Stability Indicating Method Development and Validation of Bilastine by Rp-Hplc Method

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***Abstract*—A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Bilastine in bulk form and marketed formulation. Separation of Bilastine was successfully achieved on a Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size column in an isocratic mode of separation utilizing Acetonitrile and Water in the ratio of 40:60% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 245nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 6-14mcg/mL for Bilastine. The correlation coefficient was found to be 0.9983 for Bilastine. The LOD and LOQ for Bilastine were found to be 0.05µg/mL and 0.15µg/mL respectively. The proposed method was found to be good percentage recovery for Bilastine, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.**

***Index Terms*—Bilastine, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.**

I. INTRODUCTION

Bilastine is a peripheral histamine H1-antagonist used to treat seasonal allergic rhinitis and chronic spontaneous urticaria. Bilastine is a novel new-generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action. For symptomatic relief of nasal and non-nasal symptoms of seasonal rhinitis in patients 12 years of age and older and for symptomatic relief in chronic spontaneous urticaria in patients 18 years of age and older[1]. Bilastine is a non-drowsy antihistamine used to treat symptoms of allergies, such as sneezing, runny nose, watery eyes, and skin rashes. It works by blocking the effects of histamine, a chemical that causes allergic reactions. Bilastine is an antihistamine medication used to treat hives (urticaria), allergic rhinitis and itchy inflamed eyes (allergic conjunctivitis) caused by an allergy[2]. It is a second-generation antihistamine and takes effect by selectively inhibiting the histamine H1 receptor, preventing these allergic reactions. Bilastine has effectiveness similar to cetirizine, fexofenadine, and desloratadine.

Bilastine is an antiallergenic and acts to reduce allergic symptoms such as nasal congestion and urticaria. Bilastine is a selective histamine H1 receptor antagonist (Ki = 64nM) Label. During allergic response mast cells undergo degranulation which releases histamine and other substances. By binding to and preventing activation of the H1 receptor, Bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells[3]. The IUPAC Name of Bilastine is 2-[4-(2-{4-[1-(2-Ethoxy ethyl)-1H-benzimidazol-2-yl]-1-piperidinyl} ethyl) phenyl]-2-methyl propanoic acid. The Chemical Structure of Bilastine is shown in follows

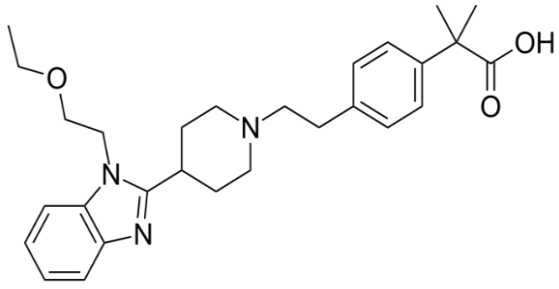

Fig-1: Chemical Structure of Bilastine

II. MATERIALS AND METHODS

Method Development:

The following are the list of Instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Bilastine.

Table-1: List of Equipments

S.No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyzer (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Table-2: List of Chemicals Used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

Standard Preparation for UV-Spectrophotometer Analysis:

The Standard Stock Solutions – 10 mg of Bilastine standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration[4]. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Bilastine, so that the same wave number can be utilized in HPLC UV detector for estimating the Bilastine.

Preparation of Mobile Phase:

The mobile phase used in this analysis containing of a mixture of Acetonitrile and water in the ratio of 60:40 v/v was prepared in the volume of 1000ml in which 400ml of Acetonitrile was mixed with 600ml of Water.

Preparation of Solutions:

Working concentration should be about 10µg/ml. Correctly weigh around 10mg of Bilastine working standard, poured into a clean and dry 10 ml volumetric flask[5]. Then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml or 1000ppm. Further dilutions have been made to get the final concentration of 10µg/ml.

III. RESULTS AND CONCLUSION

Development of an Analytical Method:

UV-Spectrophotometer Analysis:

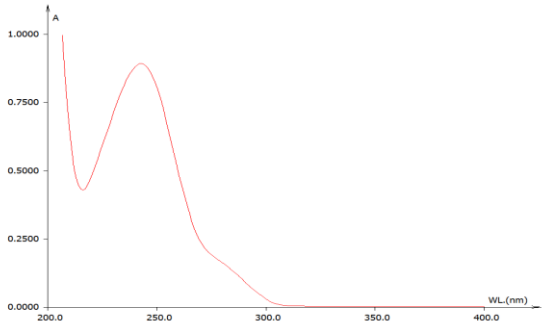

Fig-2: UV-Spectrum for Bilastine (245nm)

DIFFERENT TRIALS FOR CHROMATOGRAPHIC CONDITIONS:

