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Original Article

Stability Indicating Assay for the Determination of Bilastine in Bulk Drug and Method Development Validation by RP-HPLC Using Analytical Quality by Design Approaches

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ABSTRACT

Background: Regulatory organizations have acknowledged the need for systematic rules for understanding development as a result of the large increase in concerns and criticism regarding the quality and pharmaceutical products. Bilastine is a second generation antihistamine medication. Generally, it is used for treatment of allergic rhino conjunctivitis and urticaria (hives). **Objectives:** The current study outlines the methodical design and validation of a reversed-phase high-performance liquid chromatographic method for the estimation of Bilastine in bulk drugs using AQbD approach. **Materials and Methods:** Using Box Behnken design, the critical method parameters were methodically optimized. Risk estimation matrix was performed and Critical Analytical Attributes, Critical Method Attributes were correlated to identify risk factors of method development. A reverse phase column in isocratic elution mode with mobile phase NaH_2PO_4 buffer and methanol of different ratio and flow rate 1 mL/min was set for RP-HPLC method development. **Results:** Chromatographic separation was accomplished on INTERSIL C8 column. The optimized and predicted data from JMP PRO 14 software consist of mobile phase 0.1N NaH_2PO_4 (60%): Methanol (40%), pumped at a flow rate of 1 mL/min gave the higher desirability function of 77%. LOD and LOQ are, respectively, 0.005 mcg/mL and 0.016 mcg/mL. The Rt of Bilastine was discovered to be 1.894 min. The created method was approved and validated in accordance with ICH Q2 (R1) recommendations. **Conclusion:** The chosen models were determined to be significant with $p<0.05$. The validation parameter findings were within the permitted range. Forcefully testing the drug's stability under various stress situations revealed considerable degradation in the presence of heat.

Keywords: AQbD, Bilastine, Analytical target profile, Critical method attributes, Design of experiments, RP-HPLC, Validation.

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INTRODUCTION

The term "Quality by Design" (QbD) refers to methodology that incorporates a more thorough scientific and technical understanding of the important procedure and product qualities, develops controls and examinations according to the scientific limitations of our knowledge throughout the development and uses the knowledge gained throughout the product's life-cycle to create an environment of continuous improvement.[1] AQbD, is a new technique for optimising HPLC instrument parameters like flow rate, solvent concentration, column, length, buffer, temperature, injection volume, peak area, plate count and Rt. Ensuring the quality and dependability of analytical data requires the creation and optimisation of analytical procedures.[2,3]

Seasonal allergic rhinitis and chronic spontaneous urticaria are treated with the peripheral histamine H_1-antagonist Bilastine Figure 1. Bilastine interacts with H_1 receptor, which is activated by the release of histamine from mast cells and supresses this activation with a Ki of 64 nM, delaying the onset of allergic symptoms in terms of its chemical makeup, Bilastine has the chemical formula $C_{28}H_{37}N_3O_3$.[4] The review of literature reported several methods to analyse Bilastine either single or in combination by Reverse phase HPLC, UPLC and HPTLC, but no AQbD approach-based method development has been published. Design of experiment aids in identifying crucial method parameters, choosing the best experimental design and maximising method performance. The total quality of analytical and pharmaceutical procedures is improved with the aid of AQbD.[5,6]

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MATERIALS AND METHODS

Chemicals and Reagents

From Symed Lab Pvt. Ltd., Hyderabad, India a Bilastine of gift sample was procured. HPLC grade solvents acetonitrile, methanol and orthophosphoric acid used are acquired from Merck Specialties Ltd., an HPLC grading company, via Sd Fine-Chem Limited in India.

