH 1.2 was prepared by adding 50 mL of 0.2 M KCl solution and 85 ml of 0.2 M HCl solution and then adding water to volume. The pH 2.2 was prepared by adding 50 mL of 0.2 M KCl solution, 7.8 mL of 0.2 M HCl solution and then adding water to volume. The pH 4.6 was prepared via adding 50 mL of 0.2 M potassium biphthalate solution, 11.1 mL of 0.2 M NaOH solution and then water to volume. The pH 6.6 was prepared via adding 50 mL of monobasic potassium phosphate solution, 16.4 mL of 0.2 M NaOH solution and then water to volume. The pH 8.0 was prepared via adding 50 mL of 0.2 M Boric acid and KCl solution, 3.9 mL of 0.2 M NaOH solution and then water to volume. The pH 9.5 buffer solution was prepared by adding 50 mL of 0.2 M Boric acid and KCl solution, 32.1 mL of 0.2 M NaOH solution and then water to volume. The procedures were obtained from USP 32 chapter on Reagents: Buffer Solutions. The solutions were freshly prepared and pH's were measured at room temperature of 35˚C. 6% H_2O_2 was used for oxidative hydrolysis and double distilled deionized water for neutral hydrolysis.

The preparation of kinetic samples: Accurately weighed quantity of Bilastine (~10 mg) was transferred to a series of different 10.0 mL volumetric flasks. To each flask 5.0 mL diluent was added as co-solvent and then 5.0 mL of reagent (different pH buffer solutions, 6% hydrogen peroxide and distilled water) were added. The flasks were placed in oven at 40ºC, 60^{0}C and at room temperature; standards were withdrawn at an interval of 30, 60, 90, 120, 150 and 180 min, while samples were withdrawn at an interval 180 mint. Filter the content and 1.0 ml portion of filtrate was diluted up to 10.0 ml with diluent. Dilute further by addition of diluent to make the final concentration of 20 µg/ml of bilastine solution.

The amount of drug undegraded of sample and standard was calculated using the formula (1)

$$\boldsymbol{Drug\ undegraded} = \frac{\boldsymbol{Au_{(exposed)}}}{\boldsymbol{As_{(unexposed)}}} \times \boldsymbol{C_s} \qquad \textbf{(1)}$$

Where, Au is peak area of sample (exposed), As is peak area of standard (unexposed) and Cs is Concentration (10mg).

2.5 Determination of Degradation Constant

The degradation rate constants, k_{obs}, were calculated from the slopes of semi logarithmic plots of the drug fraction remaining versus time in accordance with Equation (2).

$$In\,[A] = -kt + In[A]_0 \qquad (2)$$

A plot of In[A] vs. time t gives a straight line with a slope of −k. Where, A_0 was the initial concentration and A was the remaining concentration of Bilastine at time t. The effect of temperature on the rate of drug degradation was determined at different pH's, oxidative and neutral hydrolysis in the mobile phase composition of Phosphate buffer: Acetonitrile (pH 5.0) (60:40). The observed pseudo first-order degradation rate constants (kobs), were calculated using Equation (2).

2.6 Kinetics of Solution State Degradation Studies

The kinetics of degraded sample was evaluated for all hydrolytic conditions. The plot of regression coefficient (r) obtained and the best fit observed indicates the order of degradation reaction. 1) Values of concentration against time (zero- order kinetics) 2) Log of concentration verses time (first- order kinetics) 3) Reciprocal of concentration verses time (second-order kinetics). Under the different conditions, where the degradation of marketed formulation occurred, the kinetics of degradation fitted graphical models of zero, first and second orders. After having submitted to various hydrolytic conditions like acidic, alkali, oxidative and neutral at two different temperatures, a reduction of drug concentration in marketed formulation according to the time of exposure to stress could also be observed. From the order of reaction, the half-life and shelf life of the marketed formulation was calculated by using formulas (3), (4) and (5)

For zero-order reaction (3)

Half- life equation: $t_{1/2} = C_0 / 2K$

Shelf-life equation: $t_{0.9} = 0.1C_0/K$

For first-order reaction (4)

Half- life equation: $t_{1/2} = 0.693/ K$

Shelf-life equation: $t_{0.9} = 0.105/K$

For second-order reaction (5)

Half- life equation: $t_{1/2} = 1/ KC_0$

Shelf-life equation: $t_{0.9} = 0.11/KC_0$

2.7 HPLC method validation

The current method developed has been validated for the analysis of bilastine and its stability studies at different pH's and temperatures using the following parameters.

