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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BILASTINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UPLC

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ABSTRACT

This paper describes a new validated Ultra Performance Liquid Chromatography (HPLC) method for the determination of Bilastine in bulk and pharmaceutical dosage form. The objective of the study is to develop a rapid, precise, accurate, robust UPLC method for the determination of Bilastine with acceptable retention time. The mobile phase consists of pH 3.5 Sodium Phosphate 10mM Buffer : Methanol : Acetonitrile (60 : 30 : 10 v/v/v) acetonitrile at a flow rate of 0.5 ml/min, with a PDA detector at 248 nm. Separation was achieved on a Phenomenex C8 column (1.7 µm; 50 mm × 2.1 mm ID) maintained at 30 °C temperature in a column oven. The method was linear between 50µg/mL – 150µg/mL concentration range. The limit of detection was 0.368µg/mL and the limit of quantification was 1.117µg/mL. The developed UPLC method achieved good precision and accuracy; suitable to be used for routine analysis of Bilastine.

KEYWORDS: UPLC, Bilastine, Method development, Robustness.

INTRODUCTION

Bilastine is a novel second generation histamine H1 receptor antagonist, used for the treatment of allergic reactions like nasal congestion and urticaria. IUPAC name is 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl)ethyl)phenyl]-2-methylpropionic acid with $C_{28}H_{37}N_3O_3$ molecular formula and 463.61 g/mol molecular weight. Histamine mediates hypersensitivity and allergic responses, which is released by mast cells upon degranulation. Bilastine acts by binding to the histamine H1 receptor, preventing its activation thereby reduce the development of allergic symptoms.

Solubility studies show that it is slightly soluble in water, acetonitrile, ethanol, soluble in methanol, and practically insoluble in dichloromethane. It's molecular structure is shown in figure 1.

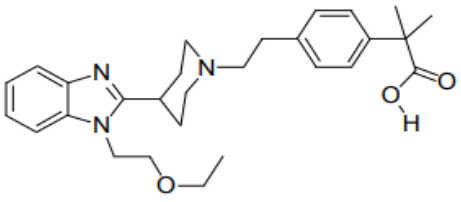


Fig 1: Molecular Structure of Bilastine.

EXPERIMENTAL

Chemicals and Reagents

Bilastine API was obtained as gift samples from Symed Labs Limited, Hyderabad. HPLC grade Methanol, HPLC grade Acetonitrile was purchased for Thermo Fischer Scientific, Hyderabad, India. Chemicals used were of analytical grade. The tablet dosage form under the brand name Bilafav of strength 20mg was obtained from local pharmacy.

Instrumentation

An Agilent Technologies Ultra Performance Liquid Chromatography (UPLC) instrument, auto sampler with PDA detector and Phenomenex C8 (50 x 2.1 mm I.D., 1.7µm) column was used. The UPLC system was operational with Open Lab EZChrom software. Spectral measurement was performed using PG Instrumentation Ltd., UV Visible Spectrophotometer.

Preparation of Solutions

Preparation of Standard Stock Solution:- Weigh accurately 10 mg of Bilastine, transfer into 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase to obtain 100 µg/mL solution.

Preparation of Sodium Phosphate (Na_3PO_4) 10mM buffer pH 3.5:- Accurately weigh 0.78 g of sodium

dihydrogen phosphate and dissolve in 900ml of HPLC water, adjust to pH 3.5 with orthophosphoric acid, dilute to 1000 ml with HPLC water.

Preparation of Sample Solution for Assay:- 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.

Selection of Wavelength:- From the above stock solution, 10 µg/mL solution was prepared and scanned in UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The UV Spectrum shows characteristic absorption maxima at 248nm for Bilastine, which was selected as working wavelength for the UPLC chromatographic method as shown in figure 2.