Methods

High-pressure liquid chromatography (HPLC), waters corporation, Massachusetts U.S.A method along with UV detector utilized for the method development of Bilastine. INTERSIL C8 column, flow rate of 1 mL/min at 275 nm was used for chromatographic separation of Bilastine mobile phase comprises of NaH_2PO_4: Methanol (60:40 v/v) at room temperature. The Box Behnken design of experiment tool is used to optimize the RP-HPLC method with utilizing JMP software.[7]

Methodology

Ishikawa diagram will help to identify the origin of the problem to find the possible cause and sub-cause affecting the CAAs (Critical Analytical Attributes) of the product. REM studies were carried out in order to determine the Critical Method Performance (CMPs) that are high-risk factors with significant effects on the CAAs. The factors with the great risk were selected by building REM with the grade of a low, medium and high.[8]

Risk assessment

Analytical Quality Risk Management (QRM) is a methodical technique to identify, monitor, communicate and analyse risks to a integrity of data over the course of the product life-cycle. Process mapping tools like Ishikawa diagrams can be used to make sure that a thorough attempt is made to reach and identify all relevant variables that may impact on data quality. Similar to sampling, sample preparation, standards, reagents and equipment operating conditions, etc. Once the variable has been discovered (Figure 2).

To evaluate parameter risks in relation to the pertinent qualities, risk matrices are used for example resolution, accuracy, precision, tailing etc. In the primary stage of development, simple matrices were built from scientific knowledge and methodological experience was utilized (Table 1).[8]

RP-HPLC Method Development

Preparation of mobile phase

The combined buffer 400 mL (40%) methanol and 600 mL (60%) of NaH_2PO_4, degassed in an ultrasonic water bath for a total of 10 min and subsequently vaccsssum filtered through a 0.45 µm.

Standard stock solution preparation

Comprised precisely weighing 20 mg of Bilastine working standard, placing into a 25 mL clean and dry volumetric flask, administering 5 mL of diluent, sonicating it for 30 min and then making up the difference with diluent. Above solution was diluted to obtain various concentrations.[9]

Detection of absorption maxima (λ_{max})

The prepared sample solution has been scanned in the UV region of 200-400 nm and the absorption maxima were found at 275 nm in methanol.

Trials and optimization of method

Here method is detected at 275 nm, injection volume 10 µL, run time of 20 min, sample and column temperature 25°c with different column, mobile phase ratio and flow rate given in the Table 2.[10]

Design of experiment

Design of Experiments (DoE) approach aims to monitor and detect changes in the output response by deliberately altering the input variables (factors) of a process through one or more tests. DoE, in its simplest form, is a method for methodically identifying cause and effect links. In the framework of creating reliable techniques for the QC setting.[10]

Box Behnken design

In this study Box Behnken design methodology, experimental studies on resilience are carried out in the vicinity of the selected ideal point. The ratio of Acetonitrile in a mobile phase, pH and concentration of ammonium acetate in the aqueous phase, were the three CPPs that were monitored during the optimization phase in addition to column temperature, mobile phase and flow rate. Later, two criteria influence the behavior of chemicals in chromatography in a known and predictable way; however, it is crucial to determine whether making a small adjustment will have an impact on the optimization's quality. The variable ranges of the factor were selected to be symmetrical with respect to the nominal value. Limits of factors and responses have taken from the method development trials. In Box Behnken design for the 3 factors and 3 responses, a total of 15 runs were obtained as shown in Table 3.

Method Validation and Solution Preparation

Preparation of Bilastine standard stock solution

100 mg of the working grade Bilastine, weighed, into 100 mL dry volumetric container. Add 30 mL of diluent and sonicating for 30 min, diluent was added to make up volume. Multiple concentrations of Bilastine were obtained by diluting the stock solution via diluents.[11]

Table 1: **Risk Estimation Matrix (REM).**

Critical Performance Attributes (CPAs)	Critical Analytical Attributes (CAAs)			Critical Method Attributes (CMAs)			
	Retention time	Peak area	Theoretical plate	Column oven temperature	Flow rate	Column type	Injection volume
Accuracy	Low	High	Low	High	Medium	Medium	High
Linearity and range	Medium	Medium	Low	High	Medium	Medium	High
Precision	High	High	Medium	Low	Low	Medium	Low
Robustness	High	High	Medium	High	High	Medium	High
Limit of the Detection (LOD)	Medium	Low	Medium	Low	Medium	Medium	Low
Limit of the Quantification (LOQ)	High	High	Low	Low	Medium	Medium	Low
Specificity	Medium	High	Medium	Low	Medium	Low	Medium

