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**ORIENTAL JOURNAL OF CHEMISTRY**

An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2023, Vol. 39, No.(3):
Pg. 736-745

# Design of Experiment Approach for Method Development and Validation of Bilastine in Pure and Pharmaceutical Dosage form using RP-UFLC

**L. SIVASANKER REDDY[1]*, R. NAGESWARA RAO[1], B. ISHAQ MOHAMMED[2], N. MADANA GOPAL[1], SHAIK MUNEER[1], Y. DASTAGIRI REDDY[3], D. CHINNA BABU[4] and K. SURENDRA[1]**

[1]Department of Pharmaceutical Analysis, Santhiram college of pharmacy, NH-40, Nandyal-518501, Kurnool, Anadhra Pradesh, India.
[2]Department of Pharmacy, University of technology and applied sciences, Al-Khuwair, Muscat, Sultanate of Oman.
[3]Department of Pharmaceutics, Santhiram college of pharmacy, NH-40, Nandyal-518501, Kurnool, Anadhra Pradesh, India.
[4]Department of Pharmaceutical Analysis, Narayana college of pharmacy, Nellore, Anadhra Pradesh, India.
*Corresponding author E-mails: shiva_s_rl@yahoo.co.in

http://dx.doi.org/10.13005/ojc/390325

(Received: May 29, 2023; Accepted: June 10, 2023)

**ABSTRACT**

Background: Bilastine is a H1 receptor antagonist, used in the treatment of allergic urticaria, seasonal rhinitis, etc. Few journals have reported the analytical related work on bilastine drugs. Objective: The objective of the work is to develop a simple, precise, rapid, and reproducible method using design of experiments (DOE) and check the optimized conditions when run on UFLC would give the best method or not. Results: The DOE software was used to select optimized conditions with minimal runs. The central composite design was the best fit, with two variables that include flow rate and column temperature. A total of 13 runs gave optimum conditions of 1.2 mL/min flow rate, column temperature of 40°C and mobile phasemethanol: buffer (pH 6.0) in the ratio of 70:30 in the binary mode using the Shimadzu C18 column on an HPLC instrument. The retention time of bilastine was found to be 5.126min, the number of theoretical plates and asymmetric factor being within the limit. The proposed method was validated as per the ICH Q2R1 guidelines. The linearity was found to be in the range of 1.25 µg/mL-10 µg/mL. The correlation coefficient was found to be within the limits i.e., $R^2$=0.999. The accuracy of the current method was being performed using the %recovery at three stages 50%, 100%, and 150% and was found to be 99.5126%, 100.2765% and 99.6714% respectively. The LOD and LOQ of bilastine was found to be 0.292 µg/mL and 0.974 µg/mL. Conclusion: The DOE software reduced the number of trials, saving both time and solvent consumption. This method can be conveniently used with confidence for regular assay, which is a simple, precise, rapid, and reproducible one for the estimation of bilastine in pure and pharmaceutical tablet dosage form using UFLC.

**Keywords:** Bilastine, $KH_2PO_4$, RP-UFLC, ICH Q2R1 guidelines, DOE and Validation parameters.

## INTRODUCTION

Bilastine is a new oral antihistaminic drug which is used for the treatment of seasonal allergic rhinitis and urticaria. The tablet dosage form of bilastine was available in 20 mg. The mechanism of action of bilastine is inhibition of the production of immune system reactions by binding on the peripheral $H^1$ receptors. Many but not all immune system reactions mediated by the release of histamine can be controlled by this drug. It has moderate to high affinity for the histamine $H^1$ receptors.

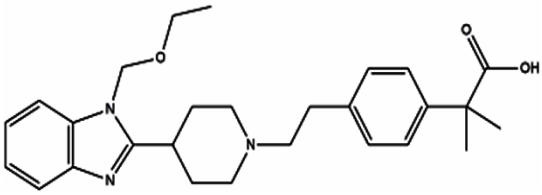


**Fig. 1. Bilastine molecular structure**

Quality by design is the trend in most of the fields of science and technology. Use of different software's which simulate the practical experiments has helped the scientific community to save time and money by minimizing the number of experiments in a project. In this study, we have used the design of experiments to run a few trials and obtain the optimum conditions to develop the method, validate the same for the drug bilastine in its pure and dosage form using high performance liquid chromatography. Many papers have published the use of such software's[1-4]. The method development and validation of the drug methotrexate using high performance liquid chromatography (HPLC) was established with the aid of design of experiments[5]. A monograph of the drug was retrieved from the "Indian Practitioner" Journal[6,7]. Very few methods were reported for the estimation of Bilastine drugs[8-12]. A few more drugs were also reported in the journals about their analytical development along with the usage of the quality by design software[13-15]. International Council for Harmonization (ICH Q2R1) guidelines were followed for method development and validation of the drug bilastine using HPLC[16]. Full factorial design for the development and validation of a RP-HPLC method for the estimation of letrozole in nano formulations was reported[17].

