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Development and validation of RP-HPLC method for the determination of Bilastine tablet

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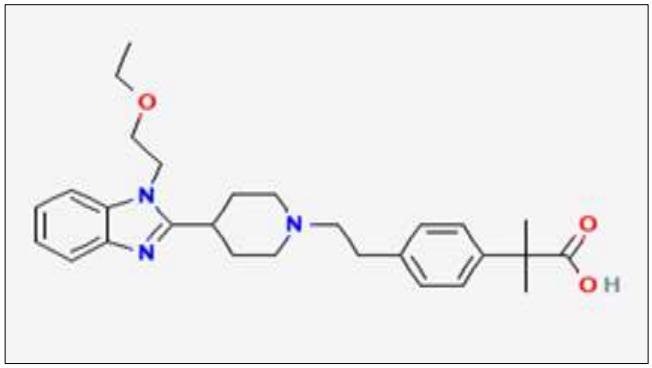
Abstract
A new RP-HPLC method was developed for the estimation of bilastine tablet and it was validated as per ICH guidelines. The chromatogram for was found to be satisfactory on symmetry Aligent C-18 (250x4.6mm) 5µ column using Buffer: (Dipotassium hydrogen phosphate): Acetonitrile (pH adjusted at 7.0 using Orthophosphoric acid) in the ratio of 50:50 v/v at a flow rate of 1ml/min at 215nm. The peak of Within limits was found well separated at 2.7 min. The retention time of bilastine were found to be 2.70 min. The system suitability parameters proved that the proposed method is suitable for estimation of bilastine. The method was found to be linear in the range of 5-20 µg/ml shows a correlation coefficient of 0.999 for a peak. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD was found to be 0.40 µg/ml and LOQ was found to be 1.42 µg/ml for bilastine. The developed method was validated for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustness.

Keywords:

Introduction
Bilastine is an antihistamine medication used to treat hives (urticaria), allergic rhinitis and itchy inflamed eyes (allergic conjunctivitis) caused by an allergy. It is a second-generation antihistamine and takes effect by selectively inhibiting the histamine H 1 receptor, preventing these allergic reactions. Bilastine has an effectiveness like cetirizine, fexofenadine, and desloratadine. Bilastine was discovered by the Spanish firm FAES Farma and received its first approval in the European Union in 2010 for the symptomatic treatment of allergic rhino conjunctivitis and urticaria. It is also approved in Canada and Australia. As of 2023, it remained unapproved for any use in the United States, although Hikma Pharmaceuticals had agreed in 2021 to begin the FDA approval process. Evidence has shown that bilastine is effective in treating skin and eye symptoms of allergic reactions, improving patient&#39, s quality of life. Bilastine meets the treatment criteria for allergic rhinitis, as published by the European Academy of Allergy and Clinical Immunology (EAACI) and the Allergic Rhinitis and its Impact on Asthma (ARIA) initiative.
The drug bilastine is CDSCO approved for symptomatic treatment of allergic rhino-conjunctivitis. On literature survey, it was found that few UV spectroscopy, HPTLC and RP-HPLC methods. The method development approaches specifically focused on pharmaceutical development in a have not been discussed it was planned to develop simple, rapid and sensitive RP-HPLC method for estimation of bilastine in tablet dosage form. Bilastine or 2-[4-(2-(4-(1-(2-ethoxyethyl)-Benzimidazole-2-yl) piperidine-1-yl) ethyl) phenyl]-2-methyl propionic acid, is a new next generation antihistamine.



Materials and Methods

Bilastine 20mg, marketed tablet formulation manufactured by Synokem Pharmaceuticals Ltd., Uttarakhand, Purchased from a local Pharmacy store.All solvents used were of HPLC grade while the other reagents were of analytical grade. HPLC (LC-CYBER-LAB), Digital balance (Wensar), pH meter (Digital pH meter instrument India), Sonicator (Ultrawave, instrument India), UV detector and Nylon membrane filter were used in the study.

Determination of working wavelength

Solution of Bilastin was scanned in the UV region and spectrum was recorded (200-400nm). The solvent used was Buffer: [Acetonitrile: Dihydrogen Phosphate buffer: 50:50 v/v] Adjusted pH with Orthophosphoric acid at 7.0. It was seen that at 215nm bilastine compounds had very good absorbance, which can be used for the estimation of compound by HPLC.

