

Research Article



Development and Validation of Stability Indicating UPLC Method for the Estimation of Bilastine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, accurate and economic Ultra Performance Liquid Chromatography method was developed for the estimation of Bilastine in bulk and tablet dosage form. The method has shown adequate separation of Bilastine from their degradation products. Separation was achieved on a Phenomenex C8 (50 x 2.1 mm ID, 1.7 μm) Column at wavelength of 248 nm, using a mobile phase pH 3.5 Sodium Phosphate 10mM Buffer : Methanol : Acetonitrile (60 : 30 : 10 v/v/v) in an isocratic elution mode at a flow rate of 0.5 ml/min. The retention time for Bilastine is found to be 1.19 min. The above drug is subjected to acidic, base, peroxide, thermal and photolytic stress environment. The stressed samples are analyzed by the proposed analytical method. Quantitation is achieved with UV detection at 248 nm based on peak area with linear calibration curve at concentration range 50 – 150 μg/mL. The LOD and LOQ are found to be 0.368 and 1.117 μg/mL respectively. The developed method was established to be rapid, accurate, precise and stability indicating as no interfering peaks at the retention time of Bilastine were observed. The developed method is therefore suitable for purpose in quality-control laboratories for quantitative analysis of drug in pharmaceutical dosage form, as it is simple and rapid with tremendous precision and accuracy.

Keywords: Bilastine, UPLC, Stability Studies, Robustness, Linearity.

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MATERIALS AND METHODS

Chemicals and Reagents

Bilastine API was obtained as gift sample from Symed Labs Limited, Hyderabad, Telangana. HPLC grade Methanol, HPLC grade Acetonitrile were purchased from Thermo Fischer Scientific, Hyderabad, Telangana, India. Analytical grade chemicals and buffers were used. Bilafav, the tablet dosage form of strength 20mg was obtained from local pharmacy.

Instrumentation

Spectral measurement was performed using Nicolet Evolution 100 Vision Pro Version 1.06, UV Visible Spectrophotometer. UPLC experiment was carried out on Agilent Technologies, with PDA detector. Data collection and processing was done using Open Lab EZ Chrom software. pH meter of Global Digital and electronic balance of Mettler Toledo were used.

Identification of Drug

The identification as well as authentication of procured drug was done by melting point determination (open capillary method) and FT-IR Spectroscopy (Bilastine – KBr pellet was prepared and IR spectrum was recorded).

Selection of Wavelength

For the wavelength of maximum absorption (λ_{max}) of the drug, 10 μg/mL standard solution was scanned within the wavelength region of 200–400 nm against methanol as blank in UV Visible Spectrophotometer. From the

INTRODUCTION

Bilastine is a new drug in the category of Antihistamines, used for the treatment of allergic reactions like nasal congestion and urticaria. Chemically, it is 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl)ethyl)phenyl]-2-methylpropionic acid. It has a molecular formula of $C_{28}H_{37}N_3O_3$ and molecular mass of 463.61 g/mol. Solubility studies show that it is slightly soluble in water, acetonitrile and soluble in methanol. It is a second-generation histamine H1 receptor antagonist, acts by binding to the receptor, preventing its activation thereby reduces the development of allergic symptoms.¹

The present work is aimed to develop a simple, accurate and robust stability indicating method with acceptable retention time in quantitative determination of Bilastine in tablets, to provide a practical approach for stability testing using UPLC to support the in process and stability analysis of Bilastine as very few researches are available in analytical domain.



absorption curve, 248nm is selected as working wavelength as maximum absorption is observed at this wavelength.

Chromatographic Conditions

The chromatographic separation was performed using Phenomenex C8 column (50x2.1mm ID 1.7µm). The mobile phase used for analysis was Sodium Phosphate 10mM Buffer (pH adjusted to 3.5 by ortho-phosphoric acid) : Methanol : Acetonitrile (60 : 30 : 10 v/v/v) in isocratic elution mode while maintaining the column temperature at 30°C. The flow rate was maintained at 0.5 mL/min, injection volume - 20µL and the run time was 5 min. The retention time of Bilastine was found to be 1.19 min. A typical chromatogram of Bilastine is shown in figure 1.