### Analytical method validation

An analytical procedure is the most important key in analytical method validation. Analytical procedures determine the characteristics of the drug product or drug substance. It also gives the acceptance criteria for the drug product or drug substance. The analytical method validation parameters include-Accuracy, Precision, Specificity, Linearity, Detection limit, Quantification limit, Robustness and Range.

## MATERIALS AND METHOD

### Materials

The drug bilastine was gifted from apcure labs, Hyderabad. Methanol and water for HPLC were bought from Merck chemicals. $KH_2PO_4$ was from SD fine chemical. The supplier of these chemicals is Bros Scientifics, Tirupati, Andhra Pradesh 517507. The Design of Experiment software was used for the prediction of optimal system suitability conditions. The entire project was done on Shimadzu UFLC (20AD), Column-C18, 4.6X 250mm, 5µm (Shimadzu Shim-Pack GIST). The filters used for the preparation of the mobile phase were Durapore 0.22µm, while the filters for the preparation ofthe sample were 0.45µm-both were manufactured by Millipore. The sonicator used was SONICA supplied by Spincotech Pvt.Ltd. The Lab solutions.

Software was used in the LC system. A trial version of Design of Experiment; File version:13.0.6.0, study type Response surface methodology, design ty2pe central composite design was used.

### Method

#### Preparation of $KH_2PO_4$ Buffer pH 6.0

A quantity of 8.5 g of $KH_2PO_4$ was weighed, transferred into a 1000 mL volumetric flask, a small volume of water was added to dissolve $KH_2PO_4$, and made up to the mark of the 1000 mL volumetric flask and adjusted the pH with 0.1N NaOH to obtain the pH at 6.0. Finally, the solution was filtered through a vacuum filter using 0.45µm membrane filter, then the solution was kept in a sonicator for 15 min to remove the dissolved gases.

#### Preparation of Mobile phase:

To prepare the mobile phase, 70 mL of Methanol and 30 mL of prepared $KH_2PO_4$ (Potassium dihydrogen Orthophosphate) buffer pH 6.0 were mixed to form in the ratio of 70:30.

**Preparation of Standard solution:**

Initially, 10 mg of bilastine Active Pharmaceutical Ingredient (API) was accurately weighed on an analytical electronic balance, transferred into a 10 mL volumetric flask and using a mobile phase, made up to the mark of the 10 mL volumetric flask. The mobile phase was prepared in the ratioof 70:30(v/v) using methanol and potassium dihydrogen orthophosphate buffer (pH 6.0). The solution was kept in a sonicator for dissolving. From the above standard solution, 0.05 mL was taken with the help of the pipette (1-100µL) and transferred into another 10 mL volumetric flask and the prepared mobile phase was added up to the mark of the volumetric flask, mixed well, filtered through 0.45 µm filter. The final concentration was found to be 5 µg/mL.

**Optimization of the method using DOE**

Initially, the trial and error methods were applied to obtain preliminary data of the method to be developed. Furthermore, the central composite design with response surface methodology was employed for theoptimization of the experimental conditions of the method. The independent factors used were two levels resulting in total 13 experimental runs were shown in Table 1. The factors selected were flow rate and column temperature while the responses included were retention time, theoretical plates, and tailing factor. The linear polynomial equations are generated from ANOVA (Analysis of Variance), depicted below.

**Table 1: Central composite design runs**

| Runs | Factors | | Responses | | |
|---|---|---|---|---|---|
| | Flow rate (ml/min) | Column temperature(°C) | Retention time | Theoretical plates | Tailing factor |
| 1 | 0.90 | 37.50 | 06.100 | 4032 | 1.172 |
| 2 | 0.60 | 40.00 | 09.032 | 5382 | 1.203 |
| 3 | 0.90 | 37.50 | 06.100 | 4032 | 1.172 |
| 4 | 1.32 | 37.50 | 04.282 | 2956 | 1.131 |
| 5 | 0.90 | 37.50 | 06.100 | 4032 | 1.172 |
| 6 | 0.60 | 35.00 | 09.290 | 6052 | 1.249 |
| 7 | 0.90 | 37.50 | 06.100 | 4032 | 1.172 |
| 8 | 0.90 | 33.00 | 06.177 | 3936 | 1.152 |
| 9 | 0.90 | 41.00 | 06.103 | 4167 | 1.147 |
| 10 | 0.47 | 37.50 | 11.459 | 5782 | 1.238 |
| 11 | 0.90 | 37.50 | 06.100 | 4032 | 1.172 |
| 12 | 1.20 | 35.00 | 04.696 | 3141 | 1.142 |
| 13 | 1.20 | 40.00 | 05.126 | 4221 | 1.140 |

The optimized factors including flow rate of 1.2 mL and column temperature of 40°C were selected as the retention time, theoretical and tailing factor was the best for these combinations.