2.7.1 Accuracy and recovery

The accuracy and recovery for the assay of bilastine were determined by analyzing pre-prepared placebo formulation samples (without drug) added with 80, 100 and 120% of the standard bilastine assay standards in triplicate at the theoretical assay levels. Sample recoveries were calculated by analyzing samples against standard preparation at the 100% target concentration.

2.7.2 Precision

In order to determine the precision of the method, repeatability and intermediate precision were applied. The intermediate precision was assessed by carrying out the analysis of at least three working solutions in three consecutive days that is called as interday. The repeatability of analytical responses is expressed in terms of relative standard deviation in (RSD%).

2.7.3 Linearity and range

Linearity and range established across the range of the analytical procedure. Linearity is generally reported as the correlation coefficients, the slope of regression line, etc. $r > 0.999$. The range of analytical method is the interval between the upper and lower level of analyte (including these levels) that have been demonstrated to be determined with suitable level of precision, accuracy, and linearity using method written. The range is normally expressed in the same unit as test results (e.g. percent, part per billion).

2.7.4 Limit of Detection and Limit of Quantification

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated experimental conditions. The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated from the equations in which the standard deviation of response and the slope of the calibration curve were used.

2.7.5 Robustness and Ruggedness

The robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Deliberate change was made in the optimized chromatographic parameters and robustness of the method was studied by evaluating system suitability parameter data after varying the flow rate, detection wavelength, pH of mobile phase and mobile phase composition etc. The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different instruments, different lots of reagents, different assay, temperatures, different days, different analysts, etc.

3. RESULTS AND DISCUSSION

3.1 Optimization of mobile phase

Various combinations of solvents were tried for optimization of mobile phase like Methanol: Acetonitrile (60:40, v/v), (55:45, v/v) and Acetonitrile: Phosphate buffer (60:40, v/v) pH 5.0 adjusted with Ortho-phosphoric acid (OPA). The mobile phase containing Acetonitrile (ACN): Phosphate buffer (40:60, v/v) pH 5 adjusted with of Ortho-phosphoric acid (OPA) and column temperature 35°C showed well defined, sharp peak with reasonable retention time at a flow rate of 1 ml/min. The retention time was found to be 4.5 min for bilastine.

3.2 Hydrolysis of Bilastine as a Function of pH and Temperature

The hydrolysis of bilastine was studied in the pH range 1.2 - 9.5 and the analysis was performed on the isocratic pump mode with the injection volume of 20µl and at a concentration of 20µg/ml.

Acidic and alkaline hydrolysis was performed by placing standard and samples with 7 different pH buffer solutions in oven at 40ºC, 60^{0}C and Room temperature for an interval of 30, 60, 90, 120, 150, 180 mints for standard and samples. In the case of marketed formulation samples are kept for 180 mints. From chromatograms it reveals that the drug was found to be susceptible to some pH condition. The pH 4.6 and 7.4 shows maximum degradation up to 60.8% and 70.3% respectively at room temperature around no significant degradation at other pH conditions **(Figures 3-4)**. At 40^{0}C the drug shows maximum degradation of 12.7% at pH 2.2 on sample and about 27.6% at pH 8.0 on marketed formulation **(Figures 5-6)**. At 60^{0}C the drug shows maximum degradation of 60.8% and 26.7% at pH 2.2 and 4.6 respectively on sample and 15.3% on marketed formulation **(Figures 7-9)**. From the observations and results it was found that as the heat increases with time the chromatographic peak is broadened due the breakage of bonds within the drug and it can affects the drugs stability within different pH conditions.