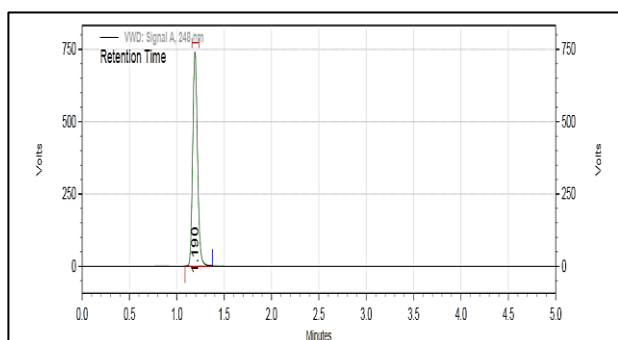


Figure 1: UPLC - Chromatogram of Bilastine

Method Validation

System Suitability

For assessing system suitability, injections of standard solutions of 100µg/mL were given for six times and chromatograms were observed. Parameters like plate number (N), tailing factor (K), retention time and peak area were calculated.

Specificity

Specificity is the degree to which the method applies to a single analyte; checked in each analysis by examining blank matrix samples for any interfering peaks. Blank and Placebo solutions were prepared, injected and the chromatograms were recorded for both the solutions.

Linearity

Linearity was demonstrated from five different concentration levels, in the range of 50 - 150 µg/ml. The calibration curve was obtained by plotting peak area v/s concentration.

Accuracy

Accuracy for the developed method was determined by Recovery Studies. To the preanalyzed sample, the reference standards of the drug i.e., 50µg/mL, 100µg/mL and 150µg/mL were added at the level of 50%, 100%, 150% respectively. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for the drug.

Precision

Intraday precision and Inter-day precision were evaluated by carrying out 6 independent sample preparations of 100µg/ml.

Limit of Detection

LOD of an analytical procedure is the lowest amount of analyte that can be detected but not necessarily quantified. The standard deviation and response of the slope are estimated from calibration curve of the analyte.

$$LOD = \frac{3.3\sigma}{S}$$

Limit of Quantitation

LOQ can be defined as the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope are estimated from calibration curve of the analyte.

$$LOQ = \frac{10\sigma}{S}$$

Robustness

The robustness of the assay method was established by introducing small changes in the chromatographic condition which included flow rate (0.4 and 0.6 mL/min), temperature (25°C and 35°C) and composition of organic phase - Buffer : Methanol : Acetonitrile (60 : 25 : 15 v/v/v and 60 : 35 : 5 v/v/v).

Assay

Preparation of Standard Solution

For the preparation of standard solution, 100 mg of Bilastine was taken in a 100 ml volumetric flask, dissolved in mobile phase and volume was made up to the mark with the same. From this solution 100 µg /ml of solution was prepared by diluting 1ml to 10ml with mobile phase.

Preparation of Sample Solution

Sample name: BILAFAV 20mg

Weigh 20 tablets and crush with mortar and pestle, then weigh a quantity of powder equivalent to 100mg of Bilastine and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 100 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

Forced Degradation Studies

Preparation of stock solution

Accurately weigh 10 mg of Bilastine in 10 ml of volumetric flask then add small quantity of diluent and dissolve it. Finally make the volume up to the mark with diluent and filter 1 ml of filtered stock solution was transferred to 10ml of volumetric flask and made up with diluent. (100µg/ml of Bilastine)

Acidic degradation

To 1 ml of stock solution of Bilastine, 1 ml of 5N HCl was added and solution was kept for 4hrs at 60°C. The resultant solution was neutralized by 1ml of 5N NaOH and then diluted to obtain 10µg/ml solution of drug. The solution was cooled, filtered with 0.45µm membrane filter and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkaline degradation

To 1 ml of stock solution of Bilastine, 1 ml of 5N NaOH was added and solution was kept for 4hrs at 60°C. The resultant solution was neutralized by 1ml of 5N HCl and then diluted to obtain 10µg/ml solution of drug. The solution was cooled, filtered with 0.45µm membrane filter and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative Degradation

To 1 ml of stock solution of Bilastine, 1 ml of 30% hydrogen peroxide was added. The solution was kept on benchtop for 5hrs. The resultant solution was diluted to obtain 10µg/ml solution of drug and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolytic Degradation

To study the effect of photolysis, 5 ml of stock solution was exposed to UV light at 1.2 million lux-hours for 4hrs in photostability chamber. 1ml of the resultant solution was diluted to obtain 10µg/ml solution of drug and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal Degradation

The drug solution was placed in oven at 105°C for 24hrs to study dry heat degradation. 1ml of the resultant solution was diluted to obtain 10µg/ml solution of drug and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

Identification of API

Identification and authentication of the procured drug was done by melting point determination and FT-IR Spectroscopy. The IR spectrum of Bilastine API is shown in figure 2.