From the model summary statistics, it was concluded that the quadratic equation best suit for this study. The equation to interpret the relation between the factors and the responses is as follows

Retentiontime = 6.10–2.34 A–0.0041B+ 0.1470AB+0.88672$A^2$+0.0214$B^2$ where A is the flow rate and B is the temperature.

Theoretical plates = 4032–1008.57A+92. 09B+437.50AB+290.69$A^2$+131.94$B^2$ Theoretical plates=4032-1008.57A+92.09B+437.50AB+290. 69$A^2$+131.94$B^2$ where A is the flow rate and B is temperature.

Taling factor=1.17–0.0402A–0.0069B+ 0.0110AB+0.0104$A^2$–0.0071$B^2$ Taling factor=1.17– 0.0402A–0.0069B+0.0110AB+0.0104$A^2$–0.0071$B^2$ where A is the flow rate and B is temperature

**System suitability**

System suitability was performed by taking six replicates of the prepared concentration, i.e., 5 µg/mL, six replicates were injected into HPLC (High Performance (or) Pressure Liquid Chromatography) setting the optimized conditions and finally the peak areas, retention times, tailings factors, theoretical plates, peak heights were noted from the chromatograms.

**Specificity**

**Preparation of standard solution**

Followed the same procedure as mentioned in the system suitability. The optimized concentrations i.e., 5 µg/mL were prepared, filtered, injected, and the peak responses were noted.

**Sample solution preparation**

10 Tablets of bilastine were taken, each tablet was weighed individually, and the average weight of 10 Tablets was calculated, i.e., 113.23 mg. The equivalent weight to 10 mg was calculated from the average weight of the 10 Tablets and label claim and the equivalent weight was found to be 56.615 mg. Next, 56.615 mg of bilastine powder was weighed accurately, transferred into a 10 mL volumetric flask, made up to the mark of the volumetric flask using mobile phase, mixed well, the

solution was filtered through 0.45 µm filter sonicated finally. Six optimized concentrations were prepared from the above stock solution. The concentrations were injected into the HPLC and the peak responses were clearly noted.

**Linearity**

**Preparation of stock solution**

An amount of 10 mg of bilastine API was accurately weighed and transferred to a 10 mL volumetric flask, the mobile phase was made up to the mark of volumetric flask, it became 1000 µg/mL, then the solution was kept in the sonicator to dissolve the bilastine completely. Then suitable dilutions were made to obtain the following concentrations of 2.5 µg/mL, 3.75 µg/mL, 5 µg/mL, 6.25 µg/mL, 7.5 µg/mL, 10 µg/mL and were filtered through 0.45 µm filter, sonicated for 5 min, then the concentrations were injected into the UFLC after that the peak responses were noted. Finally, a graph was plotted between the area on x-axis and concentrations on y-axis.

**Precision**

**Preparation of sample solution**

Followed the same procedure for the preparation of samples as mentioned in the specificity. Two types of precision were performed here, they are.

1. Intraday precision
2. Interday precision

**Intra-day precision**

Intraday precision was performed within a day at 3 stages, for every 3 h, 9AM, 12PM, and 4PM. The optimized concentration 5 µg/mL was prepared from the stock solution and six replicates of it were injected into HPLC and the peak responses were noted. The %RSD was measured fromsix replicates.

**Inter-day precision**

Interday precision was performed on 3 days consecutively. For this, one, optimized concentration (5 µg/mL) was prepared from the stock solution and six replicates of it were injected into HPLC on 3 days consecutively. The peak responses were noted individually, the %RSD was calculated.

**Accuracy**

**Preparation of sample solution2**

10 Tablets of bilastine were weighed accurately, and the average weight of 10 Tablets was calculated. The equivalent weight of tablet powder with 10 mg was calculated, i.e., 56.615 mg. Then, 56.615 mg of tablet powder was weighed, transferred into a 10 mL volumetric flask, and made up to the mark of the volumetric flask using mobile phase. The powder was dissolved and kept in a sonicator for dissolving and filtered through 0.45µm filter. To perform accuracy, a series of 50%, 100%, and 150% sample solutions were prepared.