The oxidative hydrolysis was carried out using 6%H_2O_2 at room temperature, 40^{0}C and at 60ºC. From chromatograms it reveals that the drug shows maximum degradation of 21.8% and 28.2% on marketed formulation at room temperature and 40^{0}C with no significant effect on sample **(figures 10-11)**. Neutral hydrolysis was performed by placing standard and sample of Marketed Formulation with water in oven at Room temperature, 40^{0}C and 60^{0}C. From chromatograms it reveals that the drug shows maximum degradation of 24.5% at 60^{0}C for marketed formulation and with lower degradation effect on sample **(figure 12)**.

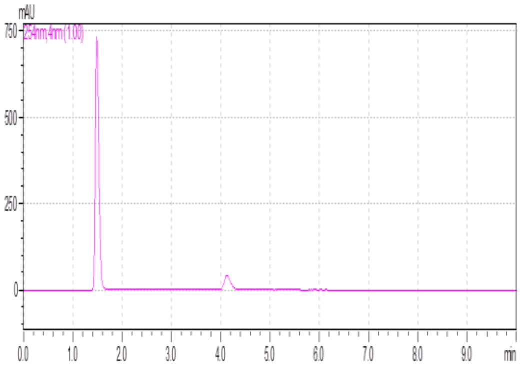

Fig. 3 Chromatogram of sample in pH buffer 4.6 at room temperature

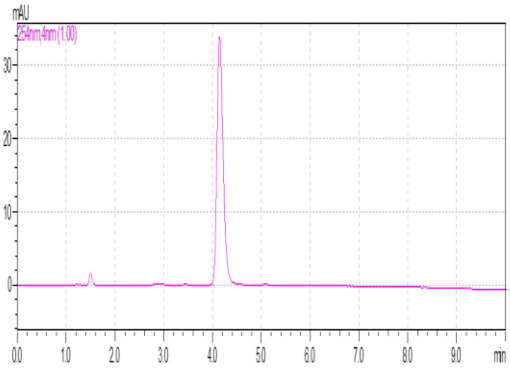

Fig. 4 Chromatogram of sample in pH buffer 7.4 at room temperature

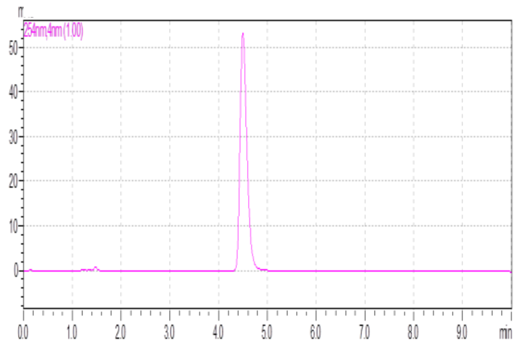

Fig. 5 Chromatogram of sample in pH buffer 2.2 at 40ºC

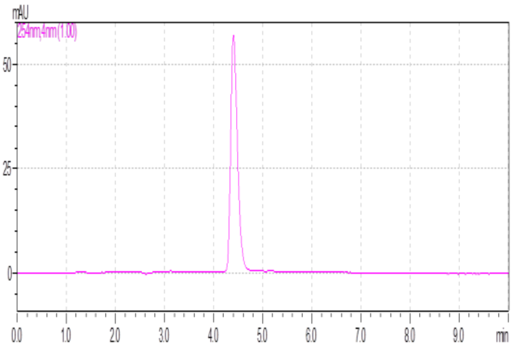

Fig. 6 Chromatogram of sample in pH buffer 8.0 at 40ºC

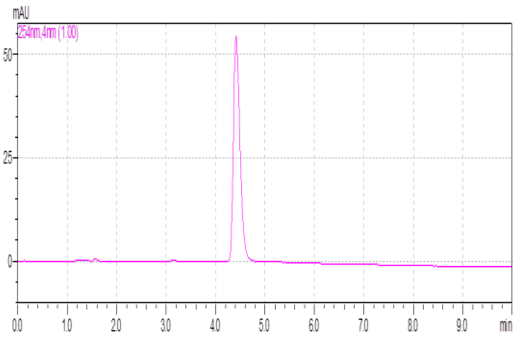

Fig. 7 Chromatogram of sample in pH buffer 2.2 at 60ºC

