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Stability indicating development and validation of RP-HPLC method for the estimation of Bilastine from bulk drug

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Abstract

Using RP-HIPLC to reliably and quantitatively estimate Bilastine was the goal of this effort. Regardless, it is an antihistaminic medication that helps with allergic reactions. With the use of an analytical tool like HPLC, a more recent and sensitive technique was created (Waters, 1525, UV-detector). Chromatographic separation was carried out using a high-quality combinational solvent system consisting of Phenomenex Luna (18-Columns) and Acetonitrile (ACN): Water (40:60). Operating in isocratic mode, with a flow rate of 1 ml/min. At an injection volume of 10µL, within the first seven minutes of operation. Chromograms were observed at a wavelength of 245 nanometers. A retention period of 2.77 minutes was recorded for bilastine, which displayed a peak area of 370133. The theoretical plate was 3334, and the tailing factor was 1.11. The linear calibration curve yielded an R2 value of 0.99984. In order to validate our method, we followed the ICH requirements and looked at things like the solubility index, repeatability, accuracy, linearity, robustness, and degradation studies. As a quality control measure, this technique was useful for quantitatively assessing Bilastine samples.

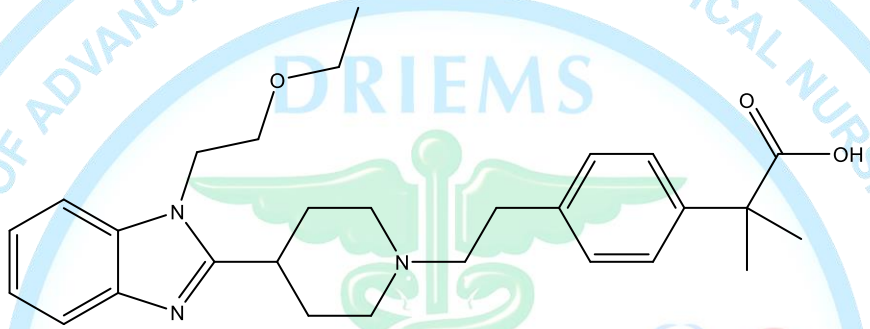
Key-Words

Anti-histaminic; RP-HPLC; Validation; Bilastine; ICH-Guidelines.

INTRODUCTION

A piperidine class of 2nd generation of anti-histaminic novel medication is Bilastine. Which prevents the activation of the H1 histamine receptor[1]. This drug is a white crystalline powder used to treat rhinoconjunctivitis and urticaria. It is known for it's rapid onset and prolonged duration of

action[2,3]. Bilastine can be estimated quantitively through precise instrumentation and accurate method (Like RP-HPLC & UV-Spectrophotometric)[4-6]. This research aims to develop a new economically sensitive and precise analytical method for the quantitative determination of Bilastine though ICH-guidelines[7,8]. In HPLC, two most prominent methods are widely accepted: normal phase HPLC (NP-HPLC) & another one is reverse phase HPLC (RP-HPLC). In reverse phase, the stationary phase is more non-polar than mobile phase. e.g. C-18 silica, RMe_2SiCl & organic miscible solvents like (Methanol, ACN)[9,10].



2-(4-(2-(4-(1-(2-ethoxyethyl)-1*H*-benzo[*d*]imidazol-2-yl)piperidin-1-yl)ethyl)phenyl)-2-methylpropanoic acid
Chemical Formula: $C_{28}H_{37}N_3O_3$
Exact Mass: 463.28
Molecular Weight: 463.62
m/z: 463.28 (100.0%), 464.29 (30.8%), 465.29 (5.2%), 464.28 (1.1%)
Elemental Analysis: C, 72.54; H, 8.04; N, 9.06; O, 10.35

Figure 1: Structure of Bilastine

Materials & Methods

An essential active pharmaceutical ingredient (API) for this research is Bilastine, which was received as a gift sample from "Syncrop Clinecare Technologies (P) Ltd. Hydrabad". We procured HPLC-grade water, ACN, and ethanol from 'Marck (P) Ltd. Mumbai'. DMSO & DMF were procured from 'CDH Fine Chemicals, New Delhi, India'. Binary HPLC system (Model 1525, Waters) equipped with 717 plus Autosamplers and Photodiode Array Detector (PDA), which was managed by Empower-2 software.