**Sample solution**

An amount of 28.3075 mg of bilastine powder was weighed, transferred into a 10 mL volumetric flask, and made up to the mark of the volumetric flask with mobile phase to get the final concentration. Pipette out 0.05 mL of the above solution into another 10 mL volumetric flask, made up to the mark with mobile phase, filtered, sonicated and six replicates of it were injected into the HPLC and the peak responses were noted clearly.

**100% sample solution**

An amount of 56.615 mg of bilastine powder was weighed, transferred into a 10 mL volumetric flask, and made up to the mark of the volumetric flask with mobile phase to get the final concentration. Pipette out 0.05 mL of the above solution into another 10 mL volumetric flask, made up to the mark with mobile phase, filtered, sonicated and three replicates of it were injected into the HPLC and the peak responses were noted clearly.

**150% sample solution**

An amount of 84.9225 mg of bilastine powder was weighed, transferred into a 10 mL volumetric flask, and made up to the mark of the volumetric flask with mobile phase to get the final concentration. Pipette out 0.05 mL of the above solution into another 10 mL volumetric flask, made up to the mark with mobile phase, filtered, sonicated and six replicates of it were injected into the HPLC and the peak responses were noted clearly.

**Limit of Detection (LOD)**

LOD=3×standard deviation/slope

**Limit of Quantification (LOQ)**

LOQ=10×standard deviation/slope

**Robustness**

The robustness was done by changing the parameters such as wavelength, flow rate, and temperature. The optimized concentrations (5µg/mL) were prepared from the stock solution. The flow rate was changed to 1.0 mL/min, 1.2 mL/min, and 1.4 mL/min, respectively. The wavelength was set to 280 nm, 282 nm, and 284 nm. The column temperatures were tuned to 37°C, 40°C and 43°C. The peak responses were noted after injecting the sample into the HPLC for all changes of flow rate, wavelength, and column temperature.

**Degradation studies (or) Stability studies**

Degradation studies were performed to know the stability period of the tablet. These studies were performed using four degradation methods, they are

1. Acid degradation method
2. Base degradation method
3. Hydrogen peroxide degradation method
4. Degradation by using UV light

**Acid degradation method**

A volume of 0.05 mL of sample solution was taken into a 10 mL volumetric flask from the prepared stock solution to this 3 mL of 0.1M HCl (Hydrochloric Acid) was added and kept it over the heating mantle at 50°C for 15 min and cooled. To this cooled solution, 3ml of 0.1N NaOH (Sodium Hydroxide) was added to neutralize the solution and made up to the mark with mobile phase, injected into HPLC and the peak responses were noted.

**Base degradation method**

The procedure for the base degradation is the same as the acid degradation procedure except for the use of acid and the base is vice versa.

**$H_2O_2$ degradation method**

A volume of 0.05 mL of sample solution was taken in a 10 mL volumetric flask from the prepared stock solution, to this 3 mL of 3% $H_2O_2$ (Hydrogen Peroxide) was added and made up to the mark of the volumetric flask using mobile phase, then the solution was kept in room temperature for 12 hours. Finally, the solution was injected into the HPLC and the peak responses were noted.

**Degradation by using UV light**

A volume of 0.05 mL of sample solution was taken from a 10 mL volumetric flask containing the stock solution and made up to the mark of the volumetric flask using mobile phase to obtain 5 µg/mL This solution was kept under UV light of 254 nm for 72 hours. After the elapsed time, the solution was directly injected into the HPLC and the peak responses were noted.

## RESULTS AND DISCUSSION

**Determination of wavelength:** The standard solution of Bilastine (10 µg/mL) was scanned in UV range of 200-800 nm using the solvent (Methanol and $KH_2PO_4$ buffer at pH 6.0 in the ratio of 70:30). The bilastine solution showed the maximum absorbance at 282 nm, which was selected as the λmax of the bilastine drug.