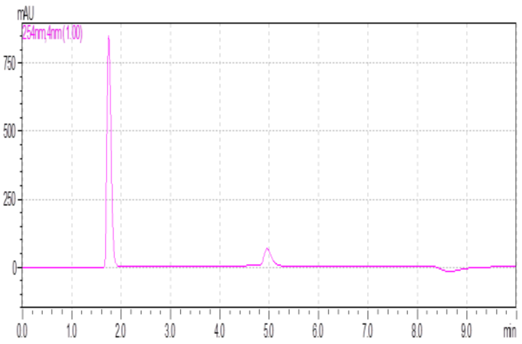

Fig. 8 Chromatogram of sample in pH buffer 4.6 at 60ºC

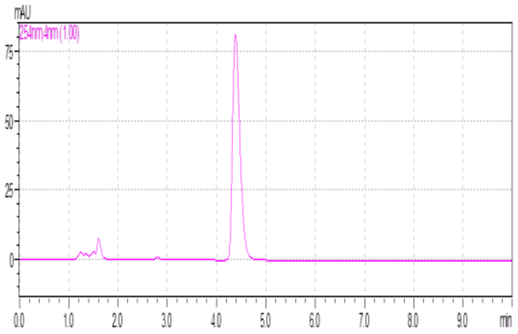

Fig. 9 Chromatogram of marketed formulation in pH buffer solution at 60ºC

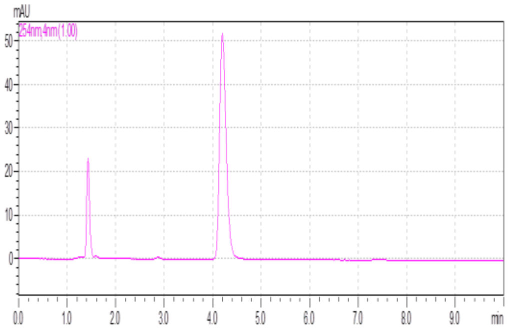

Fig. 10 Chromatograms of sample at room temperature for 6% H_2O_2

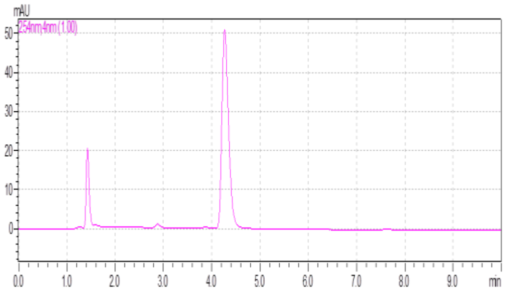

Fig. 11 Chromatograms of sample at 40ºC for 6% H_2O_2

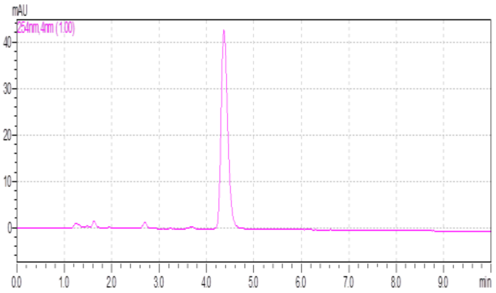

Fig. 12 Chromatograms of sample at 60ºC for neutral hydrolysis

3.3 Kinetics Of Solution State Degradation Studies

3.3.1 Determination of Degradation Constant

All the correlation coefficients (r) of the semi logarithmic plots of drug concentration versus time were >0.99, indicating the degradation of Bilastine followed pseudo first-order kinetics. Pseudo first order plots showing the degradation of Bilastine at pH 1.2, 2.2, 4.6, 6.6, 7.4, 8.0 and 9.5 and oxidative and neutral hydrolysis at various temperatures are shown in figures. **13a-13i**. Summary of rate constant for degradation of bilastine in aqueous solution at pH and temperature is given in **Table 1**.