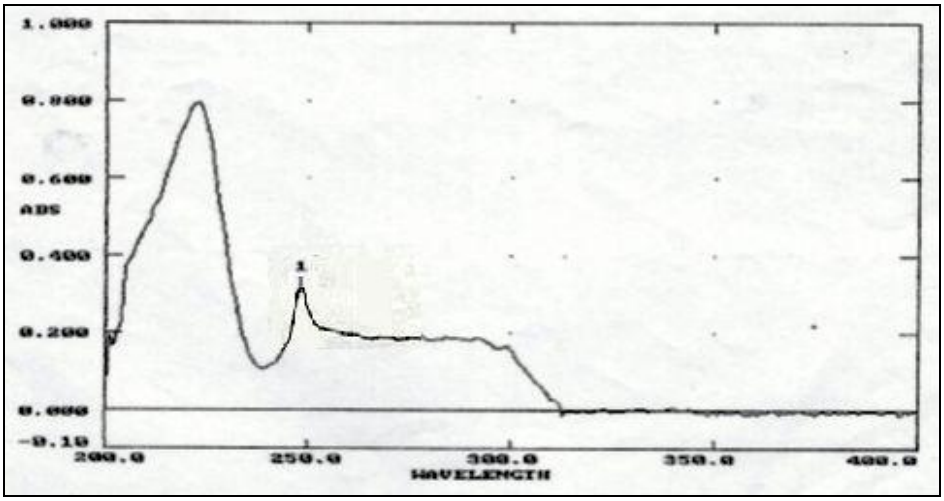


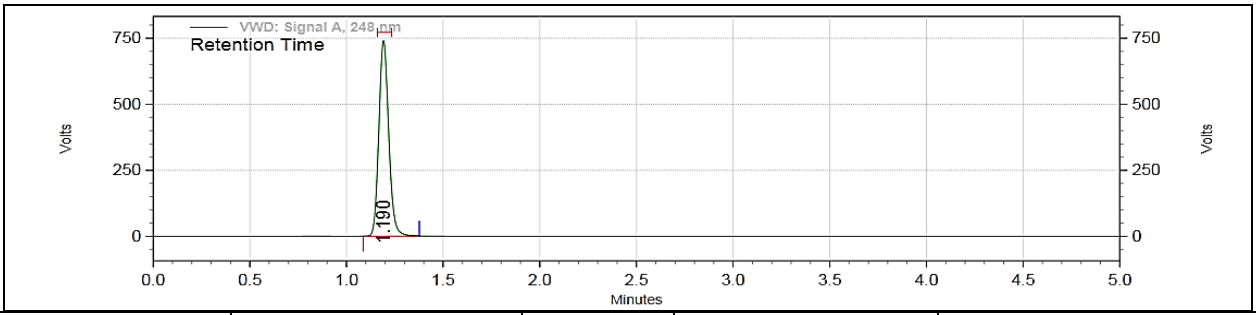
Fig.2: UV Visible Spectrum of Bilastine at 248nm.

Chromatographic Conditions

The analysis was carried out on Phenomenex C8 column (50 x 2.1mm ID, 1.7µm), the mobile phase containing pH 3.5 Sodium Phosphate 10mM Buffer (adjusted by ortho-phosphoric acid): Methanol : Acetonitrile (60 : 30 : 10 v/v/v) with isocratic elution mode and the chromatogram is given in figure 3.

Table1: Optimized Chromatographic Conditions.

Mobile Phase	**pH 3.5 Sodium Phosphate 10mM Buffer : Methanol : Acetonitrile (60 : 30 : 10 v/v/v)**
Column	Phenomenex C8 (50x2.1mm ID) 1.7µm
Flow rate	0.5 mL/min
Elution mode	Isocratic
Column Temperature	30°C
Injection Volume	20µL
Run Time	5min



Compound Name	**Retention Time (min)**	**Peak Area**	**Tailing Factor (TF)**	**Theoretical Plates (TP)**
Bilastine	1.190	41378525	1.2	4552

Fig. 3. A typical chromatogram of Bilastine.

METHOD

Validation

The developed method has been validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines.

System Suitability

Injections of standard solutions of 100µg/mL were given for six times and chromatograms were recorded. Parameters like plate number (N), tailing factor (K), retention time and peak area were calculated. The results of system suitability parameters were given in table 2.'

Specificity

The specificity of the method was evaluated with regard to interference due to presence of any other placebos. Blank and Placebo solutions were prepared, injected and the chromatograms were recorded for both the solutions. Chromatograms are presented in figures 4 and 5.

Linearity

The linearity of the method was determined by preparing five different concentrations of Bilastine in the concentration range of 50 - 150 µg/ml. The calibration curves were obtained by plotting peak area versus concentration as shown in Fig. 5. The responses from the injections are tabulated in table 3.

Accuracy

To check the degree of accuracy of the method, Recovery Studies were performed by standard addition method at 50%, 100% and 150% in triplicate. Known amounts of standard mixture of Bilastine were added to the preanalyzed sample, and were subjected to the proposed UPLC method. Results of recovery studies are shown in table 4.

Precision (Method Precision)

Method precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It was determined by injecting sample solutions of concentration 100 µg/mL for six times. Precision data is shown in table 5.