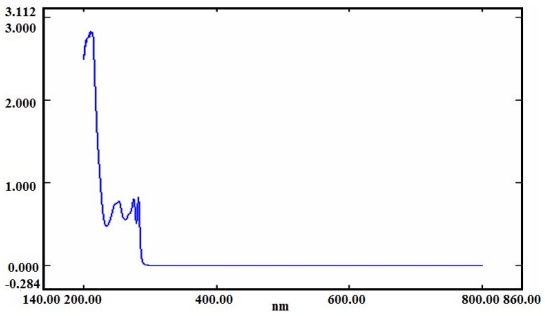


**Fig. 2. $\lambda_{max}$ Spectrum of Bilastine in mobile phase (Methanol:Buffer=70:30)**

**Table 2: ANOVA results**

| S.no | Responses | Retention time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| 1 | ±SD | 0.2085 | 261.68 | 0.0107 |
| 2 | Mean | 6.6600 | 4292.08 | 1.17 |
| 3 | %CV | 3.1300 | 6.10 | 0.9092 |
| 4 | Press | 2.1600 | 3.409E+06 | 0.0057 |
| 5 | $r^2$ | 0.9939 | 0.9525 | 0.9496 |
| 6 | Adjusted r-square | 0.9895 | 0.9186 | 0.9136 |
| 7 | Predicted r-square | 0.9566 | 0.6625 | 0.6416 |
| 8 | Adequate precision | 47.0711 | 17.5684 | 16.3531 |
| 9 | *p*-value | <0.0001 | 0.0002 | 0.0002 |

The p value is less than 0.0001, which suggests that the selected model best fits the study. The predicted $r^2$ value and adjusted $r^2$ valueare for retention time are quite closer.

