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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF BILASTINE BY RP-HPLC

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**ABSTRACT**
Current study was successfully completed with ideal for routine binary separation . The run time of study carried out with 3.8 minutes & injection volume is 20 µl. The detector used for optimization is silicon PDA and detection wavelength used for this method was 252. Elution technique carried out in the column when the temperature maintained up to 30 + 5 °C.Buffer was prepared with using 2.72 g Potassium dihydrogen orthophosphate and adjust the pH at 3.0 with OPA.Mobile Phase (Buffer: Acetonitrile- 600: 400). Methanol & Mobile Phase used as diluents.

**Keywords**: HPLC, Method development and validation, Bilastine, Linearity, Robustness.

## INTRODUCTION

Bilastine is $H_1$ antihistamine drugs that has been an effective and safe used to treat allergic conjunctivitis symptoms. The chemical formula and molecular weight of bilastine is $C_{28}H_{37}N_3O_3$, 463.622 g/mole respectively. Limited methods only available for bilastine, either alone or in combination with other compounds and related compounds, have been previously documented. Basis on the data availability present research was completed. HPLC techniques were used for various stages in the development of present method.

### Final Concentration of Both the Standard & Sample

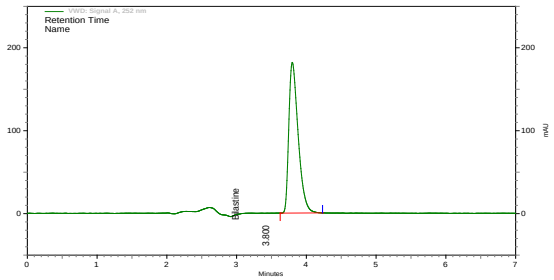
### Standard solution

Place 50 mg of Bilastine WS, accurately weighed, into weighed, into a volumetric flask with a 100-ml capacity. To produce the volume, combine after methanol dilution and dissolving. Stir mobile phase into this solution to make 25.0 ml from 100 ml of VF (100 mcg).

### Sample Preparation

20 tablets should be exactly crushed weighed, then they should be transferred into a volumetric flask containing 100 ml of methanol and diluted with a perfectly weighed quantity of tablets that have been finely crushed to contain 20 mg of bilastine, through a 0.45 filter.. Mix an additional 10.0 ml of this solution to 20.0 ml of mobile phase make final concentration to 100 mcg.

### Chromatogram: 1 optimized condition

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**METHOD VALIDATION**

**System Suitability**

Five replicate injections were used to test the system's suitability. System suitability achieved obeys the validation parameter. Area, TP, and RT's respective %RSDs were 0.63, 0.75, and 0.12 correspondingly.

**Table 1:**

| Injection .No | Area Responses | RT | TP |
|---|---|---|---|
| 1. | 42360398 | 3.630 | 4438 |
| 2. | 42577738 | 3.630 | 4389 |
| 3. | 42353769 | 3.625 | 4449 |
| 4. | 42087974 | 3.625 | 4462 |
| 5. | 41899521 | 3.620 | 4477 |
| **AVG** | 42255880 | 3.626 | 4443 |
| **SD** | 264236.16 | 0.004183 | 33.519 |
| **%RSD** | 0.63 | 0.12 | 0.75 |

**Specificity**

The selectivity of the well-known HPLC method was investigated by preventing excipient interference in the employed formulation and mobile phase components. The selectivity of the suggested techniques report was demonstrated below by comparing the chromatograms of the sample solution containing bilastine (100 mcg/mL) with the chromatograms of the standard solution, blank, and placebo. Excipients employed in the formulation and components of the mobile phase did not simultaneously obstruct the analysis of bilastine, making the method selective.

**Table: 2**

| Sample. No | Name | Area responses | RT |
|---|---|---|---|
| 1 | Blank run | 0 | 0 |
| 2 | Placebo run | 0 | 0 |
| 3 | Std run | 27579337 | 3.810 |
| 4 | Sample run | 27654432 | 3.810 |

**Accuracy**

The accuracy of the proposed strategy was assessed by producing samples spiked with 80%, 100%, and 120% of the test concentration of bilastine. The cumulative percent RSD was 0.60.

**TABLE: 3**

| S.no | Sample id | Sample area | mg of drugs taken | mcg added | mcg found | Recovery Percentage | SD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | 80% drugs | 21987443 | 90.21 | 45.83 | 45.79 | 98.91 | **0.59** | **0.60** |
| | | 22009315 | | | | | | |
| | | 22022885 | | | | | | |
| 2 | 100% drugs | 27502548 | 112.24 | 56.98 | 56.97 | 99.98 | | |
| | | 27565928 | | | | | | |
| | | 27533226 | | | | | | |
| 3 | 120% drugs | 32815279 | 134.99 | 67.90 | 67.80 | 99.85 | | |
| | | 33080221 | | | | | | |
| | | 32900295 | | | | | | |

**Precision (Method Precision)**

The accuracy of the suggested approach was evaluated using six replication analyses at a fixed concentration of the selected compounds (100 µg /ml), within the linearity range. The precision was expressed as a percentage of relative standard deviation. The results are listed below.