Preparation of solvent buffer

Dissolve 6.8gm of Potassium dihydrogen phosphate in 1000ml of milli Q water and Adjusted the pH of solution to 3.5±0.1 with dilute orthophosphoric acid. Prepare a degassed mixture of solvent buffer and Degassed Acetonitrile in the ratio of 50:50 v/v. Filtered through 0.45µ nylon membrane filter as a mobile phase.

Preparation of standard stock solution Bilastine

Weigh and transfer accurately 40 mg of Bilastine working standard into a 100ml clean and dry volumetric flask and make up with 100ml of diluent and sonicate to dissolve. From stock solution 5ml taken and make upto 50ml and again filtered and sonicated.

Sample Solution

Weighed and finely powdered not less than 20 tablets. Transferred an accurately weighed portion of the powder of Bilastin about 40mg in 100ml volumetric flak, added 100ml of diluant phase sonicated for 30 minutes. Make up the volume with diluant. Mixed well and filtered through 0.45µ nylon filter paper, discarded first few ml of the filtrate. From stock solution 5ml taken and make upto 50ml and again filtered and sonicated.

Procedure

Injected separately 20µl of the standard preparation into the equilibrated HPLC system in replicate and measure the response of the major peak due to Bilastine. Then injected 20µl of the sample preparation in duplicate and measured the response of the major peak due to Bilastine. Calculate the content of Bilastine.

Optimized Conditions

Mobile phase consisted of Dihydrogen phosphate buffer (pH 7.0) and Acetonitrile in the ratio of 50:50 v/v and chromatographic conditions include:

Column: Aligent C-18 (250x4.6mm) 5µ

Flow rate: 1.0 ml/min. wavelength: 215nm Injection volume: 20µl Run time: 6 minutes.

Validation of method

The method was validated according to ICH guidelines for accuracy, precision, linearity, LOD, LOQ and robustness.

System Suitability

System suitability of the method was performed by calculating the parameters namely, resolution and number of theoretical plates on the 10 replicate injection of standard solution into HPLC system and calculated.

Table 1: System Suitability Parameters

Inj. No.	Bilastine	
	RT	Area Response
1.	2.700	2136356
2.	2.700	21129262
3.	2.700	2113672
4.	2.700	2107774
5.	2.700	2110764
6.	2.700	2088751
Avg.		2111707.17
STD		6800.7531
%RSD		0.322

Acceptance Criteria

The % RSD for 6 replicate injections should not more than 2.0%. The system suitability parameters and % RSD for peak areas for 6 replicate injections of standard solution was found to be within limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products and matrix components. Chromatogram of blank solutions showed no peaks at the retention times of Bilastine. This indicates that the solvents and chemicals used in the formulated do not interfere in estimation of Bilastine in the Tablets.

Table 2: Specificity Data

Name of Peak	Retention time (Interference)
Diluent	No time peak observed(No interference)
Mobile Phase	No time peak observed(No interference)
Bilastine Std.	2.7 min. (No interference)

Acceptance Criteria: Diluent and Mobile Phase should not show any interference at the retention time corresponding to the peak of Bilastine.

Table 3: Results for Specificity:

S. No.	Solution	RT	Peak Purity
1.	Diluent	-	-
2.	Blank	-	-
3.	Bilastine Std.	2.7	Pass
4.	Bilastine Sample	2.7	Pass

Linearity

Appropriate aliquots of test drug were pipette out from the stock solution into a series of 50ml volumetric flasks. The volume was made up to the mark with Makeup phase. Inject 10-50µg/ml of concentration into the HPLC system and chromatographed under the optimized conditions. Evaluation was performed with the UV detector set at 215 nm and the peak areas were recorded.

Table 4: Linearity data of Bilastine

Conc. (*µ* g/ml)	Area Response		Avg. Area Response
10	1.	543505	544676
	2.	542391	
	3.	548132	
20	1.	1072575	1088019
	2.	1095169	
	3.	1096312	
30	1.	1634852	1640946
	2.	1650366	
	3.	1637620	
40	1.	2151196	2147199
	2.	2152536	
	3.	2138866	
50	1.	2733615	2729463
	2.	2722304	
	3.	2732469	
	Correlation coefficient		0.999

Acceptance Criteria:

The Correlation Co-efficient should be NLT 0.999 for peak.