Table 2: **Experimental method development components and requirements.**

Test	Mobile phase components	Column	Flow rate (mL/min)	Injection volume (µL)	Inference
1	KH_2PO_4: Methanol (50:50v/v)	SUPELCO, C_{18}, (250×4.6 mm), 5 µm.	1	10	Improper peak shape and number of theoretical plates are beyond the range.
2	KH_2PO_4: Methanol (60:40v/v)	ACE, C_{18}, 250×4.6 mm, 5 µm.	1	10	The poor peak shape and plate counts outside the limits.
3	KH_2PO_4: Methanol (80:20v/v)	INTERSIL C_{18}, 150×4.6 mm, 5 µm.	1	10	A gaussian Peak but higher retention time.
4	KH_2PO_4: Methanol (80:20v/v)	INTERSIL C_{18}, 150×4.6 mm, 5 µm.	1	10	High Retention time.
5	NaH_2PO_4: Methanol (65:35v/v)	INTERSIL C_8, 150×4.6 mm, 5 µm.	1	5	Theoretical plates are beyond the Range.
6	NaH_2PO_4: Methanol (60:40v/v)	INERTSIL C_8, (150×4.6 mm), 5µm.	1	10	All parameters are within range and good peak shape.

Table 3: **Method runs by Box Behnken design.**

Sl. No.	Factor 1	Factor 2	Factor3	Response1	Response 2	Response 3
	Flow rate(A)	Solvent Composition(B)	Injection Volume(C)	Retention Time	Theoretical Plate	Tailing Factor
1	1	40	5	1.893	3550	1.52
2	1.25	50	5	1.78	2950	1.72
3	1.25	50	5	1.89	3390	1.78
4	1.25	40	7.5	1.86	3520	1.52
5	1.25	30	10	1.98	3810	1.45
6	1	40	10	1.894	3540	1.61

Blank: The selected Mobile phase should be injected to check whether any peak appearing in the chromatogram.[12]

System suitability: This test aims to examine whether the overall testing system (RP-HPLC system) is effective for the specified use.

Specificity: For identification, purpose specificity is ability to discriminate between closely related compounds or in other means comparison to known reference sample or impurity. The blank should be injected to check whether any peak appearing in the chromatogram.[13,14]

Accuracy: Percent recovery is done by assay of known added amounts of analytic solution is utilized to describe accuracy. The 50%, 100%, 150% solutions were prepared with concentrations of 50 µg/mL, 100 µg/mL and 150 µg/mL respectively were prepared and inject into HPLC.[15]

Precision: 100 µg/mL concentration is prepared, prepare 6 vials and inject each one time.[16]

Linearity and concentration range: Different concentrations ranging from (50-150 µg/mL), were prepared with concentration of 50% to 150% and all were filled in a vial, each vial one injection.[17]

Detection and Quantification Limit: From the stock solution, 100 µg/mL concentration is prepared. Then pipette out 0.2 mL for LOD and 0.6 mL for LOQ from above solution in 10 mL volumetric flask makes up with diluent and injected to find best signal to noise ratio. A typical signal noise ratio is 3:1 for LOD and 10:1 for LOQ.[18,19]

Robustness: Robustness studies were performed by changing method parameters in system such as Flow rate (±0.1%), Ph (±0.1) and Composition of mobile phase (±5%).[20-22]

Forced Degradation Studies[23-25]

Preparation of Solutions

Stock B: Take out 5 mL of the stock solution and diluting to 50 mL with diluent.

Acid: From stock B, pipette out 1mL into 10 mL volumetric flask then add 5 mL of 0.1N HCl mix properly make up with diluent and sonicate for 30 min, inject into the HPLC.

Base: From stock B, pipette out 1 mL into 10 mL volumetric flask then add 5 mL of 0.1N NaOH mix properly make up with diluent and sonicate for 30 min, inject into HPLC.

Hydrolysis: From stock B, pipette out 1 mL into 10 mL volumetric flask then add 5 mL of water mixes properly make up with diluent and sonicate for 30 min, inject into HPLC.

Peroxide: From stock B, pipette out 1 mL from into 10 mL volumetric flask then add 5 mL of 1% H_2O_2 mix properly make up with diluent and sonicate for 30 min, inject into HPLC.