**TABLE: 4**

| S.no | Sample ID | Sample area | drugs taken | Recovery Percentage | %RSD |
|---|---|---|---|---|---|
| | spl-01 | 28268073 | 100 µg/ml | 100.631 | **0.34** |
| | spl-0 | 28414197 | | 100.08 | |
| | spl-03 | 28359845 | | 100.17 | |
| | spl-04 | 28251542 | | 100.66 | |
| | spl-05 | 28526398 | | 100.30 | |
| | spl-06 | 28568546 | | 100.98 | |

**Linearity**

The proposed method's linearity was examined by graphing peak area Vs drug concentration. The plot of peak area against the respective concentrations of bilastine was shown to be linear, linearity results from low range precision (40.2 mcg) to high range precision (60.6 mcg). Therefore, the linear range of the method for bilastine tablets is 40% to 160% with respect to assay concentration. The correlation coefficient of $R^2$ was 1.0000, which is well within the predetermined limit of NLT 0.998.

**TABLE: 5**

ANALYTICAL METHOD VALIDATION FOR THE QUANTITATIVE ESTIMATION OF BILASTINE FORMULATIONS BY RP-HPLC

**LINEARITY - BILASTINE**

Name of the Product : BILASTINE TABLETS 20mg
Label claim : Each uncoated tablet contains:

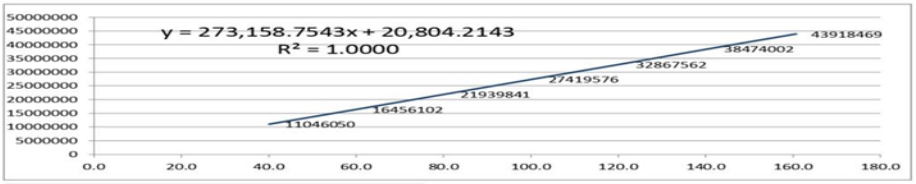
BILASTINE 20.00 mg

| | | | | | | | | | % | mcg |
|---|---|---|---|---|---|---|---|---|---|---|
| 50.20 | mg | 100 | mL | → | 2 | mL | 25 | mL | 40% | 40.2 |
| | | | | → | 3 | mL | 25 | mL | 60% | 60.2 |
| | | | | → | 4 | mL | 25 | mL | 80% | 80.3 |
| | | | | → | 5 | mL | 25 | mL | 100% | 100.4 |
| | | | | → | 6 | mL | 25 | mL | 120% | 120.5 |
| | | | | → | 7 | mL | 25 | mL | 140% | 140.6 |
| | | | | → | 8 | mL | 25 | mL | 160% | 160.6 |

*STANDARD CONCENTRATIONS AND AREA:*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Conc. (mcg/mL) (X) :** | 40.2 | 60.2 | 80.3 | 100.4 | 120.5 | 140.6 | 160.6 |
| **Observed Area (Y) :** | 11046050 | 16456102 | 21939841 | 27419576 | 32867562 | 38474002 | 43918469 |

*LINEARITY GRAPH*




| Y=MX + C | |
|---|---|
| INTERCEPT ( C ) : | 20804.214 |
| SLOPE ( M ) : | 273159 |

*Acceptance Criteria:*
1. Correlation coefficient between concentration and its response(Area) should be not less than 0.998
2. Report the Intercept
3. Report the slope of the regression line
4. Report the linearity graph
5. Report the residual sum of square.

Conclusion :
The obtained linearity results from low range precision (40.2 mcg) to high range percision (160.6 mcg) provided the correlation coefficient of 1.0000 which is well within the predeteruined limit of NLT 0.998.Hence 40% to 160 % with respect to assay concentration for assay method is linear for BILASTINE .

**Robustness (Flow Rate, Ph, nm Change)**

By making deliberate modifications to the chromatographic conditions (Flow rate 1.2 mL/min, PH 2.95, nm 254)

**TABLE: 6**

| Changes of Parameters | Area responses | Retention time | Theoretical plates (USP) | Asymmetry | % RSD |
|---|---|---|---|---|---|
| Flow rate 1.2 | 21735527 | 3.212 | 3894 | 1.16598 | 0.36 |

| | | | | | |
|---|---|---|---|---|---|
| mL/min, | | | | | |
| PH 2.95 | 28164352 | 3.805 | 3927 | 1.17668 | 0.71 |
| nm change -254 | 27011836 | 3.829 | 3992 | 1.11847 | 0.30 |

**LOD & LOQ**

This sample was dissolved using mobile phase, which was injected up to the point where the peak disappeared. The trials were conducted from 10 mcg to 0.25 mcg. It is established that the detection limit is 0.25mcg throughout this approach. For this study, the analyte was tested and quantified in six replicates at the lowest concentration.

**TABLE: 7**

| S.No | Parameters | Measured concentration |
|---|---|---|
| 1 | LOD | 1.13 |
| 2 | LOQ | 3.58 |

**CONCLUSION**

A thorough review of the literature reveals that only a few techniques have been documented for the RP-HPLC method of estimation of Bilastine-related compounds in tablet formulation. The main goal of the current research is to develop optimal conditions for the estimation of bilastine by RP-HPLC. The flow rate was set at 1.0 ml/min with a retention time of 3.8 minutes.

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