Melting point of Bilastine was observed in the range of 200 – 201°C which is similar to the reported value (200.3 °C).

The sample spectrum matches with the reference spectrum and the practical value of melting point also matches with the theoretical value. This confirms the purity of Bilastine sample.

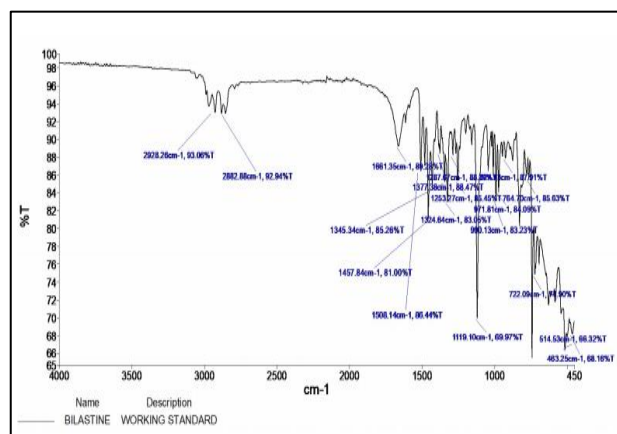


Figure 2: IR Spectrum of Bilastine API

System Suitability

The % Relative Standard Deviation for the retention times and peak area of Bilastine were found to be less than 2%. The plate count and tailing factor results were found to be well within the limit. Results of System Suitability are tabulated in table 1.

Table 1: Results of System Suitability, Accuracy, Precision

	Injection	RT (min)	Peak area	
System Suitability	Mean	1.189	415908512	
	%RSD	0.11	0.01	
Accuracy	Recovery Level	%RSD	%Recovery	
	50%	0.17%	100.52	
	100%	0.46%	99.58	
	150%	0.31%	99.82	
Precision	Intraday assay	% Obtained		% RSD
		98.56	98.25	
	Interday assay	% Obtained		% RSD
		98.90	98.82	

Specificity

Chromatograms of blank and placebo solutions showed no peaks at the retention time of Bilastine and it was observed that diluent or excipient peaks do not interfere with the Bilastine peak. The chromatogram of placebo is shown in figure 3.

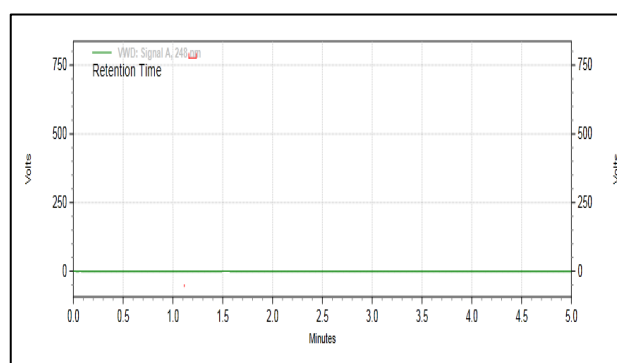


Figure 3: Chromatogram of Placebo

Linearity and Range

The calibration plot was linear over the concentration range as shown in figure 4. Correlation coefficient R^2 was found to be 0.9992 across the concentration range of 50 - 150 $\mu\text{g/mL}$. Results are tabulated in table 2.

