

Quality by Design Based Stability Indicating Quantitative Reverse Phase High Performance Liquid Chromatography Method Development and Validation for Bilastine in Tablet Dosage Form

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Pandya *et al.*: Development and Validation of Bilastine in Tablet Dosage Form by Quality by Design Approach

The objective of this study is to develop simple, rapid and sensitive reversed-phase high performance liquid chromatography method for bilastine in tablet dosage form by quality by design approach. In reversed-phase high performance liquid chromatography method, chromatographic conditions such as mobile phase composition and flow rate were optimized with the help of Design-Expert software using central composite design. In this method, bilastine was estimated using octadecylsilane column (250×4.6 mm, 5 µm) and methanol:acetonitrile (90:10 % v/v) as mobile phase and flow rate 1 ml/min which are optimized using Design-Expert software. Method was developed at 280 nm detection wavelength. The retention time was found to be 3.484 min. The proposed method was successfully applied to the determination of bilastine in tablet dosage form. High linearity of developed method was confirmed over concentration range of (20-120) µg/ml and correlation co-efficient is 0.9997. The percentage relative standard deviation for precision and accuracy of the method was found to be <2 %. The recovery was in the range of 98.8 %-99.7 %; limit of detection was found to be 0.1352 µg/ml and limit of quantification was found to be 0.4098 µg/ml. Bilastine was found to degrade under oxidation condition. There was no interference of excipient and degradation product in retention time, so the method was specific. Analytical parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization guidelines.

Key words: Bilastine, quality by design, reverse phase high performance liquid chromatography, validation, forced degradation study

The expression Quality by Design (QbD) refers to the achievement of a defined and consistent level of quality. Understanding factors and their interactions across a desired series of experiments which is called as an experimental design, is a very useful component of the QbD. QbD principles have been used to advance the product and process the quality in every industry, they have most recently been adopted by the United States Food and Drug Administration (USFDA) as a vehicle for the transformation of how drugs are discovered, developed and economically manufactured. Since, primarily initiated by the USFDA in its “pharmaceutical current Good Manufacturing Practices (cGMP) for the 21st

century”, it has become an important concept for the pharmaceutical industry that is further defined in the International Council for Harmonization (ICH) guidance^[1-5].

High Performance Liquid Chromatography (HPLC) is a separation method that uses a solid stationary phase and a liquid mobile phase^[6]. Because of its

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advantages such as rapidity, specificity, accuracy, precision and ease of automation, HPLC can be used to test most drugs in multi-component dosage types. Extraction and isolation procedures are no longer necessary with the HPLC process^[7].

Bilastine is a highly selected 2nd generation new oral H1 receptor antagonist for the treatment of sympathomimetic of allergic rhino-conjunctivitis and chronic urticaria in adult patients. Rhino-conjunctivitis and urticarial response to antihistamine treatment and bilastine is an antihistamine agents which acts by the inhibition of immune system reaction mediated histamine receptor^[8-10].

Bilastine or 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazole-2-yl) piperidine-1-yl) ethyl) phenyl]-2-methyl propionic acid, is a new next-generation antihistamine (fig. 1). It is a new piperidine molecule and belongs to the same chemical group as many new antihistamines^[11]. Within this group, it is chemically close to the piperidine-benzimidazole subgroup, which also includes molecules such as norastemizole and mizolastine.

The drug bilastine is Central Drugs Standard Control Organization (CDSCO) approved for symptomatic treatment of allergic rhino-conjunctivitis. On literature survey, it was found that few Ultraviolet-Visible (UV-VIS) spectrophotometry, High Performance Thin Layer Chromatography (HPTLC), Reverse Phase (RP)-HPLC methods and degradation studies are available for the determination of bilastine in tablet dosage form^[12-20]. According to literature survey, method development approaches specifically focused on pharmaceutical developments which have not been discussed. It was planned to develop

simple, rapid and sensitive RP-HPLC method for the estimation of bilastine in tablet dosage form. Applying more robust methods which produce consistent, reliable and quality data throughout the life cycle and in turn leads to less method incidents when used in routine environment^[21-23]. Critical attributes and method attribute parameters have been determined which have developed more robust methods^[24-26]. Stress study was carried out under the condition of acid and base hydrolysis, oxidation degradation, thermal degradation and photolytic degradation, as mentioned in ICH Q1A(R2) guidelines^[27], and the method was validated as per ICH guideline Q2 (R1)^[28]. Forced degradation study is a powerful tool for the development of stability indicating method^[29-32]. These studies help to gain a better understanding of active pharmaceutical ingredients and drug product^[33], thereby help to specify the specificity of the stability indication methods and provide insight into degradation pathways and degradation products of the drug substance^[34].