Limit of Detection and Limit of Quantitation

The detection limit is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The quantitation limit is defined as the lowest concentration of the substance that can be quantified with acceptable precision and accuracy.

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

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

The standard deviation and response of the slope are estimated form calibration curve of the analyte.

Robustness

To evaluate the robustness of the developed UPLC method, small deliberate variations in the optimized method parameters were done. The effect of ±1 mL/min in flow rate, ±5°C column temperature on the retention time and area were studied. The results of robustness were tabulated in table 7.

Ruggedness

The degree of reproducibility of the test results was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. The results of ruggedness were shown in table 8.

RESULTS AND DISCUSSION

System Suitability

The % RSD 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 satisfactory and within the limit.

Table 2: System Suitability Parameters for Bilastine.

Parameter	Bilastine	Acceptance Criteria
Retention time	1.189 min	For information
Plate count	4578	≥ 2000
Tailing factor	1.2	≤ 2
% RSD of Peak area	0.01	≤ 2

Specificity

Chromatograms of blank and placebo solutions had shown no peaks at the retention time of Bilastine; diluent or excipient peaks do not interfere with the Bilastine peak.

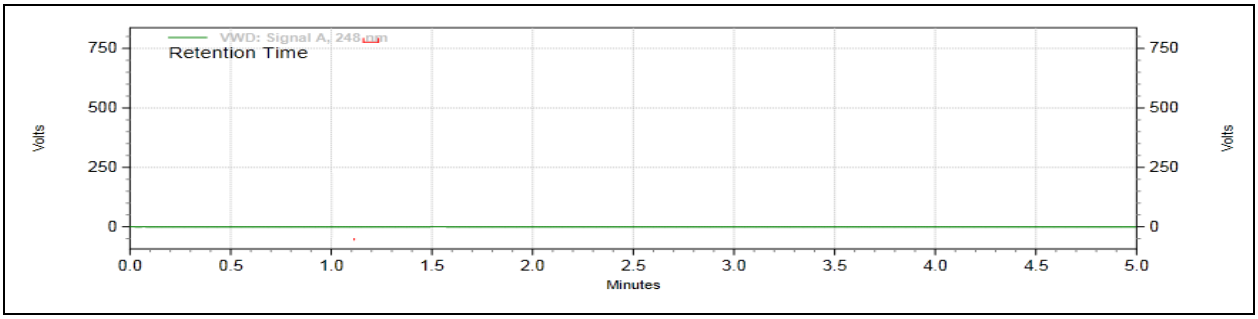


Fig. 4. Chromatogram of Bilastine for Blank.

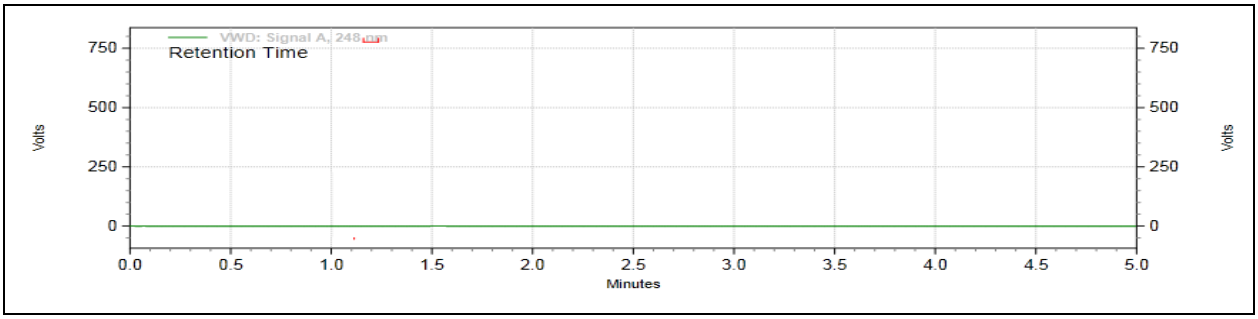


Fig. 5. Chromatogram of Bilastine for Placebo.

Linearity and Range

The calibration plot was linear over the concentration range. Correlation coefficient R^2 was found to be 0.999 with %RSD values ≤ 2.0 across the concentration range studied, was obtained from regression analysis.

Table 3: Linearity data.