Heat: From stock B, pipette out 1 mL into 10 mL volumetric flask makes up with diluent that is heated at 60°C for 30 min and inject into HPLC.

Sunlight: From stock B, pipette out 1 mL diluting agent in a 10 mL volumetric flask that is exposed to sunlight for 6 days, inject into HPLC.

RESULTS AND DISCUSSION

Method Development

Sodium dihydrogen phosphate buffer and methanol make up the mobile phase (60:40 v/v) with column flow rate of 1 mL/min, at 275 nm wavelength, the method has developed with 1.896 min retention time, 1.61 tailing and 4314 of plate count, Bilastine chromatogram is shown in the Figure 3.

Statistical optimization of selected responses of HPLC method and Prediction profiler of optimized method

Numerical optimization represents the prediction profiler Figure 4 displays a continuous correlation between various parameters and multiple responses. This shows that the greatest global desirability value of 76.48% provides the chance of achieving the desired goal for all three responses. Figure 3 is demonstrating the significance of bar representing flow rate, Theoretical plate and buffer. The data from all 5 runs was incorporated into the design to test model fit. The obtained data were statistically analysed by employing zero intercept multiple regression models. That shows Predicted R^2=0.8073 and Adjusted R^2=0.9595 for response retention time, Predicted R^2=0.9689 and Adjusted R^2=0.9943 for Theoretical plate and Predicted R^2=0.9283 and Adjusted R^2=0.9785 for response tailing are statistically significant at a significance level.

Contour profiler

Graphical optimization represents the white region in the contour profiler shown in Figure 5, provides the optimized method operational design space indicating the effect of method components on the results. The statistical parameters obtained to ensure that the CMAs factored in the design have a significant effect on the CAAs and it was found to be significant enough for optimization and prediction of the goal of the experiment.

Analytical method validation

Specificity

By examining the impact of blanks and other contaminants during Bilastine retention period, the specificity of the suggested approach was ascertained. As a result, no additional peak was discovered in the blank, revealing an elevated degree of specificity for the suggested technique.

Table 4: System suitability studies.

Sl. No.	Test sample	Peak	Rt in (min)	Area (µV*sec)	USP Plate Count	USP Tailing
1	STD-2	Bilastine	1.981	2579768	4319	1.60
2	STD-2	Bilastine	1.981	2575586	4204	1.59
3	STD-2	Bilastine	1.981	2572769	4314	1.60
4	STD-2	Bilastine	1.981	2574127	4217	1.60
5	STD-2	Bilastine	1.981	2579731	4252	1.61
Mean				2576396.1		
%RSD				0.1		

Table 5: Accuracy studies or %Recovery studies.

Spiked level	Sample Weight	Sample Area	µg/mL added	µg/mL found	% Recovery
50%	10.00	1273297	9.900	24.60	100
50%	10.00	1267116	9.900	24.58	99
50%	10.00	1266305	9.900	24.76	99
100%	20.00	2555122	19.80	49.63	100
100%	20.00	2551480	19.80	49.63	100
100%	20.00	2552796	19.800	49.76	100
150%	30.00	3849204	29.700	74.55	100
150%	30.00	3841736	29.700	74.80	100
150%	30.00	3845711	29.700	74.62	100

Table 6: Robustness studies by changing flow, pH and composition of mobile phase.

Sl. No.	Sample name	Peak	Rt in (min)	Area (µV*sec)	USP Plate Count	USP Tailing
1	FLOW-A	Bilastine	1.625	2103139	3575	1.60
2	FLOW-B	Bilastine	2.535	3289314	4572	1.57
3	pH-1	Bilastine	1.984	2580309	4333	1.60
4	pH-2	Bilastine	1.986	2576538	4321	1.58
5	COMP-1	Bilastine	2.230	3643757	4293	1.59
6	COMP-2	Bilastine	3.133	3225632	3575	1.60

System suitability: The parameters pass for the number of USP plate count was found to be 15145, USP tailing factor 1.24, %RSD and R_t was found to be 0.2% and 2.877 min, as given in Table 4.