Method Development

a. Sample Preparation

Ten milligrams of Bilastine Standards were transferred into a previously calibrated volumetric round bottle flask (10ml). Add methanol into it & allow to dissolve. Then the final volume is made up to the mark with the respective solvent (MeOH).

b. Detection of wavelength

The standard stock solution and a blank were placed in a quartz cuvette and loaded into the UV-Vis chamber to detect wavelengths (200 to 400 nm) using UV-Vis spectroscopy. The λ_{max} of Bilastine was detected at 245 nm.

The absorption spectrum is shown in Figure 2.

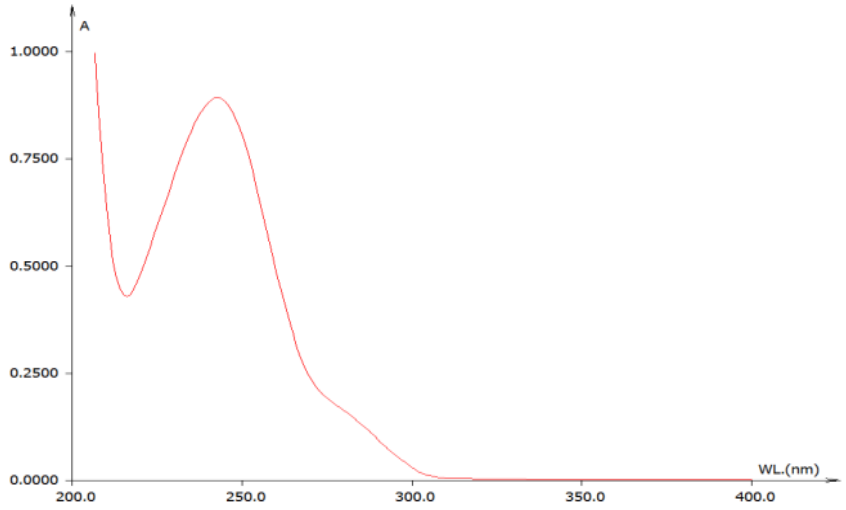


Figure 2: UV-Spectrum of Bilastine

c. Solubility index

The solubility index of the drug was investigated under various chromatographic conditions. Different ratio of ACN and water were prepared. ACN was injected at five different volumes at a flow rate of 1.0 ml/min into a symmetry C-18 column. Retention time (RT), theoretical plate, peak area, & tailing factor were recorded at a wavelength of 245nm. The results are tabulated in Table 1. Based on these results, the ACN: Water (40:60)

ratio was chosen for further study. The conditions for this study included flow rate, injection volume and running time of 1.0 ml/min, 10µl, and 7min respectively.

Table 1: Solubility index

Trial	Mobile Phase *(ACN : Water)*	Flow Rate	Wave length	RT	Peak Area	Theoretical Plates	Tailing Factor	Result
1	65 : 35	1.0 ml/min	245 nm	3.539	138731	2664	1.09	Method rejected
2	55 : 45	1.0 ml/min		2.127	174431	3251	1.87	Method rejected
3	30 : 70	1.0 ml/min		2.720	223451	3078	1.07	Method rejected
4	***40 : 60***	***1.0 ml/min***		***2.770***	***236541***	***3165***	***1.06***	***Method accepted***
5	50 : 50	1.0 ml/min		2.718	317832	3034	1.08	Method rejected

d. Chromatograms at different concentration

To get 10 parts per million, 1 milliliter of stock solution was transferred to a 10-milliliter volumetric flask and the volume was adjusted with MeOH to 10 parts per million. A 15-minute sonication was subsequently applied to the test solution. Similarly, concentrations of 12, 14, 16, and 18 ppm were generated. Chromatographic separation was accomplished using an isocratic mode and a more appropriate mobile phase, such as ACN:Water (40:60), on a Phenomenex Luna C-18 Column (250mm × 4.6mm, 5µm particle size). The flow rate was set at 1.0 ml/min. The chromatogram was obtained at 245nm and the injection volume was 10µl. Figure 3 displays the chromatographic conditions.