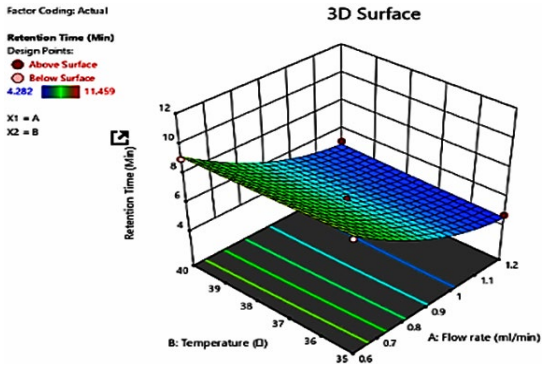


**Fig. (3a). Retention time**

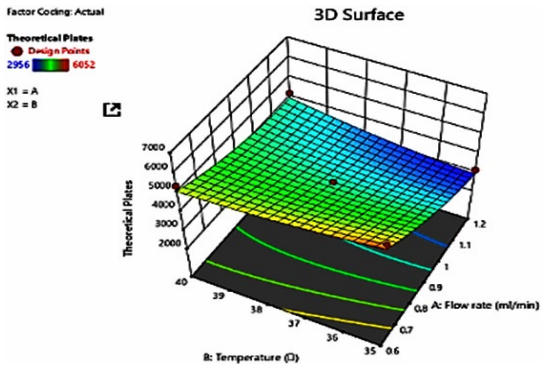


**Fig. (3b). Theoretical plates**

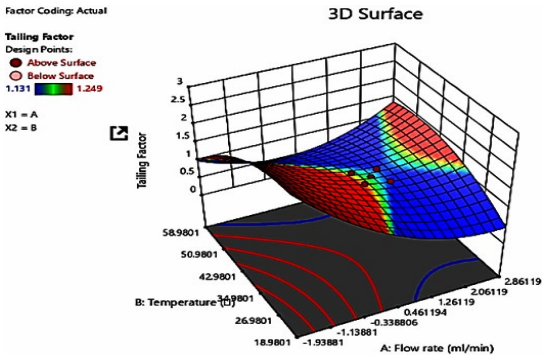


**Fig. (3c). Tailing factor**

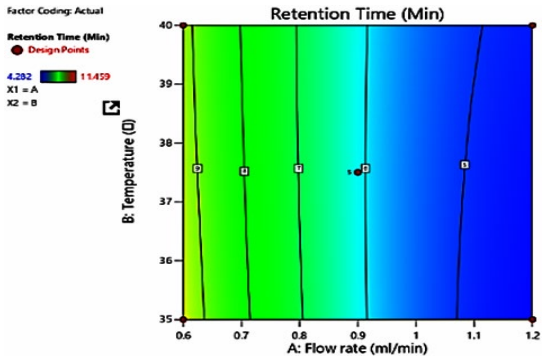


**Fig. 3. (3D plot of ANOVA results)**

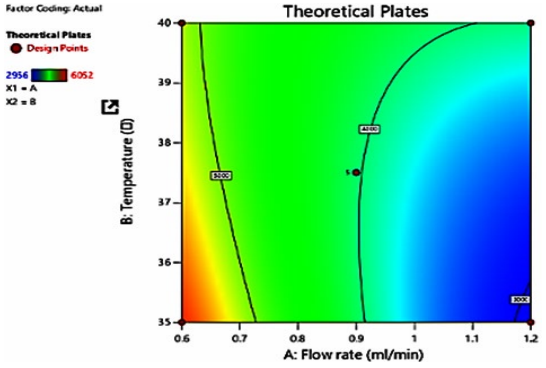


**Fig. 4. (Normal plot of ANOVA results)**

**Fig. (4a). Retention time**

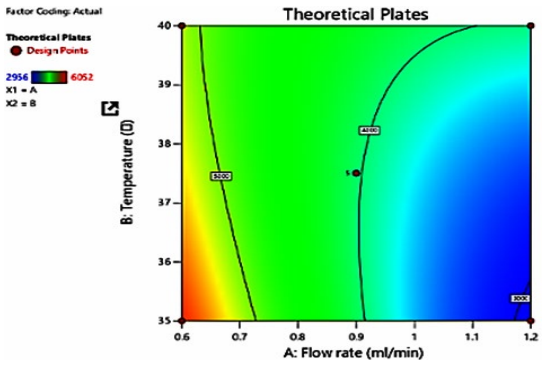


**Fig. (4b). Theoretical plates**

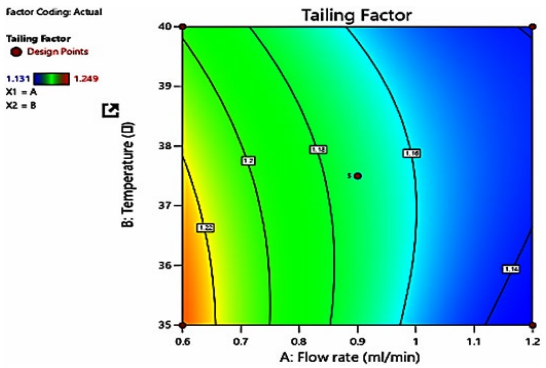


**Fig. (4c). Tailing factor**

## Analytical method development

The DOE software has shown to perform 13 runs. The trial with flow rate1.2 mL and column temperature of 40°C was selected as the optimized conditions. The peak passed all system suitability parameters. Furthermore, validation parameters were performed following ICH Q2 R1 guidelines.

Single peak was eluted, the retention time, theoretical plate, peak area, peak height, all parameters were good, so the method was optimized.

**Table 3: Optimized method**

| S. No | Parameter | Conditions |
|---|---|---|
| 1 | Mobile phase | Methanol and $KH_2PO_4$ buffer at pH 6.0 (70:30) |
| 2 | Column | C18, 4.6X250mm, 5µm (Shimadzu Shim-Pack GIST) |
| 3 | Flow rate | 1.2 mL/min |
| 4 | Column oven temperature | 40°C |
| 5 | Detection of wavelength | 282 nm |
| 6 | Run time | 6.5 min |
| 7 | Retention time | 5.126 min |
| 8 | Concentration | 5 µg/mL |
| 9 | Injection volume | 20 µL |

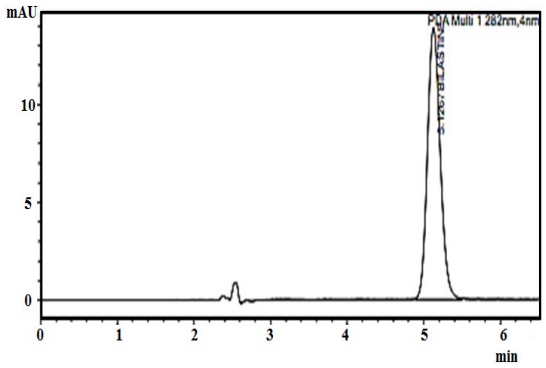


**Fig. 5. Bilastine chromatogram**

**Table 4: Linearity**

| S. No | Linearity level (%) | Concentration | Peak area |
|---|---|---|---|
| 1 | 25% | 1.25 µg/mL | 56526 |
| 2 | 50% | 2.50 µg/mL | 82783 |
| 3 | 75% | 3.75 µg/mL | 108088 |
| 4 | 100% | 5.00 µg/mL | 133849 |
| 5 | 125% | 6.25 µg/mL | 155855 |
| 6 | 150% | 7.50 µg/mL | 181249 |
| 7 | 200% | 10.0 µg/mL | 216165 |

Linearity was found inthe range of 1.25-10µg/ml.

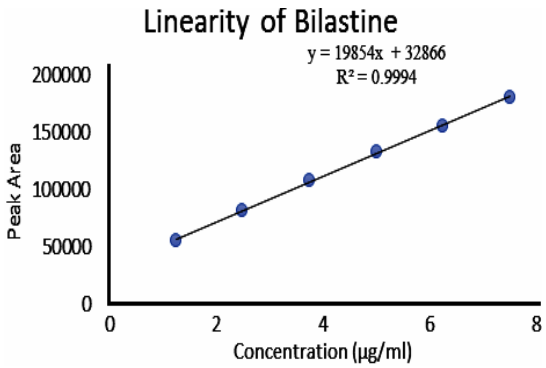


**Fig. 6. Is a graph represents the linearity of Bilastine**

A graph was plotted between the concentration on x-axis vs. peak area on y-axis and $R^2$ was obtained 0.999 in the graph which was found to be within the limit. Based upon the above limits, the linearity was passed.