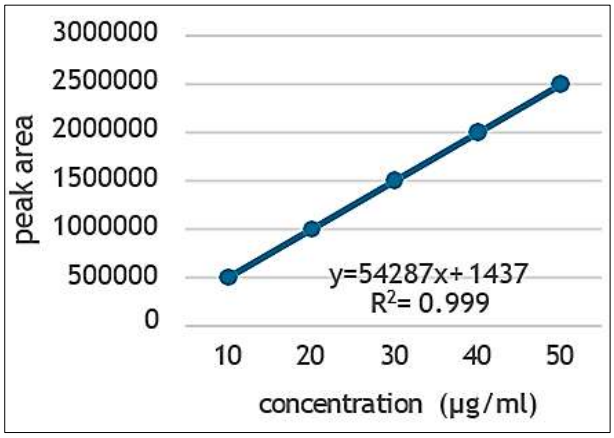


Fig 1: Calibration graph of Bilastine

System precision: System precision was done by using Bilastine of concentration about 10 µg/ml each, prepared six times and injected into the HPLC system under the optimized conditions.

Table 5: System Precision Data

Sample No.	Bilastine		
	RT	Area Response	Avg.
1.	2.70	2151196	**1654190.67**
	2.70	2176564	
	2.70	2145781	
	2.70	217479	
	2.70	2177734	
	2.70	2172275	
Avg.	2166338.1667		
STD	6285.9658		
%RSD	0.29017		

Acceptance Criteria

The % RSD should be NMT 2

Ruggedness

The ruggedness of the analytical method was conducted on different HPLC, different column and different analyst by assaying different test preparations of Bilastine tablet formulation under similar conditions. The system suitability parameters were evaluated as per test method on both the system and found to be within limits. Comparison of results

obtained on two systems show that the assay method is rugged.

Table 6: Ruggedness

% RSD from six replicate inj. of sample	Instruments Employed	
HPLC instrument	**SHIMADZU (System 1)**	**Water Alliance (System 2)**
Column	C-18 Aligent (250x4.6mm) 5 µm	C-8 Phenomenex (250x4.6mm) 5µm

Robustness

Ability to remain unaffected by small changes in parameters. The robustness of an analytical procedure is a measure of its capacity to remain unchanged by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

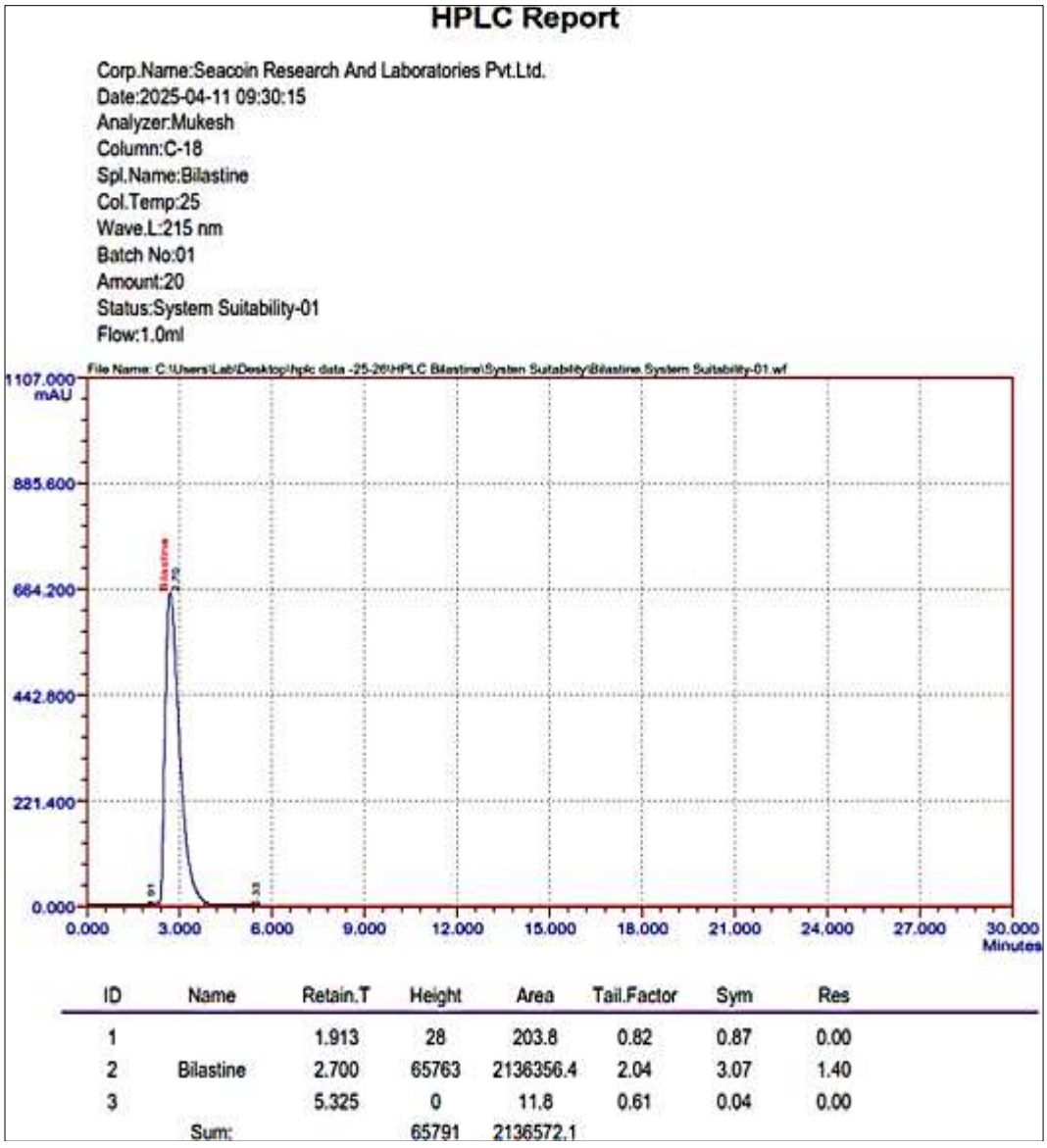
Determination: Factorial design The significant models are used to define the method robustness by predicting the effects of accumulated variations in the study factors to check that the responses remain within specification even in worst case. The robustness of the method is determined by performing the assay by deliberately altering the parameters such as flow rate±10%, pH of mobile phase±0.2, detection wavelength±5 nm, organic phase ratio±2%, column temperature±5º C, coolant temperature±1˚C, injection volume±10%, saturation time±5 minutes, spot band size±1, developing distance±1 cm, drying condition±5 ˚C etc. and check the results are not influenced much by the changes in the above parameters.

