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New RP-HPLC Method for Bilastine Estimation in Pharmaceutical and Bulk Dose Form

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KEYWORDS

Anti-histaminic, Bilastine, RP-HPLC, Accuracy, Precision.

ABSTRACT:

Bilastine is a frequently prescribed H1-antihistaminic that is authorized for the safe and efficient symptomatic management of allergic conditions, such as urticaria and rhino conjunctivitis. For the quick and precise measurement of bilastine, an Agilent Technologies LC compact 1120 module, a Shimadzu UV-1700 detector with a 20µl injection volume, and an xBridge TM C18 column were used in developing the validated Reverse Phase HPLC method. The procedure used a mobile phase made up of a 60:40, v/v% combination of methanol, 10 mM potassium dihydrogen phosphate at pH 3.5. By using the working standard solution, 10 µg/ml, satisfactory linearity shown over 5–30 µg/ml concentration range (correlation coefficient: 0.9924). Detection was done at 275 nm with a five-minute reaction time and the flow rate was maintained at 1.0 ml/min. The precision, accuracy, linearity, and robustness of the approach were confirmed by statistical validation in compliance with ICH criteria. This implies that the method for figuring out the tablet formulations is suitable.

1. Introduction

For the patients those who belong to 12 years or more then that age, bilastine which is a H1-antihistamine, is licensed to heal the various allergic disorders and severe urticaria. Allergy rhinitis is a common condition in European nations, affecting about 18% of the population[1]. According to report 10–40% of adults as well as children in US suffer from allergic rhinitis, with prevalence rates varied depending on age group[2]. Sneezing, nasal congestion, itching, and watery eyes in cases of allergic rhinitis, and hives or welts in cases of chronic urticarial disease are among the major symptoms caused by these illnesses. Acute urticaria affects about 20% of people in their lives, but chronic urticaria (CU) affects 1.8% of people[3].

Acute and chronic urticaria can both lower productivity and lower quality of life, despite their distinctions[4,5,6]. Fortunately, antihistamine therapy usually works wonders for both kinds. Second-generation antihistamines are advised first line of treatment in both acute and chronic urticaria, in accordance with current international guidelines. These medications effectively relieve symptoms like itching and hives while minimizing sedation, improving symptom control and overall quality of life for patients[7,8]. 2-[4-(2-{4-[1-(2-ethoxy ethyl)-1H-1,3-benzimidazol-2-yl]piperidin-1-yl}ethyl) phenyl]2-methyl propanoic acid, the chemical name of bilastine. The chemical formula of bilastine is $C_{28}H_{37}N_3O_3$ and molecular mass is 463.61g/mole, and the melting point is higher than 195^0C. Bilastine is a white crystalline powder[9]. The literature shows that there are limited methods available for estimating bilastine. These methods include LC-MS/MS[10], HPLC-fluorescence[11], RP-HPLC[12,13], HILIC[14], and UV-spectrophotometry[15,16].

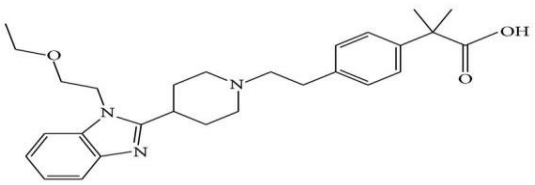

Figure 1: Chemical Structure of Bilastine[17]

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2. Methods

Instrumentation: Chromatographic study of bilastine was done by HPLC Agilent Technologies (LC compact 1120) module with UV detector.

Chromatographic conditions: After screening various combinations of mobile phases, chromatographic separation was conducted using an HPLC Agilent Technologies (LC compact 1120) module, coupled with a variable wavelength UV detector Shimadzu-UV 1700 module and a manual sampler with a 20µl injection volume. The C18 column of 5µ, 250 mm x 4.6 mm, was taken to produce separation, and EZ Chrome Elite software facilitated data collecting, validation, and storage. A UV detector was used to detect the optimized mobile phase, which included methanol and potassium dihydrogen phosphate at 60:40% (v/v) ratio. Flow rate was maintained at 1.0 ml/min.

Chemicals and Reagents: For regular analysis, a variety of chemicals were utilized, including potassium dihydrogen phosphate, methanol, ortho phosphoric acid which all are HPLC grade.