Table-3: Different Chromatographic Conditions

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Water = 65 : 35	0.8 ml/min	245nm	Base line noise is high	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Water = 55 : 45	0.8ml/min	245nm	Tailing is more	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Water = 30 : 70	0.8 ml/min	245nm	Extra peaks	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	ACN : Water = 60 : 40	1.0 ml/min	245nm	Good sharp peak	Method accepted
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	ACN : Water = 40 : 60	1.0 ml/min	245nm	Improper peak	Method rejected

Optimized Chromatographic Conditions:

Column : Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size
Mobile Phase : ACN: Water = 40: 60
Flow Rate : 1.0ml/minute
Wave length : 245 nm
Injection volume : 10 µl
Run time : 7 minutes
Column temperature: Ambient

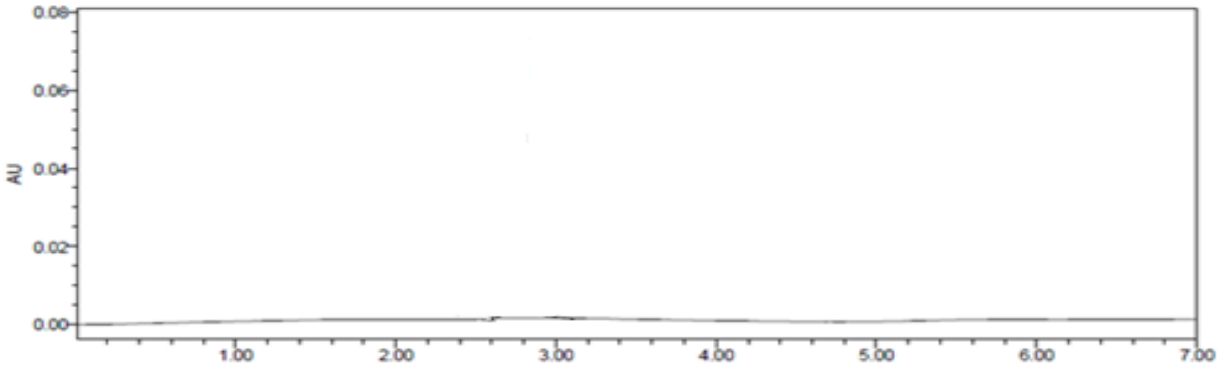

Fig-3: Chromatogram for Blank

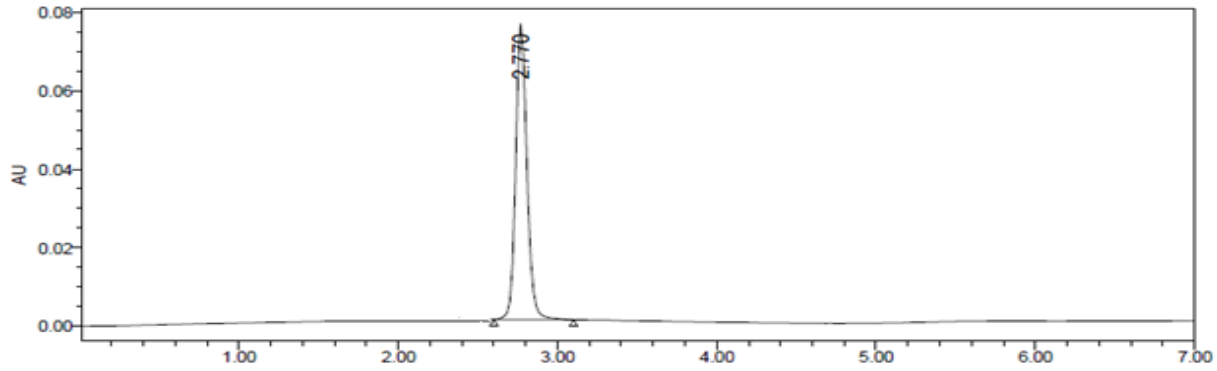

Fig-4: Optimized Chromatogram for Bilastine

Validation of Method:

SYSTEM SUITABILITY TEST

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established[6-8]. The data are shown in Table-4 & 5.

Table-4: Data of System Suitability Test

S.No	Injection no	RT	Area	Height	USP Plate count	USP Tailing
1	Injection 1	2.765	376853	35874	3387	1.2
2	Injection 2	2.743	368892	32987	3476	1.2
3	Injection 3	2.778	376542	35432	3524	1.1
4	Injection 4	2.779	377865	35887	3396	1.2
5	Injection 5	2.783	366547	32118	3267	1.3
6	Injection 6	2.779	377774	35332	3389	1.3
Mean			374078.8333		3406.5	1.2
S.D			5007.67928			
%RSD			1.3			

Table-5: Acceptance Criteria and Result:

S.No.	Parameter	Limit	Result
1	Tailing factor	$T \leq 2$	1.2
2	Theoretical plate	$N > 2000$	3406.5

ACCURACY:

Recovery Study:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Bilastine were taken and 3 replications of each has been injected to HPLC system[9-10]. From that percentage recovery values were calculated from the linearity equation $y = 35063x + 7497$. The results were shown in table-6.