## Analytical method validation

Analytical method validation parameters were performed following ICH Q2R1 guidelines and the limits; specificity: no interference; linearity: $R^2$=0.999-1.0; accuracy:98-102%; precision: Relative Standard Deviation (RSD)<2%; Detection Limit: S/N 2 or 3; Quantification limit:S/N 10; Robustness:%Assay 99-102%.

**Table 5: System suitability**

| S. No | Peak area | Retention time | Theoretical plates | Peak height | Tailing factor |
|---|---|---|---|---|---|
| 1 | 158524 | 5.117 | 4252 | 13816 | 1.14 |
| 2 | 157219 | 5.136 | 4243 | 13576 | 1.14 |
| 3 | 159711 | 5.084 | 4217 | 13959 | 1.14 |
| 4 | 159281 | 5.140 | 4227 | 13790 | 1.14 |
| 5 | 160764 | 5.154 | 4219 | 13869 | 1.14 |
| 6 | 161097 | 5.236 | 4350 | 13879 | 1.14 |
| Average | 159432.67 | 5.140 | 4251.33 | 13814.83 | 1.14 |
| STDEV | 1440.064 | 0.051 | 50.242 | 130.763 | 0.002 |
| %RSD | 0.90 | 0.99 | 1.18 | 0.95 | 0.15 |

The % RSD and standard deviation were calculated and the values were found within the limits.

## Precision

**Table 6: Intermediate precision**

| S. No | Day-1 Peak area | Day-2 | Day-3 |
|---|---|---|---|
| 1 | 157489 | 157044 | 157281 |
| 2 | 157092 | 157140 | 157175 |
| 3 | 157059 | 156852 | 155097 |
| 4 | 157646 | 157444 | 156227 |
| 5 | 157289 | 156838 | 156275 |
| 6 | 157552 | 158436 | 156249 |
| Average | 157354.50 | 157292.33 | 156384.00 |
| STDEV | 246.00 | 602.69 | 792.64 |
| %RSD | 0.16 | 0.38 | 0.51 |

The % RSD is within the limits and hence passed intermediate precision.

**Table 7: Intra-day precision**

| S. No | 9:00 AM Peak area | 1:00 PM | 5:00 PM |
|---|---|---|---|
| 1 | 150621 | 153384 | 155569 |
| 2 | 151360 | 154340 | 150217 |
| 3 | 150257 | 159664 | 159155 |
| 4 | 151570 | 158801 | 155635 |
| 5 | 153546 | 156082 | 156733 |
| 6 | 151953 | 152170 | 154311 |
| Average | 151551.17 | 155740.17 | 155270.00 |
| STDEV | 1159.12 | 3004.67 | 2963.40 |
| %RSD | 0.76 | 1.93 | 1.91 |

The intraday precision % RSD is also within limits, thus passing the test for intraday precision

### Accuracy

**Table 8: At 50% accuracy**

| S. No | Weight of sample(mg) | Peak area | Amount added | Amount found | % Recovery |
|---|---|---|---|---|---|
| 1 | 28.3075 | 79884 | 2.4958 | 2.5053 | 100.3773 |
| 2 | 28.3075 | 78570 | 2.4958 | 2.4640 | 98.7262 |
| 3 | 28.3075 | 79128 | 2.4958 | 2.4815 | 99.4273 |
| 4 | 28.3075 | 79864 | 2.4958 | 2.5046 | 100.3521 |
| 5 | 28.3075 | 78005 | 2.4958 | 2.4463 | 98.0162 |
| 6 | 28.3075 | 79724 | 2.4958 | 2.5002 | 100.1762 |

At 50% accuracy level, the mean % recovery 99.5126% is well within the prescribed range.

**Table 9: For 100% accuracy**

| S. No | Weight of sample(mg) | Peak area | Amount added | Amount found | % Recovery |
|---|---|---|---|---|---|
| 1 | 56.615 | 160440 | 4.9917 | 5.0316 | 100.7995 |
| 2 | 56.615 | 159398 | 4.9917 | 4.9989 | 100.1448 |
| 3 | 56.615 | 158985 | 4.9917 | 4.9860 | 99.8853 |

At 100% accuracy level, the mean % recovery 100.2765% is also well within the mentioned limit.