MATERIALS AND METHODS

Reagents and chemicals:

Bilastine sample was obtained from Cubic Pharmaceuticals., Ltd., while HPLC-grade methanol and acetonitrile were purchased from Merck Life Science Pvt. Ltd., Mumbai, India. Bilastine tablet (Bilagra, 20 mg) was purchased for the analytical purpose. All the other reagents and chemicals used were of Analytical Research (AR) grade which were purchased from Avantor Performance Material India, Ltd., Thane, India.

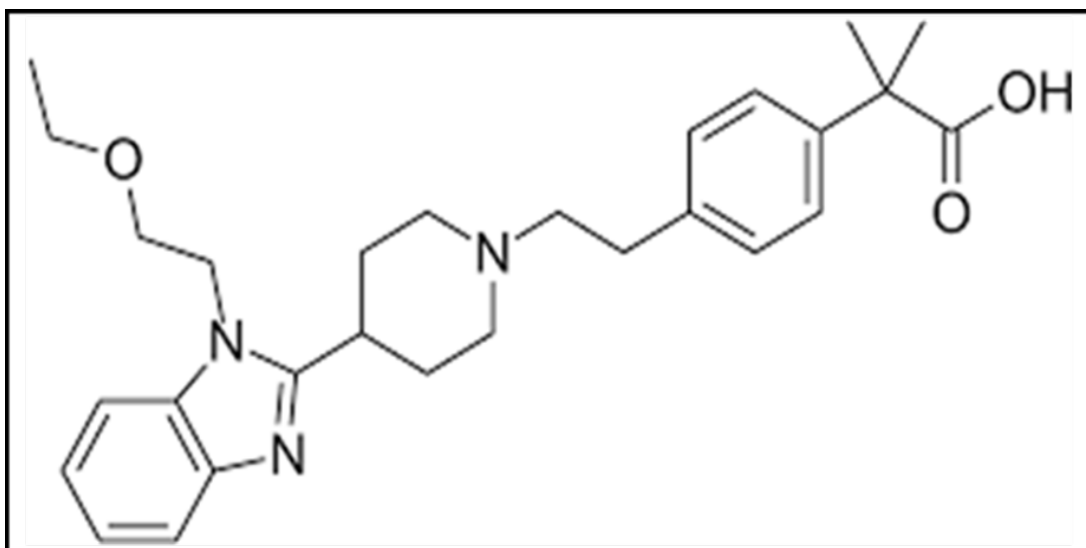


Fig. 1: Structure of bilastine

HPLC method development by QbD approach:

Chromatographic conditions and equipment:

Analysis was carried out on a Shimadzu LC_2010CHT HPLC with UV detector. The output signal was monitored and processed using LC solution software. The chromatographic column used was octadecylsilane (C18) column (250 mm×4.6 mm and 5 µm). Gradient elution process was adopted throughout the analysis. We used methanol:acetonitrile as mobile phase in the ratio 90:10 % v/v. The gradient flow of mobile phase was maintained at 1.0 ml/min; the injection volume was 20 µl and the column was maintained at room temperature. Eluted sample was monitored at 280 nm whose run time was 10 min.

Factorial design: After defining the Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs), the central composite experimental design was applied to optimization and selection of two key components, mobile phase and flow rate of HPLC method. The various interaction effects, quadratic effects of the mobile phase composition, flow rate on the Retention time (Rt) and Tailing factor (Tf) were studied using central composite statistical screening design.