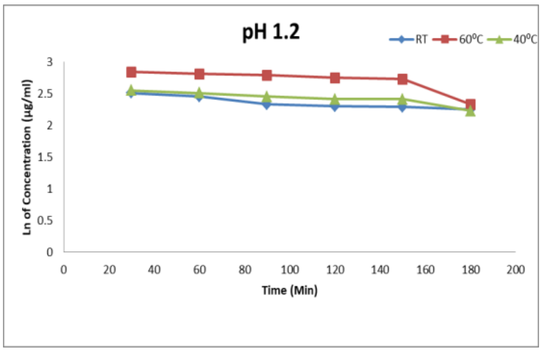

Fig. 13a Pseudo first order plots showing the degradation of drug at pH 1.2 and at various temperatures

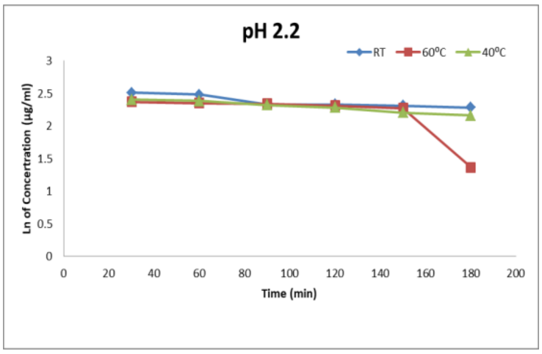

Fig. 13b Pseudo first order plots showing the degradation of drug at pH 2.2 and at various temperatures

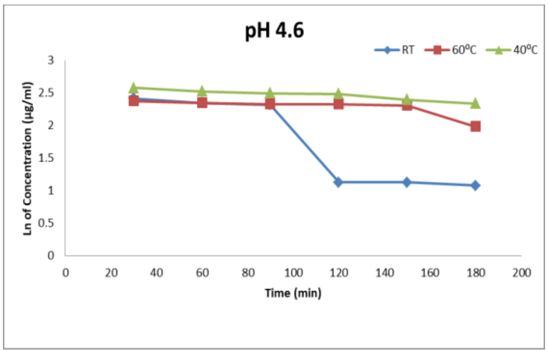

Fig. 13c Pseudo first order plots showing the degradation of drug at pH 4.6 and at various temperatures

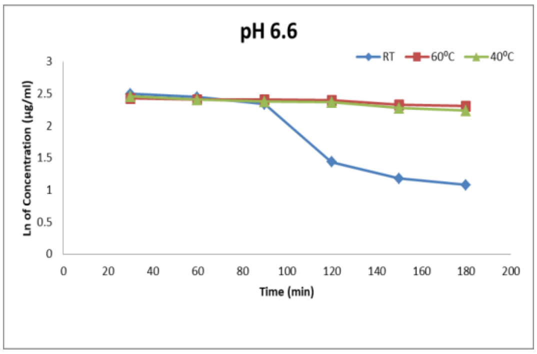

Fig. 13d Pseudo first order plots showing the degradation of drug at pH 6.6 and at various temperatures

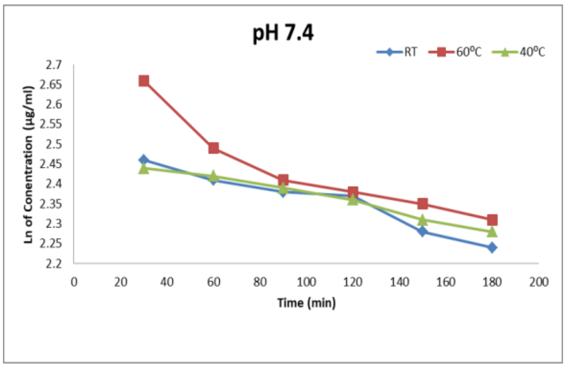

Fig. 13e Pseudo first order plots showing the degradation of drug at pH 7.4 and at various temperatures

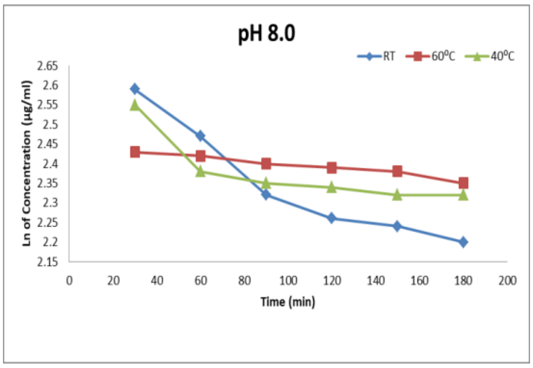

Fig. 13f Pseudo first order plots showing the degradation of drug at pH 8.0 and at various temperatures