**Table 10: At 150% accuracy**

| S. No | Weight of sample(mg) | Peak area | Amount added | Amount found | % Recovery |
|---|---|---|---|---|---|
| 1 | 84.9225 | 237957 | 7.4875 | 7.4626 | 99.6673 |
| 2 | 84.9225 | 237989 | 7.4875 | 7.4636 | 99.6807 |
| 3 | 84.9225 | 237987 | 7.4875 | 7.4636 | 99.6799 |
| 4 | 84.9225 | 237994 | 7.4875 | 7.4638 | 99.6828 |
| 5 | 84.9225 | 237958 | 7.4875 | 7.4626 | 99.6678 |
| 6 | 84.9225 | 237915 | 7.4875 | 7.4613 | 99.6497 |

At 150 % accuracy level, mean % recovery 99.6714%.

The mean % recovery was calculated and found to be within the limits (98-102%). By based upon the above results, the accuracy test was passed.

### Robustness

**Table 11: Robustness**

| S. No | Parameter | Condition | Peak area | % Assay |
|---|---|---|---|---|
| 1 | Flow rate | 1 mL | 159077 | 99.78 |
| 2 | | 1.2 mL | 159433 | 99.83 |
| 3 | | 1.4 mL | 156431 | 98.12 |
| 4 | Wavelength | 280nm | 157178 | 98.59 |
| 5 | | 282nm | 159433 | 100.00 |
| 6 | | 284nm | 156735 | 98.31 |
| 7 | Temperature | 37°C | 157767 | 98.96 |
| 8 | | 40°C | 157816 | 98.99 |
| 9 | | 43°C | 158732 | 99.56 |

The % assay was calculated fromdifferent conditions and the values were found within the limit, so the test for robustness was passed.

### Degradation studies

**Table 12: Degradation studies (or) stability studies**

| S. No | Condition | Peak area | % Assay | % Degradation |
|---|---|---|---|---|
| 1 | Acid | 143091 | 89.750 | 10.25 |
| 2 | Base | 145500 | 91.261 | 8.7390 |
| 3 | $H_2O_2$ | 143032 | 89.713 | 10.287 |
| 4 | UV | 146332 | 91.783 | 8.217 |

In the degradation studies, the optimized concentration of dosage form was degraded by different conditions. The method still can quantify the amount of drug present after degradation.

### Limit Of Detection (LOD) and Limit of Quantification (LOQ)

For LOD-Standard Deviation of system suitability=2155.81; Correlation coefficient, $R^2$= 0.994; Slope from Linearity=22113 and LOD was 0.292 µg/mL. For LOQ-Slope=22113; Standard deviation=2155.81 and the LOQ was 0.974 µg/mL.

### Assay

**Table 13: Assay**

| S. No | Peak area | % Assay |
|---|---|---|
| 1 | 159968 | 100.336 |
| 2 | 158428 | 99.370 |
| 3 | 158434 | 99.374 |
| 4 | 159071 | 99.773 |
| 5 | 159592 | 100.100 |
| 6 | 159512 | 100.050 |
| Average | 159167.50 | 99.83 |
| STDEV | 637.79 | 0.40 |
| % RSD | 0.40 | 0.40 |

The percentage of assay was found to be within the limits-98%-102%.

## CONCLUSION

A method for the estimation of bilastine in API and its tablet dosage form was developed using RP-UFLC. The method was successfully validated following ICH Q2R1 guidelines. Before physically working with the instrument, the DOE software was used to obtain the optimized conditions with an input of flow rate and column temperature as the variables. The thirteenruns were given by the software and from that we have chosen the best variable conditions satisfying the Q2R1 guidelines. The linearity was in the range of 1.25 µg/mL to 10 µg/mL, and the theoretical plates were found well

beyond 2000. The %RSD for accuracy, precision, and robustness were all found <2%, which indicates that the parameters are within the limits of the guidelines. The LOD & LOQ were found to be 0.292 µg/mL and 0.974 µg/mL, respectively. The degradation studies were also performed in the tablet dosage form. The % assay was found to be within the limits 98%-102%. The linearity range is more in the developed method when compared with the already reported methods. The mobile phase used is methanol and buffer which are comparatively cheaper than most solvents used in the literature. Thus, we can consider that this method is sensitive, economical, reproducible, and considerably rapid in the assay of bilastine in API and dosage form.

**ACKNOWLEDGEMENT**

The authors thank the principal and management of the Santhiram College of Pharmacy for providing the facilities to complete the project.

**Conflict of interest**

The authors declare that they have no conflict of interests.

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