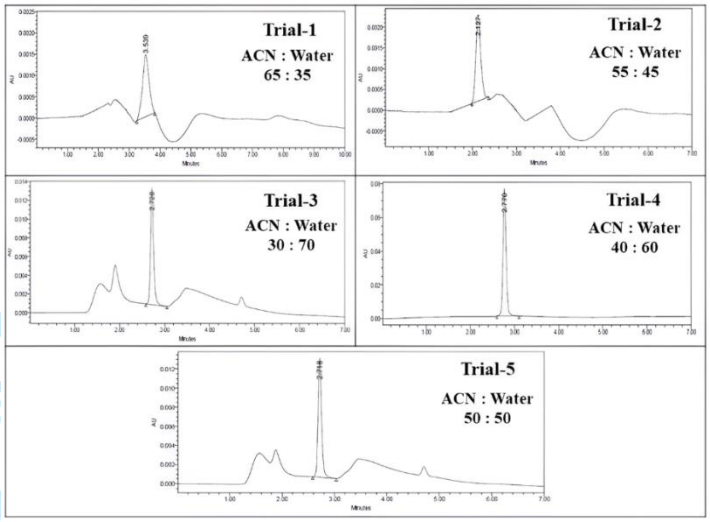


Figure 3: Chromatogram for different solvent ratio trials

e. Preparation of drug solution

Ten milligrams of Bilastine were taken into a dry, cleaned & previously calibrated 10ml volumetric flask. Then a solution with known concentration of approximately 1000 mcg/ml or 1000 ppm was prepared using mobile phase to dissolve and dilute to volume. The final concentration was made to 10 µg/ml.

Method of validation

After a suitable method development for this research, validate the same through different validation aspects mentioned in the International Conference on Harmonization (ICH) guideline.

a. Suitability Test

An important part of many analytical processes is suitability testing. The idea behind these tests is that the components that need to be examined the equipment, the analytical procedures, the electronics, and the samples that will be examined form a cohesive system. The standards for the system appropriateness evaluations were subsequently established. Table 2 displays the data.

Table 2: Suitability Study

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

b. Accuracy

The accuracy of the proposed approach was evaluated in recovery trails using pure Bilastine at three different concentrations (80%, 100%, and 120%), with three injections into the HPLC equipment per concentration. Numbers representing percentages of recovery were obtained by solving the linearity equation, y=350063x +7497. Feel free to peruse the table for the results. The findings can be found in Figure 4 and Table 3.

Table 3: Accuracy Readings

Sample ID	Concentration (µg/ml)		RT	Peak Area	Theoretical Plates	Tailing Factor	% Recovery of Pure drug	Statistical Analysis	
	Amount Injected	Amount Recovered							
S_1 : 80 %	8	7.84	2.803	282679	3032	1.122	98.10	99.646 ± S.D. = 1.76052 % R.S.D.= 1.776	% Mean Recovery = 99.686 %
S_2 : 80 %	8	8.09	2.796	291485	3541	1.15	101.24		
S_3 : 80 %	8	7.96	2.800	286887	3689	1.20	99.60		
S_4 : 100 %	10	9.82	2.768	351867	3102	1.26	98.21	Mean = 99.19 S.D. = 1.53580 % R.S.D. = 1.548	
S_5 : 100 %	10	10.09	2.784	361521	3745	1.23	100.96		
S_6 : 100 %	10	9.84	2.786	352549	3682	1.19	98.40		
S_7 : 120 %	12	11.89	2.794	424476	3254	1.22	99.10	Mean = 100.223 S.D. = 1.61317	
S_8 : 120 %	12	12.23	2.780	436546	3513	1.16	101.97		