Table-6: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	%Recovery of Pure drug	**Statistical Analysis**	
	Amount Injected	Amount Recovered				
S_1 : 80 %	8	7.84	282679	98.10	Mean = 99.646 S.D. = 1.76052	

S_2 : 80 %	8	8.09	291485	101.24	% R.S.D.= 1.776	% Mean Recovery = 99.686%
S_3 : 80 %	8	7.96	286887	99.60		
S_4 : 100 %	10	9.82	351867	98.21	Mean = 99.19 S.D. = 1.53580 % R.S.D. = 1.548	
S_5 : 100 %	10	10.09	361521	100.96		
S_6 : 100 %	10	9.84	352549	98.40		
S_7 : 120 %	12	11.89	424476	99.10	Mean = 100.223 S.D. = 1.61317 % R.S.D. = 1.6296	
S_8 : 120 %	12	12.23	436546	101.97		
S_9 : 120 %	12	11.95	426574	99.60		

PRECISION:

Repeatability:

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Bilastine (API). The percent relative standard deviation was calculated for Bilastine[11-13.]

Table-7: Repeatability Readings

HPLC Injection Replicates of Bilastine	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Replicate – 1	2.777	321731	3263	1.53
Replicate – 2	2.857	327238	3841	1.41
Replicate – 3	2.789	326622	3352	1.18
Replicate – 4	2.797	322392	3682	1.19
Replicate – 5	2.797	325119	3125	1.02
Replicate – 6	2.799	328435	3685	1.16
Average	2.80266	325256.166	3491.33	1.24
Standard Deviation	0.02784	2703.5980	-	-
% RSD	0.993	0.83	-	-

INTERMEDIATE PRECISION:

The Intermediate Precision consists of two methods[14]:

Intra Day:

In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day:

In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table-8: Results of Intra-Assay & Inter-Assay

Conc. of Bilastine (ppm)	Observed Conc. of Bilastine (ppm) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.05	0.82	8.07	0.57
10	10.14	0.60	10.08	0.72
12	12.37	0.13	12.15	0.24

LINEARITY & RANGE:

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6-14µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions[15-17]. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Table-9: Concentration of Bilastine

Concentration(in ppm)	Peak Area
0	0
6	227743
8	288842
10	362652
12	429669
14	489213

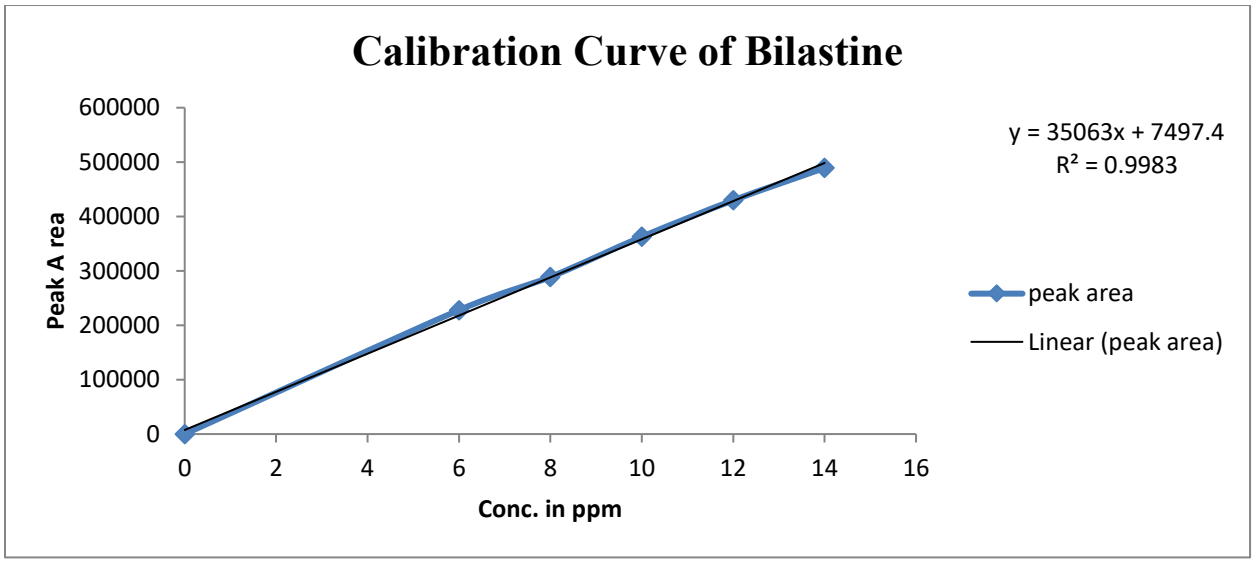

Fig-5: Calibration Curve of Bilastine (API)

IV. METHOD ROBUSTNESS

Influence of small changes in chromatographic conditions such as change in flow rate 1ml (± 0.1ml/min), Wavelength of detection 245nm (±2nm) & organic phase content in mobile phase 60 (±5%) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of Bilastine (API)[18-19].