Preparation of mobile phase: The mobile phase was created by adding 1.36086 grams of potassium dihydrogen phosphate and 300 ml of water which was composed of methanol and potassium dihydrogen phosphate at a ratio of 60:40, v/v%. Orthophosphoric acid was used to bring the pH down to 3.5, and then added water up to the volume of 1000 ml. Following 10-minute degassing period in an ultrasonic water bath, the mixture was vacuum-filtered via a 0.22 µm filtration.

Preparation of the standard solution: Bilastine then dissolved in 50 ml of volumetric flask with methanol serving as the diluent to prepare standard stock solution of 1 mg/ml. After degassing for ten minutes in an ultrasonic water bath, the mixture was vacuum filtered via a 0.22 µm filtration.

Preparation of working standard solution: The aforesaid solution was diluted using HPLC-grade water as the diluent to prepare working standard solution of concentration 10 µg/ml.

Preparation of sample stock solution: 0.05 gram of Bilastine were taken in 50 ml of volumetric flask using HPLC-grade water as the diluent to prepare sample stock solution of concentration 1 mg/ml. After degassing for ten minutes in an ultrasonic water bath, the mixture was vacuum filtered via a 0.22 µm filtration.

Method Validation: According to ICH criteria, the developed technique for estimating bilastine has been verified, considering parameters like system compatibility, robustness, limits of detection and quantification (LOD & LOQ), specificity, selectivity, linearity, accuracy, and precision.

1. Precision: Through the analysis of numerous samples from a homogeneous sample, the analytical method's precision was evaluated. Peak area and peak symmetry characteristics were measured for reproducibility within the day, three times each and the intermediate precision of two days at one concentration level to verify reproducibility of the analysis. Data obtained from the six injections were expressed as a percentage of RSD both within and between trial days[18].

2. Linearity: Concentrations from 5-30 µg/ml of bilastine were analyzed to determine the linearity of the method, and corresponding areas were graphically displayed[19].

3. Accuracy: To assess the accuracy of the devised approach, a recovery of bilastine was conducted. This required applying conventional addition approach to ascertain the bilastine recoveries. Bilastine standard solutions of 80%, 100% and 120% concentrations were combined with pre-quantified sample solutions containing 20µg/ml in known volumes. A calibration curve was then used to determine the amount of bilastine that had been recovered[20].

4. Robustness: By purposefully adjusting parameters including flow rate and detecting wavelength, the robustness of the approach was assessed[21].

5. Limit of detection and limit of quantification: LOD and LOQ were computed using a signal-to-noise ratio of 3:1 for the LOD and 10:1 for the LOQ in accordance with ICH recommendations[22].

Statistical analysis: The Windows operating system's Microsoft Excel 2010 software was utilized to calculate the linearity results through the application of linear regression. Next, the percentage RSD for each value was determined.

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3. Results

Using C18 column and the mobile phase made up of a 60:40, v/v% combination of methanol, potassium dihydrogen phosphate in the ratio of 60:40. The chromatographic separation was effectively done. Flow rate was maintained at 1.0 ml/min throughout the investigation, and it was found that 275 nm was the ideal wavelength for detecting the analyte.

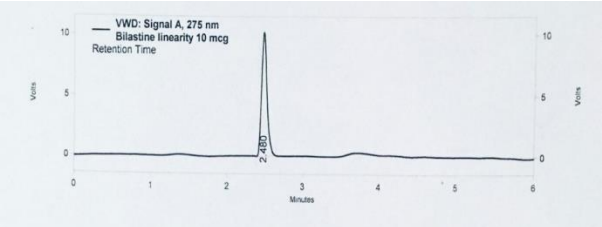

Figure 2: Chromatogram for Bilastine

Specificity & selectivity

The selectivity test was conducted to assess the method's ability to differentiate the analyte from excipients. Standard solution of bilastine, commercial product solution, and blank solutions were sequentially run in the instrument. Results indicated that no detectable signal was produced by components other than the drug bilastine, as depicted in figure 4.

