*Original Research Article*

# Development and Validation of RP-HPLC Method in Simultaneous Estimation of Bilastine and Montelukast as Tablet Dosage Form




## ABSTRACT

**Aim**: For a concurrent evaluation of Bilastine and Montelukast drugs in a tablet dosage form, a primary, explicit method has been brought about in this study.

**Place and Duration of study:**University College of Technology, Osmania University, Hyderabad, Telangana, between

**Methodology**: In this work, an Inertsil ODS C18, 5 m (4.6 x 250mm) was utilized for chromatogram, and as a mobile phase, phosphate buffer and acetonitrile in 30:70 ratio was opted to pump at 1ml/min flow rate via column. The buffer pH was maintained at 4.6, and the temperature was made ambient for the evaluation. Eventually, the wavelength for Bilastine and Montelukast was noticed at 260nm. Further, the retention time for both the opted drug was discerned at 2.319mins and 4.299 min respectively, Even the percentage purity of these drugs was observed at 100.6% & 100. 3% correspondingly. Later the system suitability criteria i.e., tailing factor, theoretical plates for Bilastine were revealed at 1.3 and 5117.5, whereas Montelukast was at 1.4 and 3877.8, further with the resolution at 9.0.

**Results**: Finally, the validation of the method was determined such that the linearity range was observed well at 1- 5 µg and 100-500 µg concentration series, the correlation coefficient ($r^2$) was noticed at 0.999 for both, and then the %mean recovery was at 100.1% and 100.4%, for %RSD repeatability it was 0.31 and 0.38, whereas for %RSD in intermediate precision it was at 0.12 and 0.15 respectively. The LOD values were obtained as 2.94 and 3.03, and LOQ values were obtained as 9.87 and 10.1.

**Conclusion**: Themethodvalidation was discovered to be precise, robust& repeatable, allowing the study to proceed on RP-HPLC an approachable technique for the evaluation of Bilastine and Montelukast as pharmaceutical dosage form on a daily basis, due to their rapidity, clear-cut results, durability, sturdiness, and reproducibility.


**Keywords:**Bilastine, Montelukast,RP-HPLC, Simultaneous estimation, Method development,Validation

## 1. INTRODUCTION

A current new antihistamine called Bilastine discerns highly for H1 histamine receptor with rapid onset but long-term action period, which comes under the selective Histamine of H1 receptor Antagonist (Ki = 64Nm) [1]. At the time of sensitive feedback pole, cells release histamine and other substances by undergoing degranulation. Bilastine, by binding to and protecting against the activation of the H1 receptor, can slow the progression of acute symptoms by releasing histamine from mast cells[2]. IUPAC name of Bilastine is "2- [4-(2- 4- [1-(2-ethoxyethyl)-1 H-1,3-benzodiazol-2-yl] piperidin-1-yl ethyl) phenyl] -2-methylpropanoic acid". The Chemical Solution of Bilastine is $C_{28}H_{37}N_3O_3$. Bilastine has a Molecular Weight of 463.622 $g.mol^{-1}$. Bilastine is soluble in the natural solvent chloroform at a concentration of about 30 mg/ml.

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Montelukast is a leukotriene receptor villain that partly is used in daily asthma therapy, to prevent the workout caused by bronchoconstriction, as well as in hay fever treatment[3]. Cells such as pole cells, eosinophils release CysLT (CysteinylLeukotrienes) especially LTD4, LTC4, LTE4, and even Eicosanoids. Once these CysLT binds accordingly with CysLT receptors such as the type- I CysLT receptors present over the smooth muscular tissue cells of breathing air passage, Macrophages' air passage & even pro-

inflammatory cells such as eosinophils, few myeloid stem cells can aid in asthma pathophysiology and boosting of allergic rhinitis[4]. IUPAC Call is 2- [1-(methyl) cyclopropyl] acetic acid. Chemical Formula is $C_{35}H_{36}ClNO_3S$. Molecular Weight is 586.183 $g.mol^{-1}$. The physical properties of Montelukast salt are its hygroscopic nature, optical activity, white to beige colored powder. Montelukast sodium is highly soluble in ethanol and methanol, but is slightly soluble in water and acetonitrile[5].