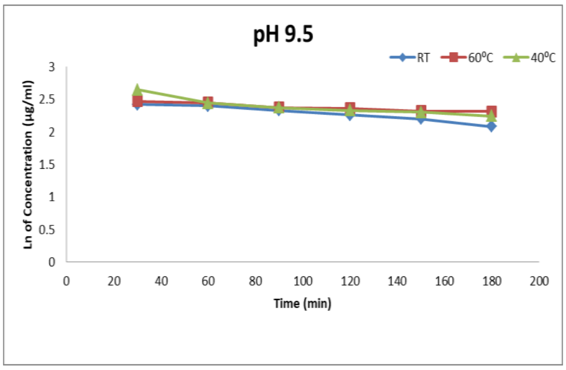

Fig. 13g Pseudo first order plots showing the degradation of drug at pH 9.5 and at various temperatures

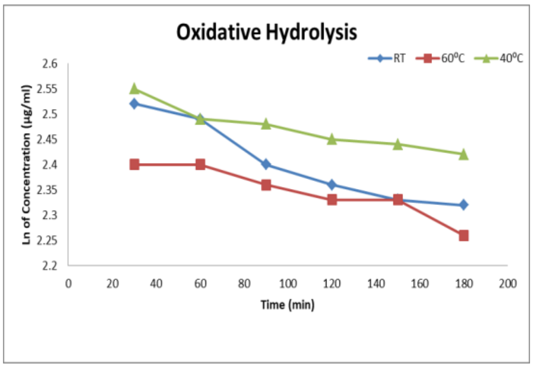

Fig. 13h Pseudo first order plots showing the degradation of drug in oxidative hydrolysis and at various temperatures

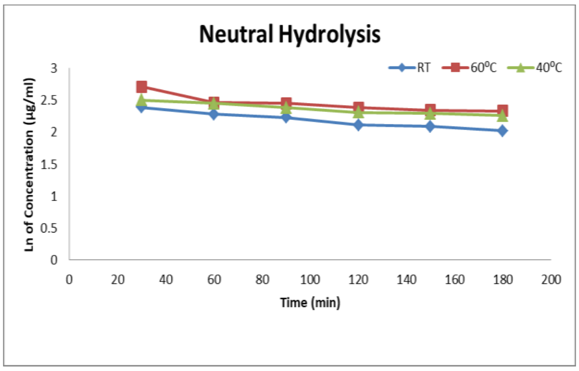

Fig. 13i Pseudo first order plots showing the degradation of drug in neutral hydrolysis and at various temperatures

3.3.2 *Kinetics parameter of Degradation*

From the observation and results of degradation of various hydrolysis at room temperature, 40^{0}C and 60^{0}C in oven, the graph was plotted and regression coefficient was observed and decided the order of kinetics. The observation and results of degradation kinetics rate constant, half-life and shelf life are given in **Table 1**.

Table 1 Observations and Results - Kinetic parameters of Degradation at Room temperature, 40°C and 60°C