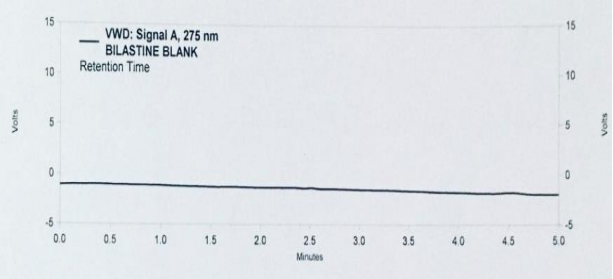

Figure 3: Specificity chromatogram of blank

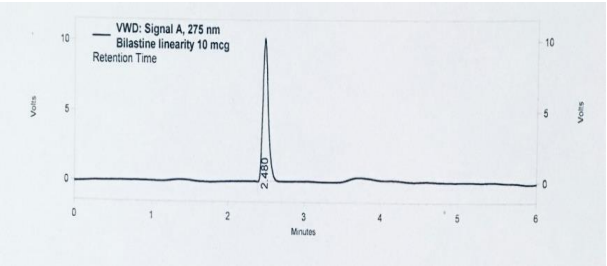

Figure 4: Specificity chromatogram of sample

Linearity

To achieve final concentrations of 5-30 µg/ml, multiple aliquots of standard stock solution of bilastine were poured into volumetric flasks of 10 ml and diluted using the mobile phase. Calibration graph for each chemical was prepared by scheming the obtained peak regions against the appropriate concentrations. The obtained linearity regression coefficient (R^2) values were found to be 0.9924. The linearity equation derived for bilastine was y = 49078x + 205677. Figure 6 illustrates the linearity graphs for bilastine.

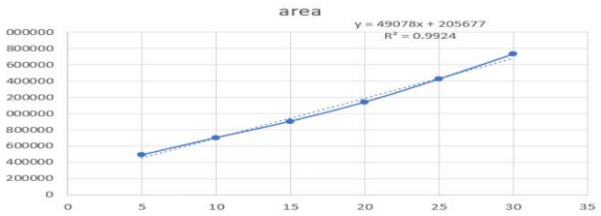

Figure 5: Calibration curve of bilastine

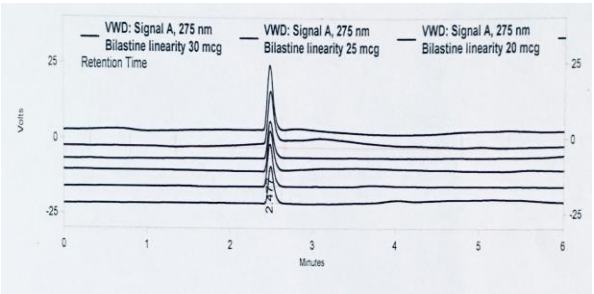

Figure 6: Linearity Chromatogram of bilastine

System suitability testing results

Table 1: System suitability testing

Theoretical plates (USP)	Capacity factor	Asymmetry (Tailing factor)	S/N (6 σ)
5896	0.00115	1.42869	113.177559

Accuracy

To assess the accuracy of the devised approach, a recovery of bilastine was conducted. This required applying conventional addition approach to ascertain the

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bilastine recoveries. Bilastine standard solutions of 80%, 100% and 120% concentrations were combined with pre-quantified sample solutions containing 20µg/ml in known volumes. A calibration curve was then used to determine the amount of bilastine, and table 2 shows the mean percentage recovery data.

Table 2: Observed data for accuracy

SL. NO	Level in %	Mean Peak area	% Recovery	% Mean Recovery	% RSD
1	80%	980321	98.62	100.36	1.22%
		1003704	101.62		
		997698	100.86		
2	100%	1194786	100.76	100.34	0.41%
		1203921	101.69		
		1202671	101.57		
3	120%	1392649	100.77	100.60	0.76%
		1400074	101.40		
		1379231	99.63		

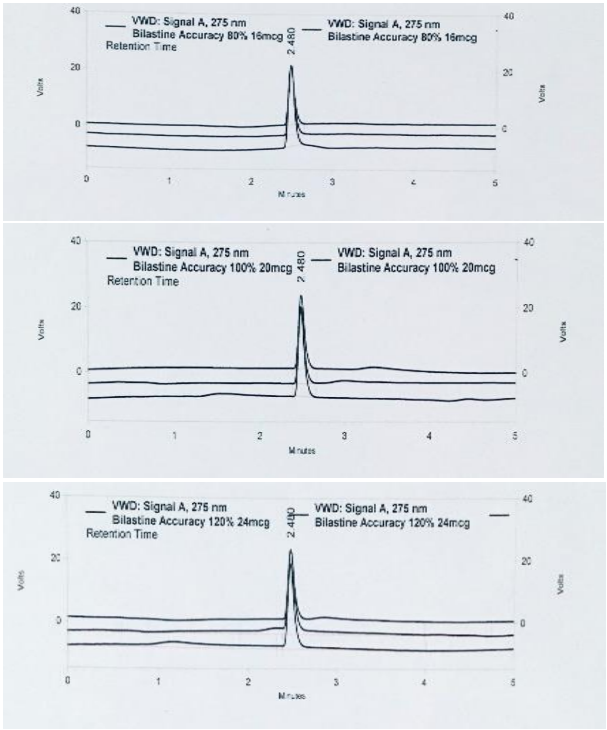

Figure 7: Chromatogram for accuracy 80%, 100%, 120%.