A 2-factor, mobile phase composition and flow rate at 2 different levels, design was used with Design Expert® software (Version 11.0, Stat-Ease Inc.,) which was the best suited response for 2nd order polynomial exploring quadratic response.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{21} B^2 A + \beta_{12} A^2 B$$

Where A and B are independent variables coded for levels, Y is the measured in response associated with each combination of factor level. β_0 is an intercept and β_1 to β_{22} are regression coefficients derived from experimental runs of the observed experimental values of Y. Inter-action and quadratic terms respectively represent the terms AB, A^2 and B^2 .

Since multivariable interaction of variables and process parameters have been studied, the factors were selected based on preliminary analysis. As independent variables, mobile phase and flow rate

compositions were chosen (Table 1). The dependent variables were Rt and Tf as dependent variables for proposed independent variables.

Using the Central Composite Design (CCD) approach, these method conditions were assessed. At the 1st step, the conditions for Rt and Tf were evaluated; for bilastine, this resulted in distinct chromatographic conditions and the proven acceptable range from robust regions was depicted as the deliberate variations in the method parameters which do not affect the quality. This ensures that the method does not fail downstream during validation testing. If the modeling experiments do not have the desired response, the variable needs to be optimized at different levels until the responses are within the acceptable ranges. The best suited chromatographic conditions shall be optimized using the Design-Expert tools.

Risk assessment: The optimized final method is selected against the attributes of the method like that of the developed method which is efficient and will remain operational throughout the product's lifetime. Risk based approach based on the QbD principles set out in ICH Q8 and ICH Q9 guidelines was applied for the evaluation of method to study the robustness and ruggedness. The parameters of the method or its performance under several circumstances such as various laboratories, chemicals, analysts, instruments, reagents and days were evaluated for robustness and ruggedness studies.

Selection of optimization design: The most important factors were further examined by use of response surface design methodology. CCD could predict the optimum condition with lowest runtime, therefore saving time and cost; thus this technique was considered for study. Number of experiments to be executed for 2-factors and two levels for different experimental design are shown using the below given formula.

$$2^{(K)} + 2K + C = 2^2 + 2(2) + 5 = 13$$

Where, K=number of factors and C=number of centre points

TABLE 1: CODED VALUES FOR INDEPENDENT VARIABLES

Factor	Coded values given factor	Levels		
		-1	0	1
Mobile phase	A	85:15	90:10	95:05
Flow rate	B	0.8	1	1.2

Preparation of standard and test solutions:

Preparation of stock solution: Stock solution for optimization experiments and method validation were prepared by accurately weighing 10 mg of bilastine and dissolving it in 100 ml of methanol by gentle stirring to yield final concentration of 100 µg/ml.

Preparation of working standard solutions: Working standard solution was prepared by accurately transferring the (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) aliquots of the standard stock solution in a series of 10 ml volumetric flask. The volume was made up to mark mobile phase to obtain concentration of 20-120 µg/ml.

Preparation of sample solution: 10 Bilagra (20 mg bilastine) tablets of marketed formulation were taken, whose average determined weight of the content is 113.4 mg. 56.7 mg weight equivalent to 10 mg bilastine was transferred to 10 ml volumetric flask and dissolved in methanol. The solution was sonicated and filtered through Whatman filter paper.

Forced degradation study: Forced degradation studies of the drug were carried out under conditions of acid hydrolysis (0.1 N Hydrochloric acid (HCl) at 60°), alkali hydrolysis (0.1 N Sodium hydroxide (NaOH) at 60°), oxidative degradation, thermal degradation and photolytic degradation.

Method validation:

Specificity: It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be a present. Specificity of the method was evaluated by comparison between chromatogram of standard and test solutions. There should be absence of any interfering peak with peak of analyte.

System suitability test parameters: System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were the chromatographic retention time, peak area, theoretical plate number and Tf. The repeatability of these parameters was checked by injecting the solution of bilastine six times.

Linearity and range: Aliquots of working standard solutions (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) were transferred into series of 10 ml volumetric flask and diluted up to mark with methanol. These yielded solution of 20, 40, 60, 80, 100 and 120 µg/ml of it. An aliquot of 20 µl of each solution was injected under

operating chromatographic condition. Calibration curve of area vs. respective concentrations was plotted and found out correlation co-efficient and regression line equation for bilastine. Each response was average of 3 determinations.