LOD and LOQ

The LOD was found to be 0.40 µg/ml and LOQ was found to be 1.42 µg/ml for bilastine.

Results and Discussion

The working condition for the HPLC and UV method was established for Bilastine Drug and then was applied on pharmaceutical dosage forms (a Bilastine tablet was used) A simple reverse phase High Performance Liquid Chromatography has been developed and subsequently validated. The separation method was carried out by using a mobile phase consisting of Potassium dihydrogen phosphate buffer (pH 3.5±0.1) (0.05M): Acetonitrile 50:50 v/v)



ID	Name	Retain.T	Height	Area	Tail.Factor	Sym	Res
1		1.913	28	203.8	0.82	0.87	0.00
2	Bilastine	2.700	65763	2136356.4	2.04	3.07	1.40
3		5.325	0	11.8	0.61	0.04	0.00
	Sum:		65791	2136572.1			

Fig 3: Chromatogram for system specificity of bilastine

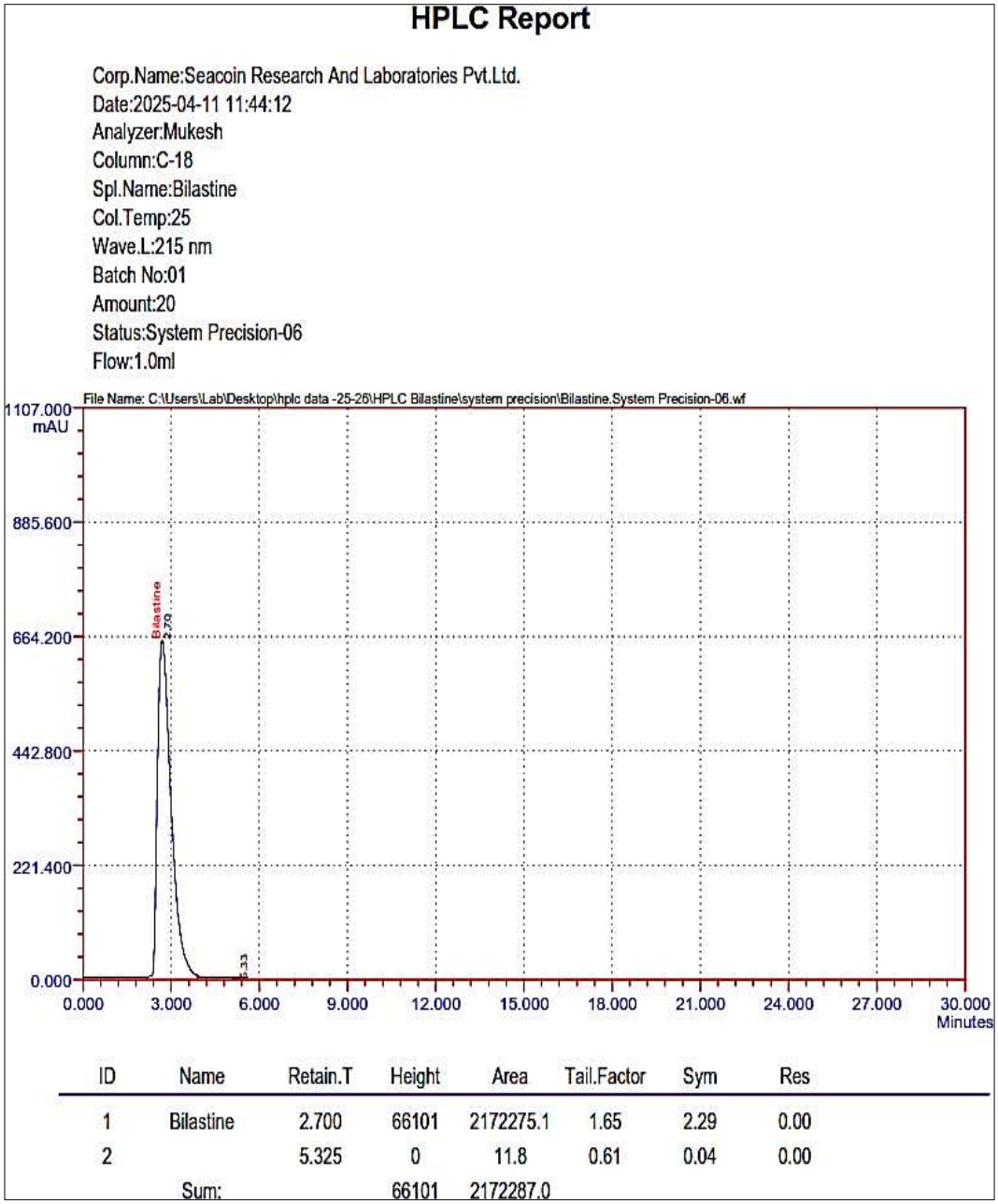


Fig 4: Chromatogram for system precision of bilastine

The deduction was carried out by using UV detector at 215nm. The column was Agilent C 18 (250X4.6mm) 5µ. The flow rate was selected as 1.0ml/min. The retention time of Bilastine was found to be 2.70. The number of theoretical plates of 4254 which indicates the efficient performance of the column. These parameters represent the specificity of the method. From the linearity studies, specified concentration levels were determined. It was observed that Bilastine was linear in the range for the target concentration by RP-HPLC. The linearity range of Bilastine 10% to 50% µg/ml was found to obey linearity with a correlation coefficient (r^2) of 0.999. The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The ruggedness study was also performed. The robustness studies were performed. These are all comes under the specified limits and passes. The analytical method validation was carried out by UV and RP-HPLC as per ICH guidelines which are mentioned below as follows in table 7.