Precision

Through the analysis of numerous samples from a homogeneous sample, the precision of the analytical approach was evaluated. Peak area and peak symmetry characteristics were measured for reproducibility within the day, three times each and the intermediate precision of two days at one concentration level to verify reproducibility of the analysis. Tables 3 and 4 show the results of the six injections that were given, which were expressed as a percentage of RSD both within and between trial days.

Table 3: Inter day precision

Serial number	Sample	Peak area	
		Day 1	Day 2
1	I	824128	803421
2	II	838633	829654
3	III	834696	815976
4	IV	818651	800547
5	V	817604	811321
6	VI	824342	821002
Average		826342.33	813653.5
SD		8547.0921	10930.09
%RSD		1.03%	1.34%

Table 4: Intraday precision

Serial Number	Sample	Peak area	
		Morning	Afternoon
1	I	794512	839100
2	II	787209	820773
3	III	793215	823365
4	IV	804531	845826
5	V	789213	843271
6	VI	797215	833354
Average		794315.833	834281.5
SD		6173.3475	10390.518
%RSD		0.78%	1.25%

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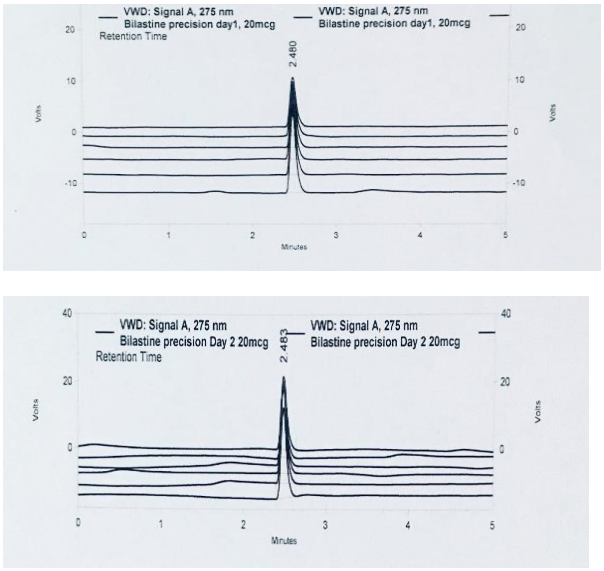

Figure 8: Chromatogram showing interday precision- Day 1 & Day 2

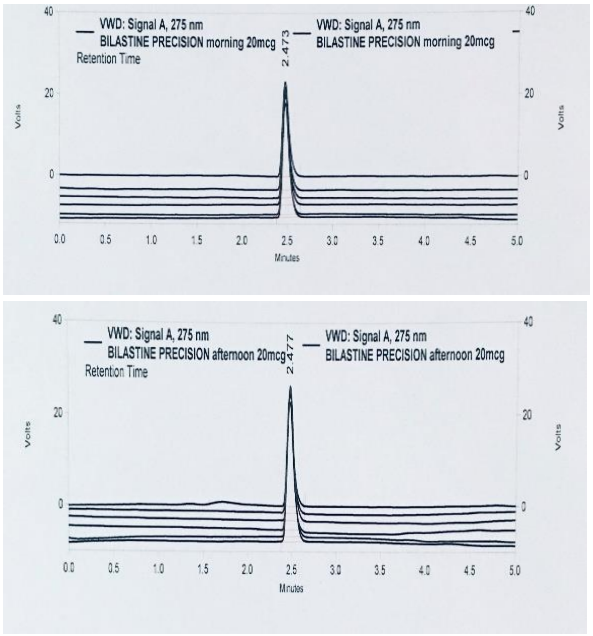

Figure 9: Chromatogram showing intraday precision- morning & afternoon

Robustness

Table 6 presents the results of the robustness investigation, which reveal that there was no significant change in the elution sequence or resolution for any of the components. The peak areas and RSD stay unchanged inside the 2.0% bound.