Precision: It was categorized into two types, intra- and inter-day precision. Intra-day precision was determined by analyzing of bilastine standard solutions in the range 40, 60 and 80 µg/ml of it triplicate in a day; % Relative Standard Deviation (RSD) for bilastine was calculated. Similarly, inter-day precision was determined analyzing bilastine standard solutions in the range 40, 60 and 80 µg/ml on 3 different d; % RSD for bilastine was calculated.

Accuracy: Accuracy was determined by calculating recovery of bilastine by the standard addition method. The known amounts 0.32, 0.4 and 0.48 ml of working standard solutions of bilastine were added to 0.4 ml test solution of bilastine in vial. Each solution was injected in triplicate and the recovery was calculated from regression equation of calibration curve by measuring the peak areas.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ of the drug were calculated using following equations according to ICH guidelines. LOD was $3.3 \sigma/s$ and LOQ was $10 \sigma/s$; where σ is the standard deviation of regression line and S is the slope of the calibration curve.

Robustness: The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing 4 small changes in flow rate (1 ± 0.2 ml/min), mobile phase (90 ± 5 ml) and injection volume (20 ± 5 µl). Then each sample solution was injected and percentage assay with system suitability parameters were checked.

RESULTS AND DISCUSSION

Wavelength measurement was determined. Standard spectra of bilastine were scanned between 200-400 nm. It is evident that bilastine show an absorbance at 280 nm. Chromatogram for standard and test bilastine has been depicted in fig. 2 and fig. 3.

Method optimization by QbD approach execution of experimental design and responses were evaluated (Table 2). Analysis of model was carried out using Analysis of Variance (ANOVA) for Quadratic model for response of Rt of bilastine was studied (Table 3). Lack of Fit (F-value) of the model denoted 520.68, implying that the model is significant. There is only a 0.01 % chance that F-value could vary due

to noise. Similarly, $p < 0.050$ indicate are found to be significant. In this case A, B, A^2 , B^2 are significant model terms. F-value of 0.76 implies that it is not significant relative to the pure error. There is a 57.20 % chance that F-value this large could occur due to noise. Non-significant lack of fit is good.

Further, ANOVA for quadratic model for response of Tf of bilastine was studied (Table 4). F-value of the model denoted 355.64, implying that the model is significant. There is only a 0.01 % chance that large F-value could occur due to noise. $p < 0.0500$ was found to be significant. In this case B, AB, A^2 and B^2 are significant model terms.

F-value of 0.28 implies the value is not significant relative to the pure error. There might be 84.06 % chance of occurrence of error due to noise. Non-significant lack of fit is considered to be good. Then 3D plot of the effect of interaction terms A and B on response variable of Rt and Tt were determined (fig.

4 and fig. 5).

Optimization of model was carried out according to the solution suggested by design expert software, where the mobile phase composition included 10:90 and the flow rate was 1 ml/min. 20 μ l injection volume was selected as optimized chromatographic condition because shorter migration distance minimizes the use of stationary phase results into economy of analysis.

Forced degradation study found that bilastine was marginally degraded in acid and thermal conditions and stable in alkali oxidative and photolytic conditions. Results of forced degradation study have been presented in Table 5.

Specificity of the method was evaluated by comparison between chromatograph of standard and test solutions. There was no any interfering peak in the test sample of chromatogram. It reveals that developed method is specific for bilastine. Linearity and range bilastine was determined and was found to be in the range of 20-120 μ g/ml (fig. 6).