S_9 : 120 %	12	11.95	2.775	426574	3166	1.08	99.60	% R.S.D. = 1.6296	

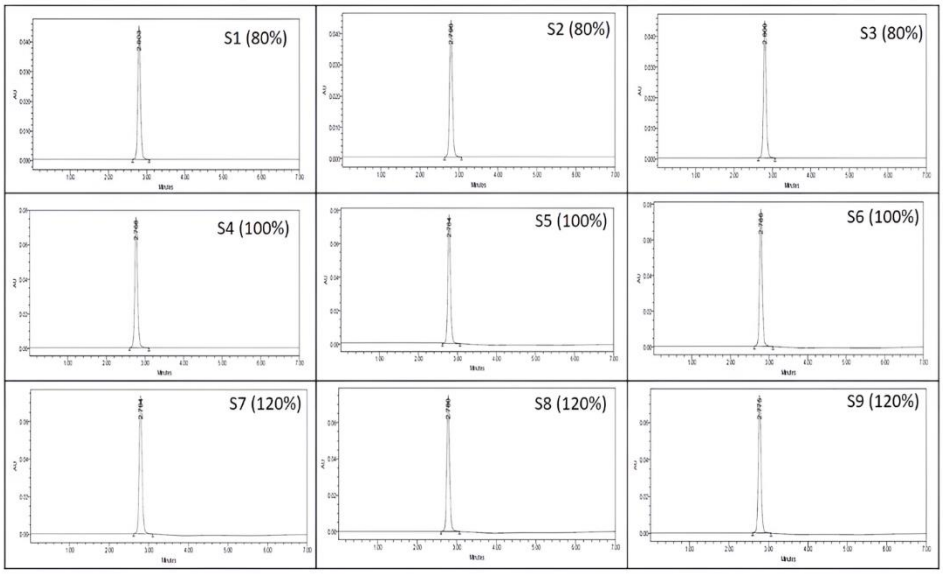


Figure 4: Chromatogram for Accuracy

c. Precision

I. Repeatability

The accuracy of each method was evaluated using the peak areas and retention times obtained from six independent determinations of a fixed dosage of the drug Bilastine (API). The results are shown in Table 4 and Figure 5.

Table 4: Repeatability readings

HPLC Injection Replicates of Bilastine	Retention Time	Peak Area	Theoritical 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**	**-**	**-**

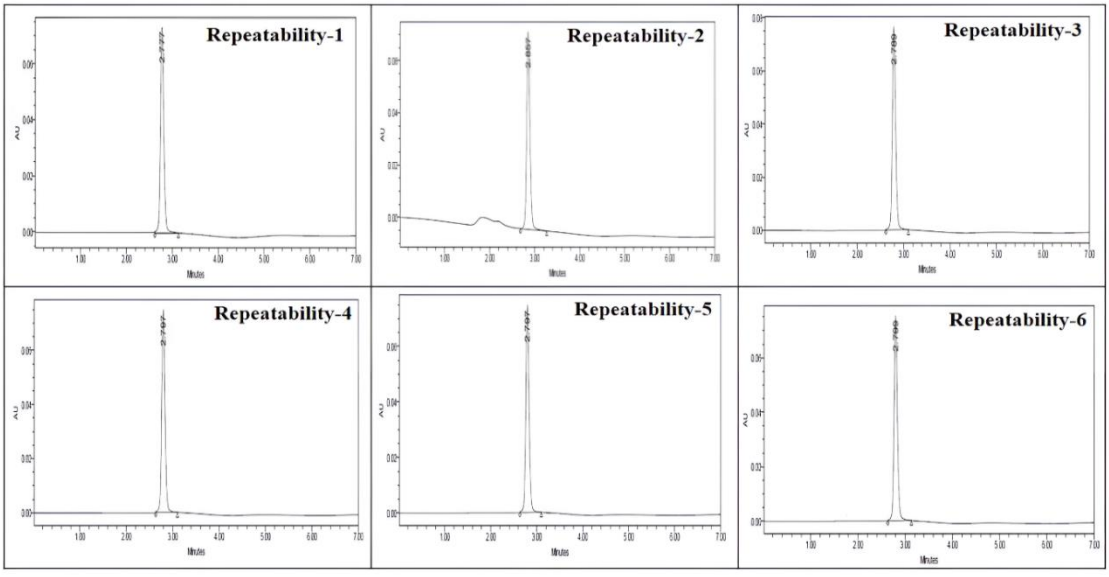


Figure 5: Chromatogram for Precision

II. Intermediate Precision

The two different approaches were used for the intermediate precision.

i. Intra day

The first one was Intra day i.e., the involves the injecting volume of 80%, 100%, and 120% concentration at a various time during the same day.

ii. Inter day

The second one was Intra day method. In this method, the solutions (80%, 100% and 120%) were injected at varying times on different days.

d. Linearity & Range

The analyte was diluted and sonicated in a series of concentrations ranging from 6-14 µg/ml with mobile phase. The HPLC system was chromatographed under the optional conditions after 10µl injections of each concentration were made from these solutions. A calibration curve was created and presented by Figure 6,7 and Table 5.