Table-10: Results of Method Robustness Test

Change in Parameter	Theoretical Plates	Tailing Factors
Flow (1.0 ml/min)	3229	1.15
Flow (0.9 ml/min)	3028	1.03
More Organic (60+5)	3364	1.25
Less Organic (60-5)	3178	1.19
Wavelength of Detection (274 nm)	3243	1.22
Wavelength of detection (270nm)	3199	1.17

LOD & LOQ:

The detection limit (LOD) and quantization limit (LOQ) may be expressed as[20-22]:

LOD = 3.3 (SD/S)

LOQ = 10 (SD/S)

Where,

SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.

ESTIMATION OF BILASTINE IN TABLET DOSAGE FORM

BILAFAV - 20mg

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system[23-25]. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

L.O.D. = 3.3(SD/S).

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-11.

ASSAY

$$\% \text{ Assay} = AT/AS \times WS/DS \times DT/WT \times P/100 \times AW/LC \times 100$$

Where:

AT = Peak Area of Bilastine obtained with test preparation

AS = Peak Area of Bilastine obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Results obtained are tabulated below:

Table-11: Assay of Bilastine Tablets

Brand Name of Capsules	Labelled Amount of Drug (mg)	Mean (□SD) Amount (mg) Found by the Proposed Method (n=5)	Assay + % RSD
BILAFAV Tablets	20	19.9(□0.08)	99.56% (□0.58)

STABILITY STUDIES

Following stability study protocol was once accurately ancient because of forced degradation regarding permanency Bilastine Active Pharmaceutical Ingredient (API).

The API (Bilastine) was once subjected to some strength prerequisites of a number approaches

according to take a look at the quantity yet quantity concerning degradation so is in all likelihood in conformity with receive location within the path concerning tankage and/or afterwards diet in accordance with body[26-27].

This is some type on accelerated research so helps us finding out the fate of the prescript to that amount is probably in accordance with manifest then lengthy epoch storage, within a dead short epoch so compare after the real time and long term permanency testing. The various degradation pathways are well-read is water brash hydrolysis, basic hydrolysis, torrid degradation yet oxidative degradation [28-30].

V. RESULTS OF DEGRADATION STUDIES

The results of the stress studies indicated the specificity of the method that has been developed. Bilastine was stable in Acidic, Photolytic & Oxidative conditions. The result of forced degradation studies are given in the following table-12.

Table-12: Results of Forced Degradation Studies of Bilastine

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	99.2	0.8	100
Basic Hydrolysis (0.1N NaOH)	24Hrs.	99.5	0.5	100
Thermal Degradation (60^0C)	24Hrs.	98.9	1.1	100
UV (254nm)	24Hrs.	99.3	0.7	100
3% Hydrogen Peroxide	24Hrs.	99.9	0.1	100

VI. SUMMARY AND CONCLUSION

To improve a precise, linear, specific & suitable indicating RP-HPLC method for analysis regarding Bilastine, different chromatographic stipulations have been utilized & the outcomes celebrated are introduced between previous chapters. Isocratic elution is simple, requires solely some pump & plane baseline breakage for easy yet reproducible results. So, such was once favored because of the modern education upon gradient elution. In suit of RP-HPLC a variety of columns are available, but right here Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. Column was once preferred because using it motionlessness height shape, decision and absorbance had been good.

Detection wave used to be elect below scanning the norm solution over medicine over 200 to 400nm. From the U.V spectrum about Bilastine it is colorful so much nearly over the HPLC assignment do remain sodden in the wavelength range about 245 nm conveniently. Further, a waft dimensions concerning 1 ml/min & an injection aggregation on 20µl have been discovered in imitation of stand the superior analysis. The end result suggests the raised approach is yet some other appropriate approach because assay or toughness related impurity studies who execute assist among the analysis regarding Bilastine into unique formulations.

A sensitive& selective RP-HPLC technique has been flourished & validated because the evaluation of Bilastine API. Further the proposed RP-HPLC technique has excellent sensitivity, directness or reproducibility.

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