Sr No.	Conditions	Order			Rate Constant (k)			Half Life ($t_{1/2}$)			Shelf Life ($t_{0.9}$)		
		Room	40⁰C	60⁰C	Room	40⁰C	60⁰C	Room	40⁰C	60⁰C	Room	40⁰C	60⁰C
1	pH 1.2	First	Pseudo First	Pseudo First	6.11×10^{-4}	1.7×10^{-3}	2.8×10^{-3}	1134.20	407.64	247.5	172.13	61.76	37.5
2	pH 2.2	First	Pseudo First	Pseudo First	5.3×10^{-4}	1.3×10^{-3}	5.6×10^{-3}	1307.54	533.07	123.76	198.11	80.76	18.75
3	pH 4.6	Pseudo First	Pseudo First	Pseudo First	7.4×10^{-3}	1.3×10^{-3}	2.1×10^{-3}	93.64	533.07	330	14.18	80.76	50
4	pH 6.6	First	Pseudo First	Pseudo First	4.6×10^{-3}	1.2×10^{-3}	6.5×10^{-4}	150.65	577.5	1066.15	22.82	87.5	161.53
5	pH 7.4	Pseudo First	Pseudo First	First	1.02×10^{-2}	9.05×10^{-4}	7.8×10^{-4}	69.3	770	888.46	10.5	116.66	134.61
6	pH 8.0	First	First	First	9.1×10^{-4}	5.2×10^{-4}	1.8×10^{-4}	761.53	1332.69	3850	115.38	201.92	583.33
7	pH 9.5	Pseudo First	First	Pseudo First	1.8×10^{-3}	9.2×10^{-4}	9.27×10^{-4}	385	753.26	753	58.33	114.13	114.130
8	Oxidative Hydrolysis	First	First	Pseudo First	4.5×10^{-4}	2.9×10^{-4}	8×10^{-4}	1540	2389.65	866.25	233.33	362.06	131.25
9	Neutral Hydrolysis	First	First	First	9.5×10^{-4}	5.4×10^{-4}	8.3×10^{-4}	729.47	1283.33	834.93	110.52	194.44	126.50

3.3.3 *pH-Rate Profile of Bilastine*

Based on the pH of the solution Bilastine can exists in three different forms, cation DH^+ , free base/neutral species DH and anion D^-. A pH-rate profile of Bilastine over the pH range 1.2 - 9.5 at 37°C was observed with maximum solution stability at pH around 4.6. Therefore, as seen in **Figure 14**, the slope is positive at higher pH for this specific base catalysis. Around pH 4.6, a minimum of natural logarithm of k_{obs} is observed suggesting an acidic catalytic effect. Degradation rate profiles are, however, often complex for molecules such as Bilastine that have multiple ionization sites within the molecule. This is because the observed degradation rate at any specified pH is composed of contributions from acid and base on all existing species of the drug in the solution **(Figure 14)**. The effect of pH on the degradation of Bilastine in aqueous solution and constant µ (0.3) at 37°C is shown in plots of $\log k_{obs}$ versus pH **(Figure 14)**.

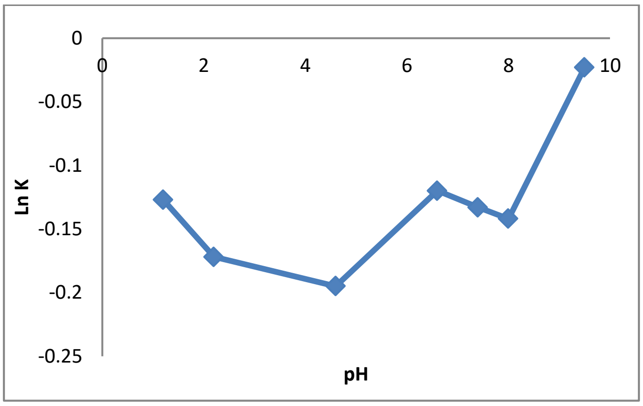

Figure 14: pH-rate profile of Bilastin

4. VALIDATION OF PROPOSED METHOD

4.1 Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. Results are shown in **Table 2.**

Table 2 Observation and Results of Recovery study

Sr. no	Weight of Tab taken equivalent (in mg)	Amt of Pure Drug added (mg)	Total Amount Recovered (mg)	% Recovery
1	239.1	5.0	5.03	100.6
2	239.2	10.0	9.96	99.63
3	239.2	15.0	14.82	98.86
Mean				**99.697**
±SD				**0.87**
%RSD				**0.87**

4.2 Precision

Precision of proposed method was expressed as SD and %RSD of series of measurements. Precision of estimation of Bilastine by proposed method was ascertained by replicate analysis of homogeneous samples of tablets. Results are shown in **Table 3**.