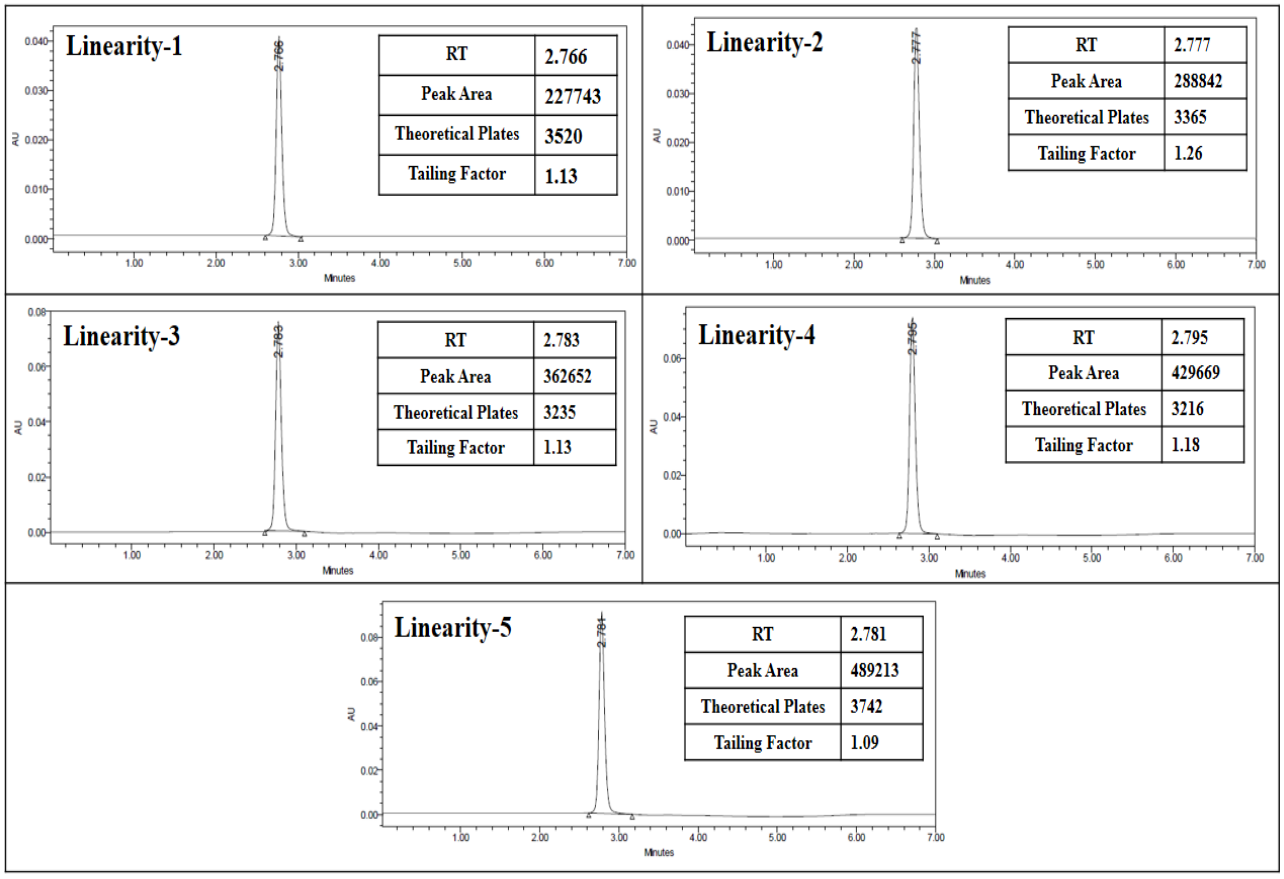


Figure 6: Chromatogram for Linearity

Table 5: Concentration of Bilastine on Calibration Curve

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

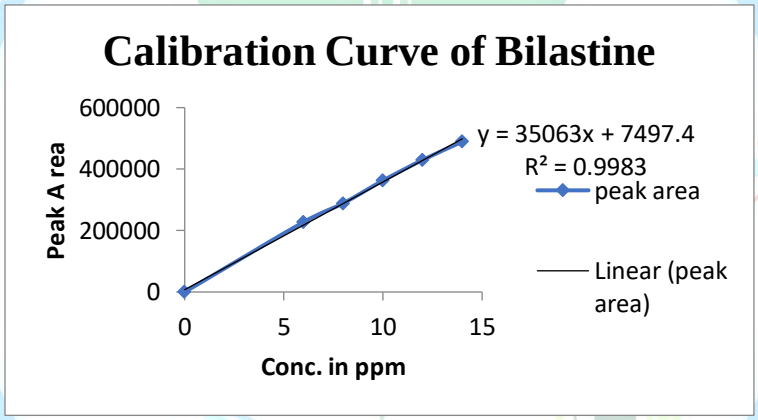


Figure 7: Calibration Curve of Bilastine (API)