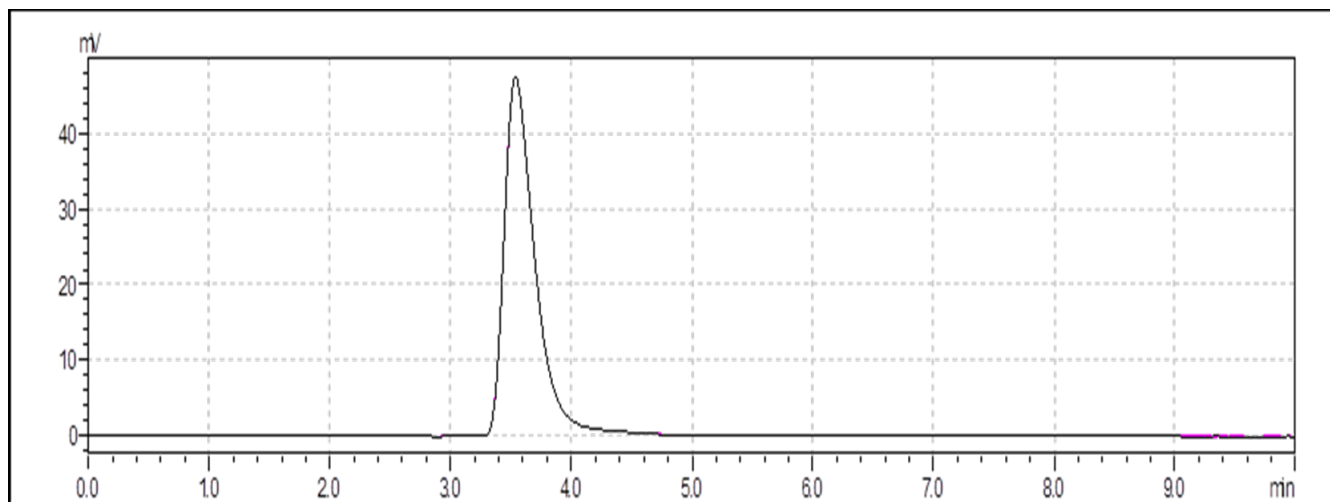


Fig. 2: Chromatogram of bilastine using 40 μ g/ml of standard solution

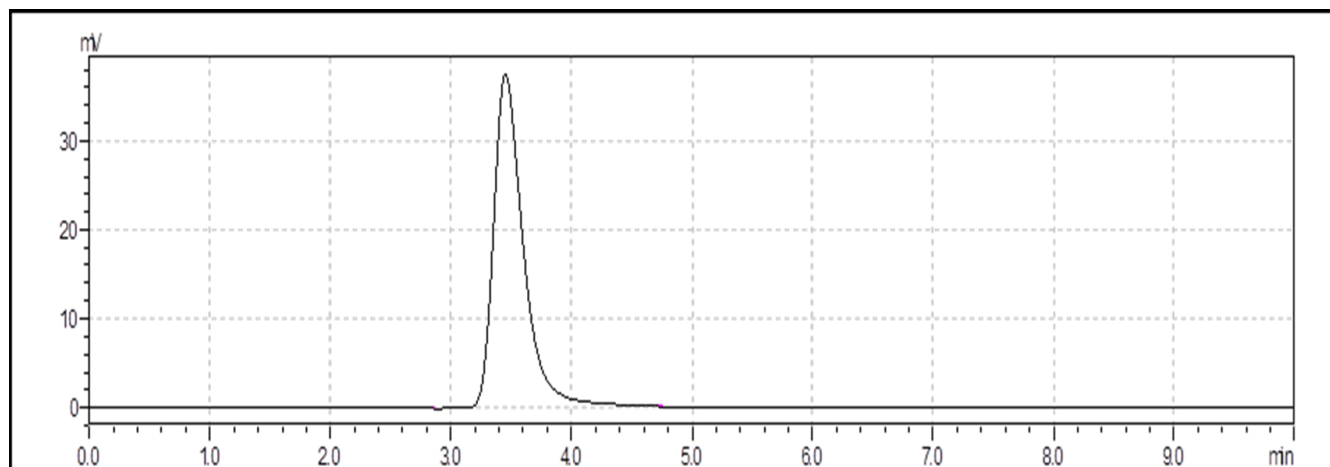


Fig. 3: Chromatogram of bilastine using 40 μ g/ml of test solution

TABLE 2: EXECUTION OF EXPERIMENTAL DESIGN AND RESPONSES

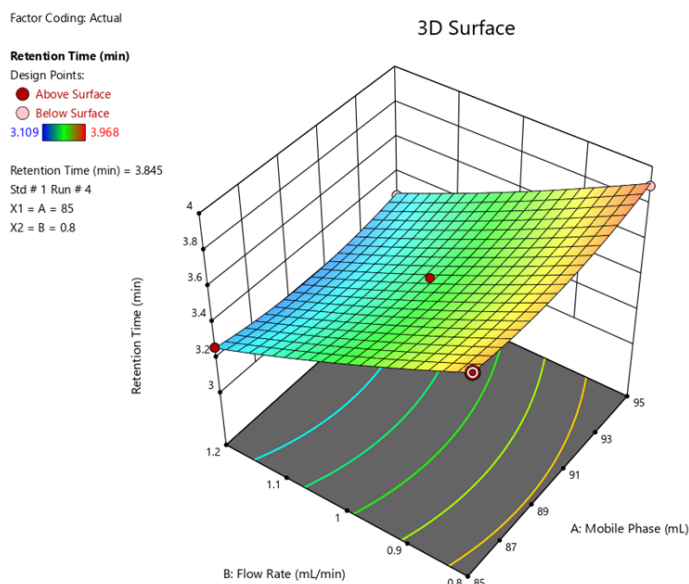
Standard experiment no	Run rate	Factor 1 A: Mobile phase composition (ml)	Factor 2 B: Flow rate (ml/min)	Rt (min)	Tf
5	1	82.92893	1	3.583	1.601
6	2	97.07107	1	3.666	1.607
2	3	95	0.8	3.899	1.836
1	4	85	0.8	3.845	1.849
9	5	90	1	3.484	1.507
8	6	90	1.282843	3.109	1.225
7	7	90	0.717157	3.968	1.932
10	8	90	1	3.485	1.501
4	9	95	1.2	3.269	1.382
3	10	85	1.2	3.268	1.309
12	11	90	1	3.488	1.503
11	12	90	1	3.491	1.502
13	13	90	1	3.527	1.549

TABLE 3: ANOVA TABLE FOR RESPONSE Rt

Source	Sum of squares	Rt	Mean square	F	p
Model	0.7651	5	0.153	520.68	<0.0001