Accuracy: Accuracy was determined by injecting a concentration of 50%, 100% and 150% with each three injections. Accuracy at three different concentrations were in the limit of 97-103%, so the developed method passes this parameter as shown in Table 5.

Precision

The concordance of data between the series of measurements was studied to determine the precision of a system and approach. Six injections at 100% concentration were used in this instance. With a precision %RSD of 0.057%, the suggested approach offered a high level of precision.

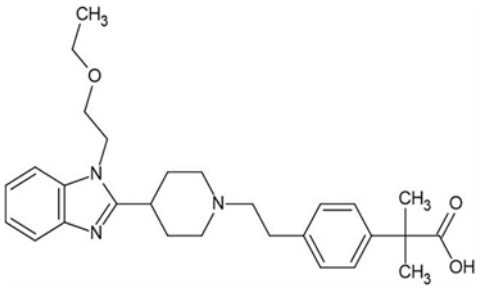

Figure 1: Chemical Structure of Bilastine.

Linearity and Range

Bilastine solutions with concentration ranging from 50 to 150 µg/mL were injected at 275 nm wavelength. From the graph it shows that R^2 was found to be 1 this states that the Bilastine passes linearity over the range of 50% to 150% as shown in Figure 6.

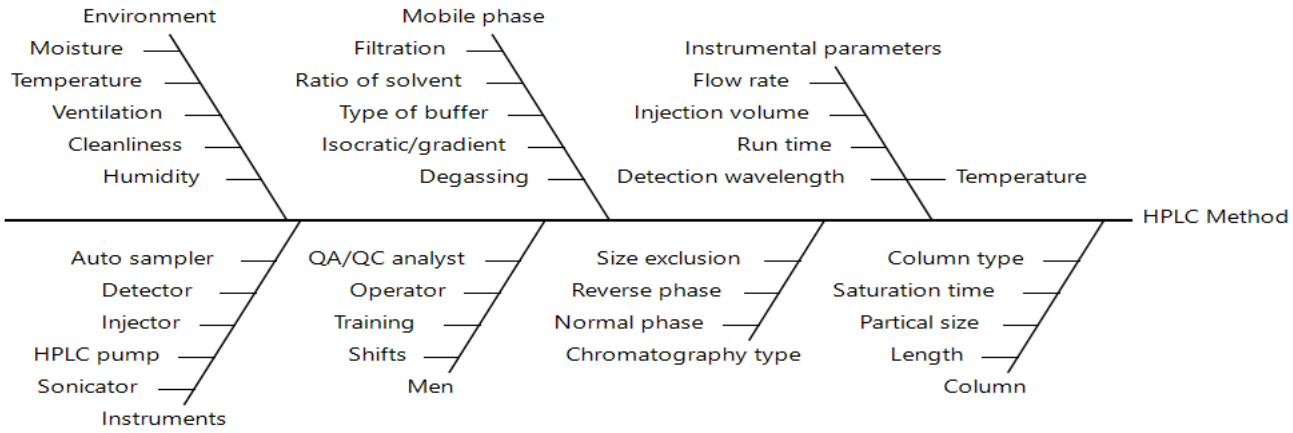

Figure 2: Ishikawa fishbone diagram (depicted for risk).

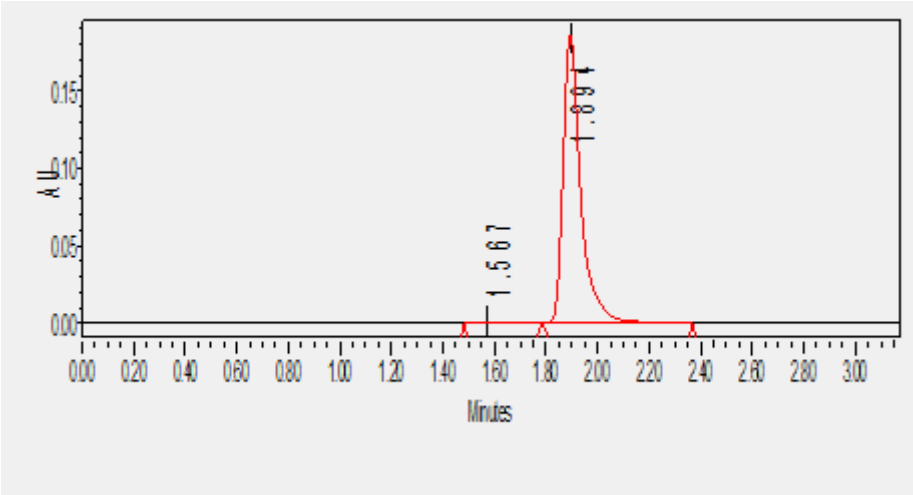

Figure 3: Chromatogram of Bilastine.