e. Robustness

In order to determine the method's resilience, the effect of small changes to the chromatographic parameters, like a 1 ml flow rate change (±0.1 ml/min), a 245 nm wavelength of detection change (±2nm), and a 60 nm organic phase content change (±5%), were studied. The results, which had an RSD of less than 2%, also provided support for the established RP-HPLC method for analyzing Bilastine (API).

Estimation of Bilastine in Tablet (BILAFAV 20mg)

This medication, Bilafav 20 mg, is made by Cipla Pharmaceutical Limited and was purchased from the pharmacy. With each film-coated pill comes 20 milligrams of Bilastine (API). Following the directives of the Indian Pharmacopeia (2018), twenty Bilafav tablets were consumed in order to ascertain the mean weight. Triturating the tablets with a mortar and pestle to obtain a powder followed the determination of weight. A volumetric flask that had been cleaned, dried, and calibrated was then filled with 8 milliliters of mobile phase and 10 milligrams of the triturated powder. To get rid of any air bubbles, the mixture was set in the sonicator for 15 minutes after a good mixing. The last volume was filled during the mobile phase. To get a concentration of 10 ppm, mix 10 ml of the mobile phase (MeOH) with a milliliter of the stock solution that was properly pipetted out. Prior to sonication, pass the mixture through a 0.45 µm membrane filter for further filtration. This technique was repeated multiple times with varying ppm concentrations (12ppm, 14ppm, 16ppm, and 18ppm) of the produced solution fed into the HPLC autosampler. In order to determine the assay %, we used formula 1 and recorded the findings in the table.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots [1]$$

Where; AT-Peak area of test sample, AS-peak area of standard, WS-working standard (mg), WT-sample weight, DS- dilution for standard, DT-dilution for sample, P-working standard purity (%). The results were tabulated in the Table 6.

Table 6: Assay of Bilastine Tablets

Brand name of Tablets	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 Study

Degradation of Bilastine (API) was a part of the stability study methodology. To determine how much and how quickly bilastine would degrade when stored or consumed, it was subjected to various stress settings. In comparison to real-time stability testing, this expedited stability analysis

sheds light on the drug's probable degradation pathway in a shorter amount of time. Hydrolysis under pressure, whether acidic or basic, thermal, oxidative, or photosynthetic, is an important area to investigate.

i. Acid Hydrolysis

After meticulous measurement, ten milligrams of the pure medication was transferred to a dry, clean, and calibrated volumetric flask. After adding 30 ml of 0.1 N HCl, the flask was refluxed in a water bath set at 60°C for 4 hours. Diluting with 0.1 N NaOH neutralized the mixture once it cooled to room temperature. The mobile phase was used to get the total amount down to 10 ml. To begin injecting the solution into the HPLC system, we first utilized this mobile phase as a blank. Then, we tweaked the mobile phase compositions for optimal performance. Repeated experiments with the same concentration of 0.1 N HCl allowed us to detect the deterioration curve.

ii. Basic Hydrolysis

An accurately measured ten milligram dose of the medication was put to a sterile, calibrated, dry volumetric flask. It was then refluxed for four hours at 60°C in a water bath after 30 ml of 0.1N NaOH solution was added to it. Using a 0.1 N HCl solution, the sample was neutralized after cooling, and the concentration was adjusted to 10 µg/ml with the use of the mobile phase. Once it was optimized, it was injected into the HPLC system as a blank, taking into account the mobile phase. For the purpose of observing its deterioration profile, the same concentration of 0.1N NaOH was used repeatedly. The results are documented in the chromatograms..

iii. Thermal Degradations

A volumetric flask that was clean, sterile, and dry was used to hold 10 mg of pure medication. This was then refluxed in a water bath set at 60°C continuously for 6 hours after adding 30 ml of HPLC grade water. After the medicine has become soluble, let the mixture cool and reflux. A concentration of 10 µg/ml was achieved by employing the mobile phase. After that, the mobile phase, which served as a blank, was injected into the HPLC apparatus after the solution.