Table 2: Results of Linearity

S. No	Concentration ($\mu\text{g/mL}$)	Area
1	50	19061043
2	80	33056236
3	100	44024641
4	120	54032671
5	150	70024268

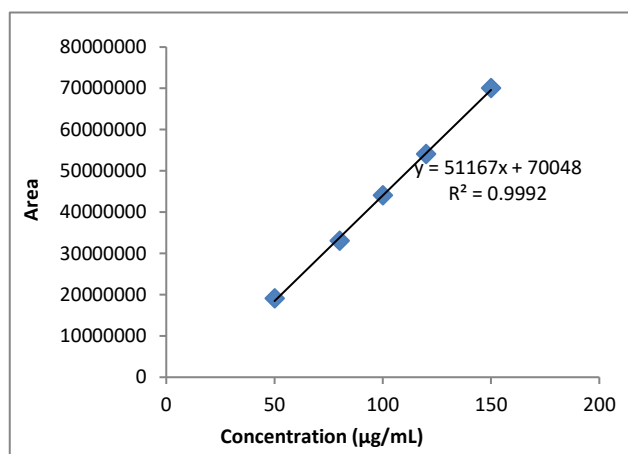


Figure 4: Calibration Curve for Linearity

Accuracy

Accuracy of the proposed method was assessed by performing Recovery Studies. The percentage mean recovery of Bilastine was found to be 99.97% and %RSD was found to be less than 2. Results of Accuracy are shown in table 1.

Precision

Intraday precision and inter-day precision were evaluated by carrying out six independent sample preparations (100 $\mu\text{g/mL}$ concentration). %RSD were found to be less than 2%. Results of Precision are shown in table 1.

Limit of Detection and Limit of Quantitation

The LOD and LOQ which produced requisite precision and accuracy were found to be 0.368 $\mu\text{g/mL}$ and 1.117 $\mu\text{g/mL}$ respectively.

$$\text{LOD} = \frac{3.3 \times 6112}{51167} = 0.368 \mu\text{g/mL}$$

$$\text{LOQ} = \frac{10 \times 6112}{51167} = 1.117 \mu\text{g/mL}$$

Robustness

Robustness was assessed by making deliberate variations in method parameters of flow rate, temperature and composition of organic phase. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of Bilastine. The results are shown in table 3.

Table 3: Results of Robustness

Chromatographic Changes			RT (min)	Peak Area
Flow rate	- 1 mL/min	0.4 mL/min	1.473	41906763
	+ 1 mL/min	0.6 mL/min	0.973	41579667
				%RSD = 0.55
Column Temperature	- 5°C	25°C	1.143	41553243
	+ 5°C	35°C	1.137	41534121
				%RSD = 0.03
Organic Phase (Methanol : ACN)	- 5; + 5(v/v)	25 : 15 (v/v)	0.933	41572364
	+ 5; - 5(v/v)	35 : 5 (v/v)	1.503	41580278
				%RSD = 0.39

Assay

The tablet formulation under the brand name Bilafav of strength 20mg was used for assay. The procedure was repeated for 5 injections. The drug content was estimated to be 100.4%

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

Stability Studies

Drug sample of Bilastine was subjected to stress conditions and it was found that maximum degradation was observed in peroxide hydrolysis and minimum degradation was observed in photolytic degradation. Results of Stability Studies/ Forced Degradation Studies are shown in table 4.

Table 4: Degradation data of Bilastine

Degradation Condition	Peak Area	% Recovery	% Degradation
Unstressed Sample	42609769	100	-
Acid Hydrolysis	41118427	96.5	3.5
Alkaline Hydrolysis	41800183	98.1	1.9
Peroxide Hydrolysis	40266231	94.5	5.5
Thermal	42268890	99.2	0.8
Photolytic	42354111	99.4	0.6



CONCLUSION

A simple, fast, accurate and precise stability indicating UPLC analytical method has been developed and validated for the quantitative analysis of Bilastine in bulk drugs and tablet dosage forms. The analytical method was found to be stable and sensitive, without any degradation products. Forced degradation studies were carried under various stress conditions and it was observed that under peroxide hydrolysis condition, the degree of degradation of Bilastine was higher i.e., 5.5% and under photolytic degradation condition, the degree of degradation of Bilastine was found to be as low as 0.6%. From the above experimental results and parameters it was concluded that, the newly developed method for estimation of Bilastine was found to be simple, fast, precise, accurate sensitive and reproducible and hence it can be effectively proposed that the developed method can be applied for the analysis of routine quality control samples and samples obtained from stability studies.

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