Table 7: The analytical method validation was carried out by UV and RP-HPLC as per ICH guidelines which are mentioned below

S. No.	Parameters	Limit	Observations	Passes/Fails
1.	Specificity	No interference at retention time of the peak	No interference at retention time of the peak	Passes
2.	Linearity	Correlation coefficient(r^2) NLT 0.999	Bilastine-0.999	Passes
3.	Precision	% RSD NMT 2.0	Bilastine-0.290	Passes
4.	Accuracy	% Recovery range 98- 102%	Within limits	Passes
5.	Ruggedness	RSD NMT 2.0%	Within limits	Passes
6.	Robustness	%RSD NMT 2.0	Within limits	Passes

Summery and Conclusion

A simple and effective HPLC method for Bilastine was developed and validated in combined tablet dosage form as pre ICH Guide lines. UV Detector and Agilent C 18 (250x4.6mm) 5µ column, injection of 20µl is injected and eluted with the mobile phase of Potassium dihydrogen phosphate buffer with pH 3.5: Acetonitrile in the ratio 50:50 v/v which was pumped at a flow rate of 1.0ml at 215nm. The peak of Within limits was found well separated at 2.7 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustness. The analytical method validation of Bilastine by RP-HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Bilastine tablet dosage forms.

Future scope

The analytical method validation of Bilastine by RP-HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Bilastine tablet dosage forms.

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