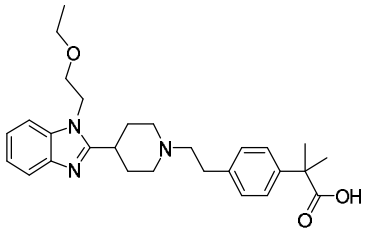


**Figure 1:BilastineStructure**

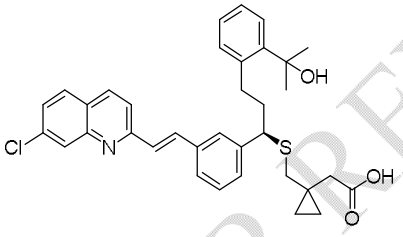


**Figure 2: MontelukastStructure**

In the analysis of Bilastine and Montelukast in both pure and other drug combined forms there are many works of literature works with different techniques have been reported, such as RP-HPLC[6-10], RP-UPLC [11], UV [12]. Because of the requirement for an appropriate and economical RP-HPLC method for daily analysis of both Bilastine and Montelukast as tablet dosage form, as being accessible, specific, accurate, and cost-efficient analytical technique, this has been considered for the current study. Further, the validation of the suggested above method was performed by keeping the ICH standards.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Reagents

Bilastine and Montelukast were bought on the market. Whereas an analytical grade $KH_2PO_4$ was procured from Finerchem limited, then Merck supplied Orthophosphoric acid & water, Methanol required for HPLC analysis were bought from 'Lichrosolv- Merck.'

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### 2.2. Conditions for chromatographic analysis and equipment

A Waters 2695 HPLC system was used to perform chromatography that contains an auto-sampler, a UV detector, and a software application named Empower 2. At 260 nm, the analysis was performed with the column Inertsil ODS C185 m (4.6 x 250mm), dimensions at $25^0$C temperature level. The enhanced mobile phase contains Phosphate buffer 4.6 pH and also Acetonitrile (30:70). The flow rate at 1ml/min was conditioned at a run time of about 12 min.

### 2.3. Preparation of solutions

#### 2.3.1. Preparation of Phosphate buffer with pH of 4.6

About 6.8 gm of $KH_2PO_4$ was dissolved in a 1000 ml volume beaker, then till 1000 ml it was diluted with HPLC water, and with orthophosphoric acid, the pH is re-adjusted to 4.6.

**2.3.2. Mobile Phase preparation**
For the preparation of the mobile phase, 300 mL phosphate buffer with pH of 4.6 and about 700 mL of ACN in the ratio of 30: 70 were taken, and by using an ultrasonic water bath, it was degassed at around 5 mins. Then this service is infiltrated in vacuum purification by using a 0.45-micron filter.

**2.3.3. Diluent (Blank)Preparation**
As diluent mobile phase can be used.

**2.4. Preparation of the individual Bilastine Standard**
The weighed 10mg of Bilastine according to the prescribed standard was added to a 100 ml clean volumetric flask along with 20ml DMF (Dimethyl Formamide). Later, it is dissolved by sonicating, then by using the stock solution, i.e., diluent, it was made tillfull mark. The diluent was then pipetted into a 10 ml volumetric flask and diluted to the mark with the stock solution.

**2.5. Preparation of the individual Montelukast Standard**
The weighed 10mg of Montelukast according to the prescribed standard was added to a 100 ml clean volumetric flask along with 20ml DMF. Later, it is dissolved by sonicating,then by using the stock solution, i.e., diluent,it was made up to meet the standard.The diluent was then pipetted into a 10 ml volumetric flask and diluted to the mark with the stock solution.

**2.6. Preparation of Sample Solution :( Tablet)**
About ten tablets were taken into mortar and pestle to obtain the fine powder, with weight equivalent to 20 mg of Bilastine& 10 mg Montelukast was taken in a 100mL neat completely dry volumetric flask along with the addition of 70mL Diluents and is dissolved by sonicating then by using the stock solution, i.e., diluent it was made till mark. A ml of diluent was pipetted directly into a 10 ml Volumetric flask and diluted to the mark with the stock solution.