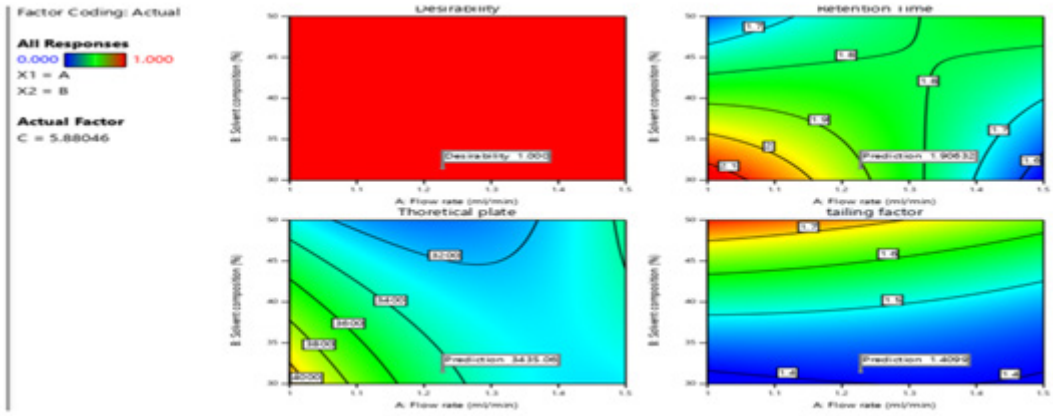

Figure 4: Prediction profiler with maximized desirability.

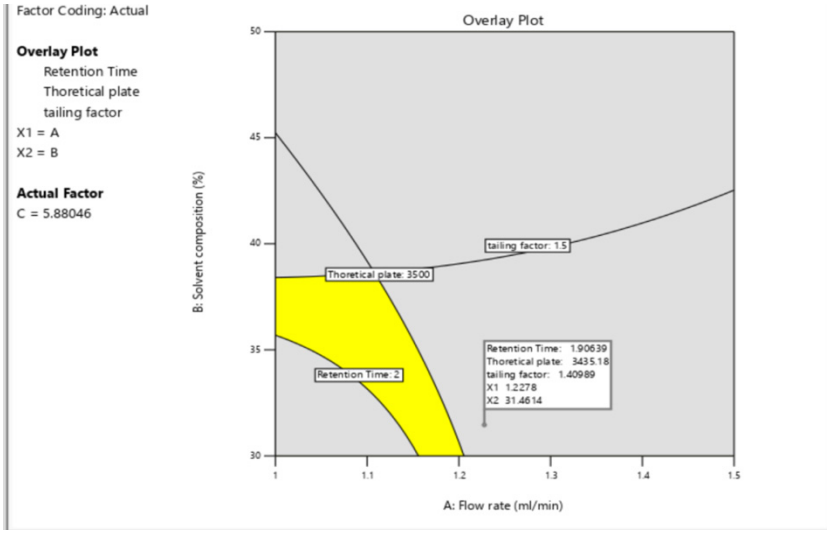

Figure 5: Profiler showing effect of method components on the responses.