A-mobile phase	0.0037	1	0.0037	12.64	0.0093
B-flow rate	0.7331	1	0.7331	2494.72	<0.0001

TABLE 4: ANOVA TABLE FOR RESPONSE Tf

Source	Sum of squares	Tf	Mean square	F	p-significant
Model	0.5198	5	0.104	355.64	<0.0001
A-mobile phase	0.0006	1	0.0006	2.01	0.1997
B-flow rate	0.4969	1	0.4969	1699.88	<0.0001

**Fig. 4: Effect of interaction term A and B on response variable Rt (3D plot)**

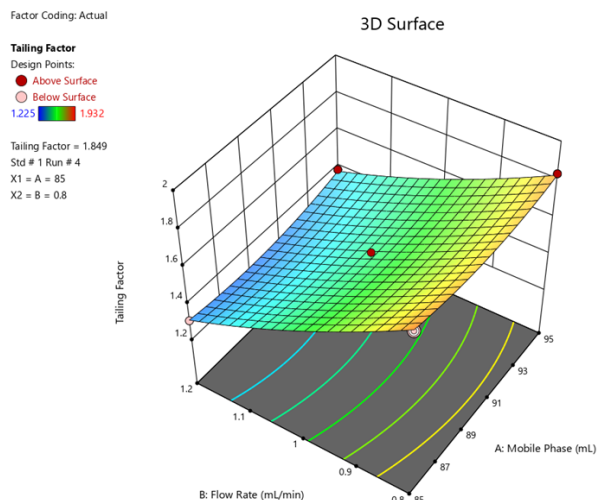


Fig. 5: Effect of interaction term A and B on response variable Tt (3D plot)

TABLE 5: RESULT OF FORCED DEGRADATION STUDY OF BILASTINE

S. no	Stress type	Condition	No. of peaks	% degradation
1	Acid hydrolysis	0.1 N HCl at 80° for 30 min	1	7.25
2	Alkali hydrolysis	0.1 N NaOH at 80° for 2 h	-	-
3	Oxidative degradation	3 % (v/v) H ₂ O ₂ at 80° for 30 min	1	5.24
4	Thermal degradation	At 70° for 8 h	-	-
5	Photolytic degradation	UV 280 nm for 24 h	-	-