**2.7. Procedure**
20µL of the sample was infused into the chromatographic system, and the area peaks for Bilastine and Montelukast were measured. The formulae are then used to calculate the percent Assay.

## 3. RESULTS AND DISCUSSION

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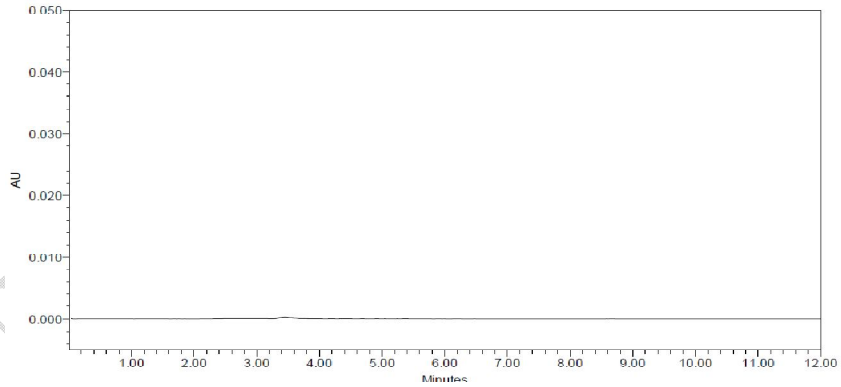


**Figure 3:Blank chromatogram**

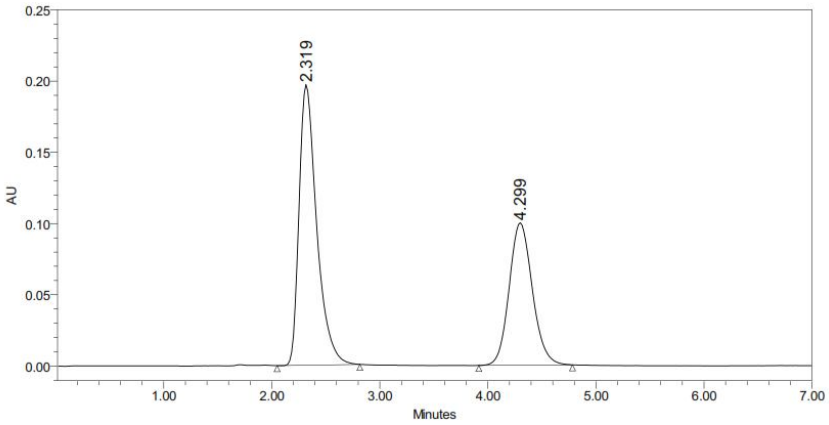


**Figure 4:Standard chromatogram**

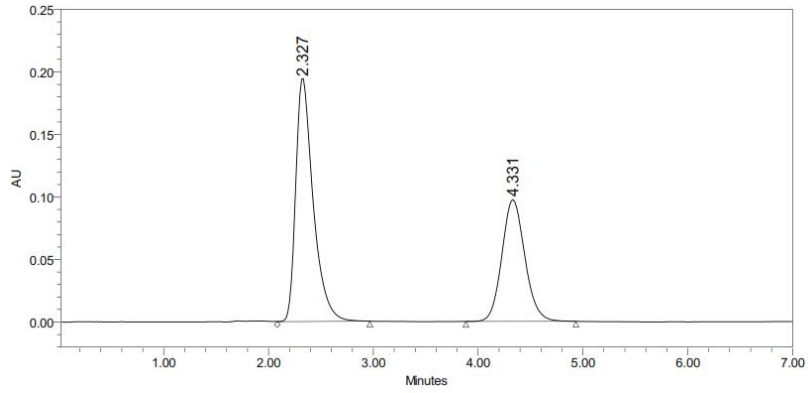


**Figure 5:Sample chromatogram**

**3.1. Validation of the Analytical method**

The created chromatographic approach was checked for linearity, suitability with system, precision, accuracy, durability as well as effectiveness based on ICH standards.