Table 5: Observed data of robustness

Serial number	Parameter	Conditions	Retention time (mins)	Peak area
1	Flow rate	0.9 ml	2.767	1798876
		1.1 ml	2.253	1719737
2	Detection wavelength	274 nm	2.483	1918606
		276 nm	2.483	1918606

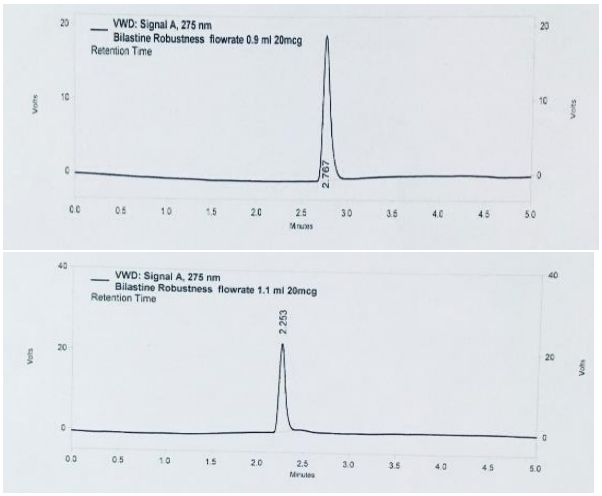

Figure 10: Chromatogram of flow rate 0.9 ml/min & flow rate 1.1 ml/min

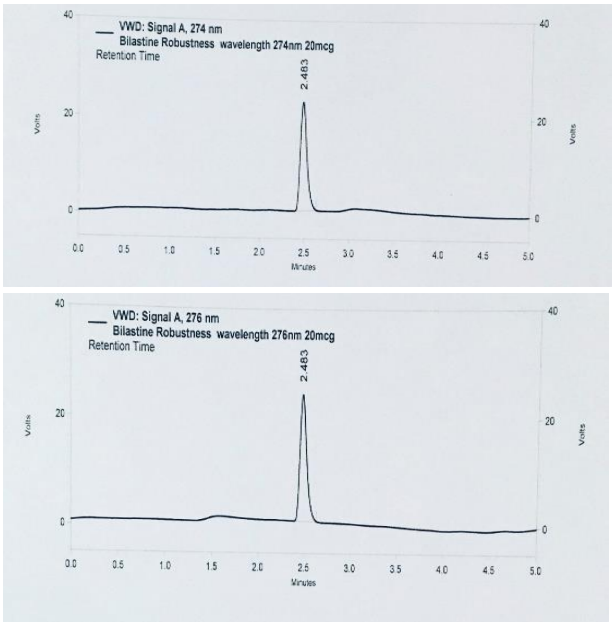

Figure 11: Chromatogram for wavelength 274 nm & 276nm

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Limit of detection and limit of quantification

It was found that the LOD and LOQ values were respectively, 0.8835 and 0.2650 µg/ml. The approach demonstrated linearity throughout the concentration range under investigation, according to the results of the regression analysis, making it appropriate for the detection and quantification of bilastine over a wide concentration range.

Parameters	**Bilastine**
Linearity range (µg/ml)	5-30
Regression equation	y=49078x + 205677
Slope	546839
Intercept	158464
Correlation coefficient	0.9924
Limit of detection (µg/ml)	0.2650
Limit of quantification (µg/ml)	0.8835

4. Discussion

In summary, the developed HPLC method for bilastine analysis exhibited robustness and precision, as indicated by the system suitability test and precision study results. The percentage RSD values fell between the approved 2% bounds for both intra- and inter-day intervals. Linearity analysis showed a high correlation coefficient ($R^2 = 0.9924$), meeting the required criteria. Accuracy assessment demonstrated satisfactory mean recovery values of approximately 100%, indicating minimal interference from matrix components. Deliberate adjustments to the flow rate and detection wavelength further demonstrated the robustness of the approach, and the fluctuations in retention times remained within reasonable bounds. In conclusion, It was found that the LOD and LOQ values were respectively, 0.8835 and 0.2650 µg/ml, satisfying the predetermined parameters.

5. Conclusion

Following ICH criteria, the established RP-HPLC technique for bilastine determination in this study demonstrated to be straightforward, sensitive, accurate, and exact. Acceptable linearity, robustness, accuracy, and precision were shown during validation. Without excipient interference, the approach demonstrated appropriateness for bilastine determination in both bulk and tablet dosage forms, suggesting its potential for regular pharmaceutical analysis.

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