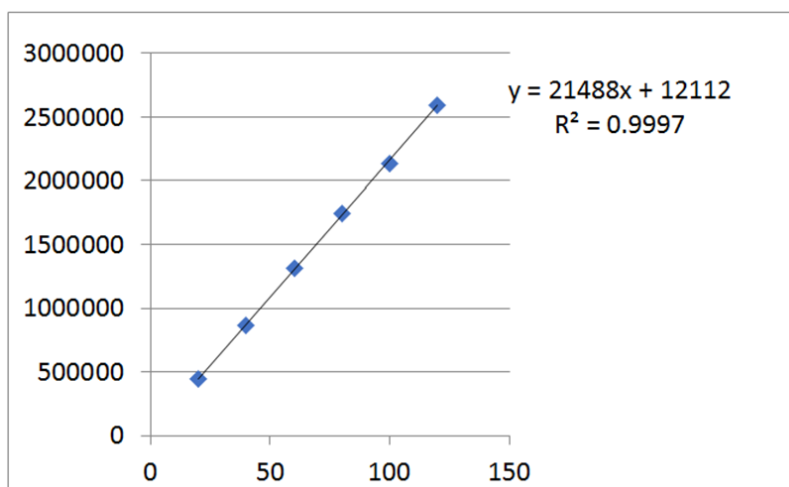


Fig. 6: Calibration curve of bilastine

Note: (◆): Area and (—): Linear (area)

Precision was calculated using % RSD; intra-day and inter-day % RSD of bilastine was found to be 0.116631 %-0.295779 % and 0.107392 %-0.491767 %, respectively. Similarly, accuracy of the method was confirmed by recovery study from marketed formulation of bilastine at three levels (80 %-120 %) of standard addition. Percentage recovery for bilastine was found to be 98.8 %-99.7 % (Table 6). LOD and LOQ was found to be 0.1352 µg/ml and 0.4098 µg/ml respectively.

Robustness was determined using typical variations studied under these parameters such as flow rate, mobile phase composition and injection volume. According to data comparison the developed method was estimated to be robust. Analysis of marketed formulation was studied and tested. Applicability of the proposed method was tested by analyzing the available tablet formulation Bilagra (20 mg). All validation parameters of bilastine results have been presented in Table 7.

TABLE 6: RECOVERY DATA FOR BILASTINE

Formulation level	Amount of test solution (µg/ml)	Amount of standard added (µg/ml)	Amount recovered mean±Standard Deviation (SD)*	%	% RSD
80 %	40	32	71.74±0.108	98.8	0.15
100 %	40	40	79.76±0.122	99.7	0.153
120 %	40	48	87.35±0.145	99.3	0.166

Note: *Average of three determinations

TABLE 7: SUMMARY OF VALIDATION PARAMETERS FOR RP-HPLC METHOD

S. no	Parameters	Bilastine
1	Specificity	Specific
2	Linearity range	20-120 µg/ml
3	Regression line equation	Y=21488x+12112
4	Correlation co-efficient	0.9997
5	Precision (% RSD)	
	Repeatability	0.116631-0.295779
	Inter-day precision	0.107392-0.491767
6	% assay	99.72 %
7	LOD	0.1352 µg/ml
8	LOQ	0.4098 µg/ml

In conclusion, QbD approach has been successfully applied for development for RP-HPLC method for estimation of bilastine. Primarily, goals of the method have been clarified based on process understanding. The experimental design describes the investigation of the key components including mobile phase composition and flow rate. The interrelationships are studied and optimized conditions are obtained from each combination of conditions. Here, a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the method to meet their intended purpose is done. It gives symmetric peak shape and reasonable retention for it. The method was validated accordance to ICH guidelines. The developed and validated RP-HPLC method for estimation of bilastine in bulk and tablet dosage form has found to be specific, accurate and precise. Hence method can be used successfully for the routine analysis of bilastine in bulk and tablet dosage form.

Acknowledgement:

The authors are thankful to the Shri Sarvajani Pharmacy College, Mehsana, Gujarat, India, for providing necessary infrastructure facilities. The authors are also highly grateful to the Dr. C. N. Patel sir for the generous gift sample of pure bilastine.

Conflict of interests:

The authors declare no competing interests.

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