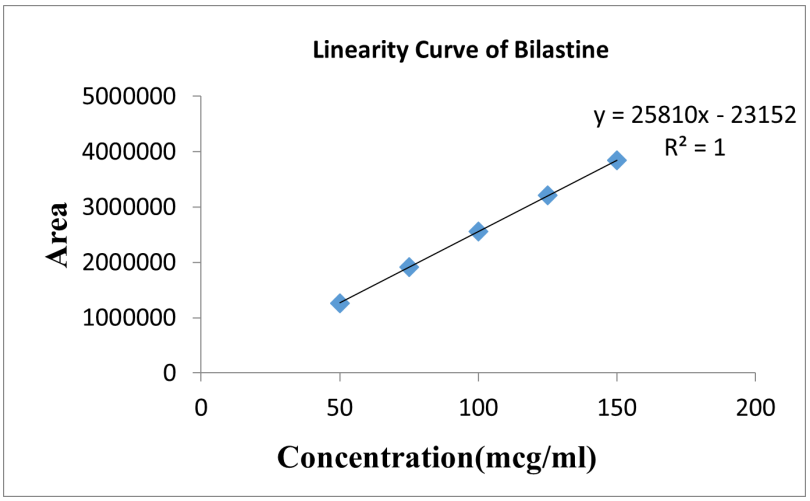

Figure 6: Graphical representation of linearity curve of Bilastine.

Table 7: Forced degradation studies.

Stress	Sample Area	% Assay
Acid stress	2277337	88.73
Base stress	2355176	91.76
Peroxide stress	2428173	94.61
Heat stress	2329183	90.75
Sunlight	2458836	95.80
Hydrolysis stress	2552498	99.45

Detection and Quantitation limit

LOD and LOQ were determined by S/N ratio that was found to be 3.2 and 10.7 respectively. The minimal amount of sample that can be detected has been identified using LOD and it was discovered to be 0.005 µg/mL. The minimal quantity of sample that was capable of being quantified was discovered to be 0.016 µg/mL, or LOQ.

Robustness

All of the results meet system suitability requirements, demonstrating the new method's robustness and lack of substantial deviations even when flow rate, pH and mobile phase composition were slightly altered given in Table 6.

Studies on forced degradation

These were conducted under a number of stressful circumstances, such as acid, base, peroxide, heat and sunlight to study the degraded products will interfere with study. From the degradation data, it was found that Bilastine showing some degradation in stress condition, such as acid (11.24%), base (8.24%), peroxide (5.39%), heat (9.25%) and sunlight (4.2%) and hydrolysis stress (0.55%), but degradation was not above 20% of limit in all conditions (Table 7). Additionally, it was discovered that deteriorated products did not obstruct the primary peak. Hence the inference of the results is analysed as: Acid>Heat stress>Base stress>Peroxide stress>Photo stress> Hydrolysis stress

CONCLUSION

For estimation of Bilastine, a simple RP-HPLC method was developed using an analytical quality by design approach. The Crucial Method Parameters (CMPs) that were selected were the column temperature, flow rate, column type and injection volume. The three most important quality attributes are peak area, theoretical plates and retention time. The CMPs were carefully optimized through the usage of Box Behnken design. Ideal chromatographic conditions are achieved with a mobile phase of 0.1 NaH_2PO_4 (60%) and methanol (40%) injected at a flow rate of 1 mL/min. Retention time was found to be 1.894 min and theoretical plates as well as asymmetry were within the bounds. The developed procedure was validated in compliance with the guidelines provided by ICH Q2 (R1) and was discovered that the drug was sensitive in acidic conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AQbD: Analytical quality by design; **HPLC:** High performance liquid chromatography; **UPLC:** Ultra performance liquid chromatography; **HPTLC:** High performance thin layer chromatography; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **UV:** Ultraviolet; **QC:** Quality control; **%RSD:** Percent relative standard deviation; **Rt:** Retention time.

SUMMARY

The study aimed to establish a reliable method for estimating Bilastine concentration in bulk drugs using a systematic AQbD approach. Key findings include optimization of critical parameters through Box Behnken design, with chromatographic separation achieved using RP-HPLC. The optimized conditions resulted in a desirability function of 77%, LOD and LOQ of 0.005 mcg/mL and 0.016 mcg/mL respectively, and a retention time of 1.894 minutes for Bilastine. Validation according to ICH guidelines confirmed method reliability, with statistically significant models ($p<0.05$). However, stability testing revealed notable degradation under heat stress, highlighting the importance of further investigation into formulation or packaging improvements. Overall, the study presents a robust method for Bilastine estimation, meeting regulatory standards while identifying areas for potential refinement.

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