Oxidation with (3%) H_2O_2

A sterile, clean and dry volumetric flask (100ml) was taken along with 10 mg of pure drug was placed with 30 ml of 3% H_2O_2 and added a Some mobile phase were to facilitate the solubility. The flask was then left undisturbed in darkness for 24 hrs. Then the final volume was adjusted up to the mark. Then this solution was subsequently injected into the HPLC system by following the mobile phase as the blank.

i. Photolytic degradation

Ten milligrams of the pure drug were placed over a sterile petri plate and placed in a UV-chamber for 24 hrs. at a wavelength of 245 nm. The drug solution (10 µg/ml) was prepared by using a UV-exposed drug. This solution then analysed with the help of HPLC against blank.

The results of degradation study were tabulated in the Table 7 and Figure 8.

Table 7: 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

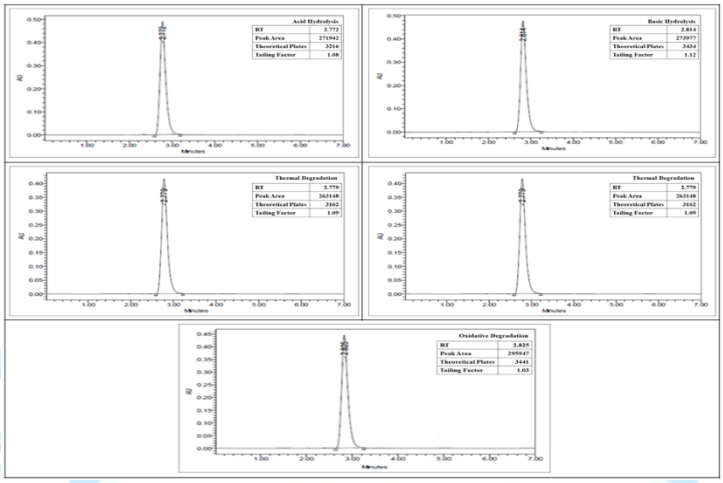


Figure 8: Chromatogram showing degradation

RESULTS

The analytical method of RP-HPLC was used to quantify bilastine, with an optimized mobile phase composed of acetonitrile and water in a ratio of 40:60. With detection at 245 nm, the technique demonstrated a flow rate of 1.0 ml/minute. Even though the run only took seven minutes, the chromatographic peak displayed a theoretically outstanding plate count and symmetry. With mean percentage recovery values of 99.686 ± 1.76%, 99.19 ± 1.54%, and 100.223 ± 1.61%, respectively, for 80%, 100%, and 120% sample solutions, these values were within the acceptable range of 98-102%. Accuracy was demonstrated by %RSD values of 1.776%, 1.548%, and 1.629%, respectively. Compliance with ICH criteria was achieved with a %RSD of 0.83% for peak area and 0.993% for retention time (RT). Inter- and intra-day variations demonstrated %RSDs and standard deviations below 2%, indicating that the measurements were accurate. Within the concentration range of 6-14 µg/ml for Bilastine, the calibration curve showed remarkable linearity ($R^2 = 0.998$). You can see the regression equation here: $y = 35063x + 7497$.

DISCUSSION

The % Purity of BILAFAV tablets containing Bilastine was determined to be 99.56 ± 0.58%, indicating high purity of the active pharmaceutical ingredient (API) in the formulation. Degradation Studies; Bilastine remained stable under various stress conditions including acidic,

basic, thermal, photolytic, and oxidative conditions. Assay results under these stress conditions ranged from 98.9% to 99.9%, confirming the stability of Bilastine. The RP-HPLC method provided robust analytical results for the determination, quantification, and stability assessment of Bilastine, highlighting its suitability for pharmaceutical analysis and quality control purposes.

CONCLUSION

The developed RP-HPLC method is precise, accurate, linear, specific, and suitable for the stability-indicating analysis of Bilastine. It can also be used for assay and impurity studies in various formulations. The method is preferred due to its simplicity, reproducibility, and effectiveness in achieving good peak shape, resolution, and absorbance, which may be preferred by the scientific community.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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