Table 3 Observations and Results of Precision study

Sr. no.	Wt. of tablet powder taken (mg)	% labeled claim
Precision 1	239.1	98.87
Precision 2		100.09
Precision 3		100.68
Precision 4		99.24
Precision 5		101.81
Mean		**100.13**
±S.D.		**1.17**
%RSD		**1.17**
Intraday precision		
0 h	239.2	99.31
3 h		100.19
6 h		101.61
Mean		**100.37**
±S.D.		**1.16**
%RSD		**1.15**
Inter day precision		
Day 1	239.1	99.54
Day 3		100.6
Day 7		100.03
Day 10		99.23
Mean		**99.85**
±S.D.		**0.59**
%RSD		**0.59**

4.3 Linearity and Range

The plot of concentration vs AUC so plotted was found to be linear and is shown in fig. 15 The observation and results are given in Table 4**.**

Table 4 Observation of Linearity and Range

Sr. No.	% of level	Area (µV)
1	80	4149274
2	90	4279465
3	100	4605331
4	110	4800257
5	120	4874694
Correlation Coefficient	**0.980**	

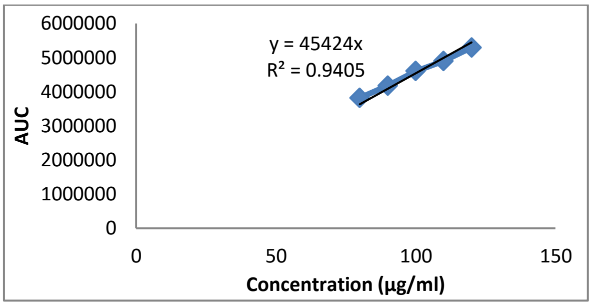

Fig. 15 Plot of Linearity and Range of Bilastine

4.4 Limit of Detection and Limit of Quantification

The standard deviation of Y-intercept and slope of calibration curves were used to calculate the LOD and LOQ for all the drugs. The results are given in Table 5**.**

Table 5 Result of LOD and LOQ

Sr. No.	**LOD(µg)**	**LOQ(µg)**
1	3.94	11.94

4.5 Robustness and Ruggedness

Deliberate change was made in the optimized chromatographic parameters and robustness and ruggedness of the method was studied by evaluating system suitability parameter data after varying the different chromatographic conditions. The observation and results of robustness and ruggedness study are given in the Table 6 and 7 respectively.

Table 6 Observations of robustness study

Sr. No.	Deliberate Condition	Retention Time (min)	Theoretical Plate	Tailing Factor
1	Standard condition	4.217	3603.41	1.409
2	Organic phase change (+10%)	2.867	3255.24	1.406
3	Organic phase change (-10%)	11.69	3845.97	1.427
4	Wavelength (249)	4.204	3794.19	1.413
5	Wavelength (259)	4.205	3740.99	1.404
6	Flow rate (1.2 mL/min)	3.463	3824.09	1.404
7	Flow rate (0.8 mL/min)	5.257	3825.05	1.400
			Mean	**1.40**
			±SD	**0.0086**
			%RSD	**0.61**

Table 7: Results of Ruggedness Study

Sr. No.	% Label Claim*	
	Analyst-I	**Analyst-II**
1	98.92	100.04
2	100.5	99.67
3	99.69	99.52
Mean	**99.70**	**99.74**
±SD	**0.79**	**0.26**
%RSD	**0.79**	**0.26**

*Each is a mean of two replicates

5. CONCLUSION

The method was found to be accurate, precise, rugged and robust. The results indicate that the pH, temperature, ionic strength and oxidation greatly influence the stability of bilastine and the degradation behavior of bilastine followed pseudo-first-order kinetics. The pH rate profile can be rationalized by the different catalytic effects of various components in bilastine solution. Bilastine was most stable in neutral, alkaline, lower temperature conditions and lower ionic strength. Investigating of suitable storage conditions for bilastine should consider the influence of pH, temperature, ionic strength and oxidation. This study was a typical example of the development of a stability-indicating assay established following the recommendations of ICH guidelines. The information obtained from the degradation kinetics will be helpful for understanding the stability of bilastine and providing a reference for further studies of bilastine clinical applications.

CONFLICT OF INTEREST- None

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