S. No	Concentration (µg/mL)	Peak Area
1	50	19072054
2	80	33067347
3	100	44035752
4	120	54043782
5	150	70035278

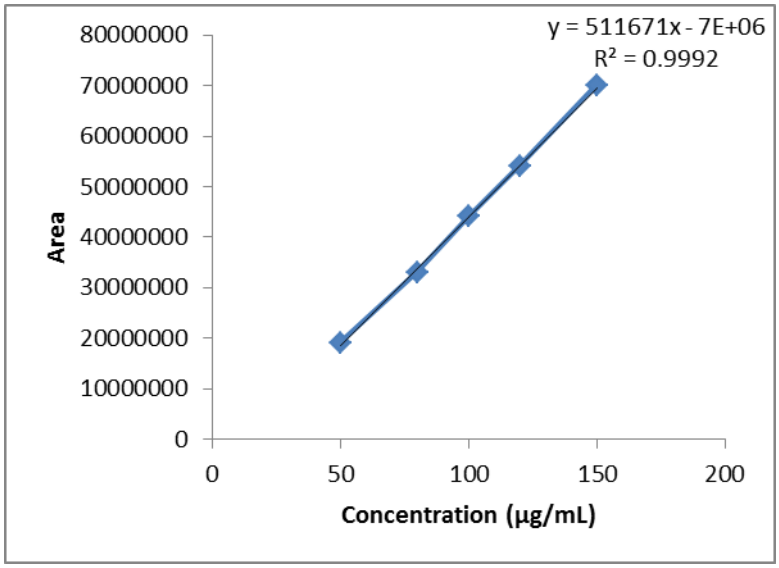


Fig. 5. Calibration curve of Bilastine.

Accuracy

Accuracy of the method was determined by Recovery Studies. The percentage mean recovery of Bilastine was found to be 99.97% and %RSD was found to be less than 2.

Table 4: Results of Recovery Studies.

Recovery level	Amount added (µg/mL)	Amount found (µg/mL)	Area	%RSD	%Recovery	% Mean Recovery
50%	25	75.13	1907557	0.17	100.52	99.97
100%	50	99.79	4590044	0.46	99.58	
150%	75	124.86	7018016	0.31	99.82	

Method Precision

The % RSD for 6 sample determinations of Retention time and Peak area of Bilastine was found to be less than 2% which satisfies the acceptance criteria.

Table 5: Results of Precision.

S. No	Retention Time (RT)	Area
1	1.137	41524111
2	1.143	41524112
3	1.143	41575241
4	1.140	41584722
5	1.143	41557445
6	1.137	41635221
Average	1.14	41566808
S.D	0.0029	41966.685
%RSD	0.26	0.10
Concentration used is 100 µg/mL		

Limit of Detection and Limit of Quantitation

The LOD and LOQ that produced requisite precision and accuracy were found to be 0.368µg/mL and 1.117µg/mL

$$LOD = \frac{3.3 \times 6112}{51167}$$

$$LOQ = \frac{10 \times 6112}{51167}$$

Table 6: LOD and LOQ of Bilastine.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Bilastine	0.368	1.117

Robustness

The results for robustness were in favor of (% RSD < 2%) the developed UPLC method for the analysis of Bilastine.

Table 7: Results of Robustness.

Chromatographic Changes			RT (min)	Peak Area	TF	TP
Flow rate	− 1 mL/min	0.4 mL/min	1.473	41906763	1.0	4942
	+ 1 mL/min	0.6 mL/min	0.973	41579667	1.3	4247
				%RSD = 0.55		
Column Temperature	− 5°C	25°C	1.143	41553243	1.4	4467
	+ 5°C	35°C	1.137	41534121	1.1	4496
				%RSD = 0.03		

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Table 8: Results for Ruggedness.

	Analyst 1		Analyst 2	
	Standard	**Sample**	**Standard**	**Sample**
% Obtained	99.90	98.56	99.82	98.25
% RSD	0.06		0.22	

Assay

The assay procedure was repeated for 5 injections. The drug content was estimated to be 100.4%; the results of tablet dosage form.

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

Table 9: Result for Assay.

Drug	Brand name	Label claim (mg)	Mean standard area	Mean sample area	% Assay
Bilastine	Bilafav	20	54223207	54491918	100.4

CONCLUSION

A simple, fast, accurate and precise UPLC analytical method has been developed and validated for the quantitative analysis of Bilastine in bulk and pharmaceutical dosage form. The results obtained show the developed method to be cost effective, rapid (shorter retention time), simple, accurate (the value of %RSD less than 2), precise and can be successfully employed in the routine analysis of the drug in bulk and tablet dosage form. The simplicity and reproducibility of the proposed method fulfills the objective of this research work.

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