**3.2. System suitability specifications**

In order to assess the system viable criteria like tailing factors, retention time, 'USP' theoretical plate count, then with the help of the column at a circulation rate of 1.0 ml / 1 min for about 12 min the mobile phase is passed, in order to equilibrate column at required temperature level as shown in the Table-1. By injecting about 20 µlof criterion right into Inertsil ODS C185 m, (4.6 x 250mm) the chromatographic splitting is achieved, then the mobile Phase of composition Sodium Phosphate buffer 4.6 pH; Acetonitrile (30:70) was allowed to pass along with column at circulation flow of 1.0 ml per minute. Retention time, tailing variable as well as 'USP' theoretical plate count of the particular technique as depicted in table 1.

**Table 1: Parameters of System Suitability**

| Parameters | Bilastine | Montelukast |
|---|---|---|
| Retention time | 2.327 | 4.331 |
| USP Plate count | 5117.5 | 3877.8 |
| USP Tailing | 1.3 | 1.4 |

**3.3. Assay of pharmaceutical formulation:**The recommended verified technique was efficiently put on figuring out Bilastine and Montelukast in their tablet dosage type. The obtained result Bilastine and also Montelukast that was comparable with the respective labeled quantities as shown in Table2.

**Table 2: Results of Bilastine and Montelukast Assay**

| | Label Claim (mg) | % Assay |
|---|---|---|
| **Bilastine** | 20 | 100.6 |
| **Montelukast** | 10 | 100.3 |

**3.4. Linearity and range**

The linearity research work was carried out to have a focus on levels of 100 ppm – 500 ppm and one ppm – 5 ppm. Each degree was directly infused into the chromatographic system. At each level, the area is used in calculating the relationship coefficient. The injection of each level into the chromatographic system is completed, and the peak area is measured.A chart was plotted with peak area (on the Y-axis) vs. concentration (on the X-axis), and the correlation coefficient was calculated.outcomes are depicted in table 3,4 and graphs in figure 6,7.

**Table 3: Linearity results for Bilastine**

| S.NO | SAMPLE NAME | RT | AREA | HEIGHT |
|---|---|---|---|---|
| 1 | Linearity 1 | 2.309 | 1810101 | 145867 |
| 2 | Linearity 2 | 2.322 | 2044873 | 176895 |
| 3 | Linearity 3 | 2.324 | 2367122 | 206674 |
| 4 | Linearity 4 | 2.336 | 2602248 | 228475 |
| 5 | Linearity 5 | 2.340 | 2869772 | 259345 |

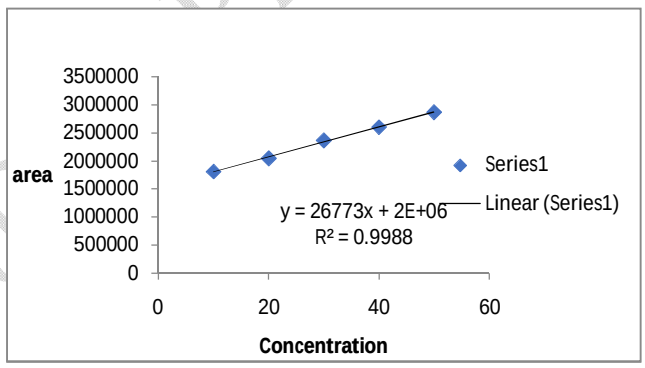


**Figure 6: Linearity graph for Bilastine.**

**Table 4: Linearity results for Montelukast**

| S.NO | SAMPLE NAME | RT | AREA | HEIGHT |
|---|---|---|---|---|
| 1 | Linearity 1 | 4.304 | 1164173 | 74586 |
| 2 | Linearity 2 | 4.323 | 1342555 | 87689 |
| 3 | Linearity 3 | 4.214 | 1556824 | 101999 |
| 4 | Linearity 4 | 4.524 | 1774565 | 117084 |
| 5 | Linearity 5 | 4.218 | 1956421 | 129409 |

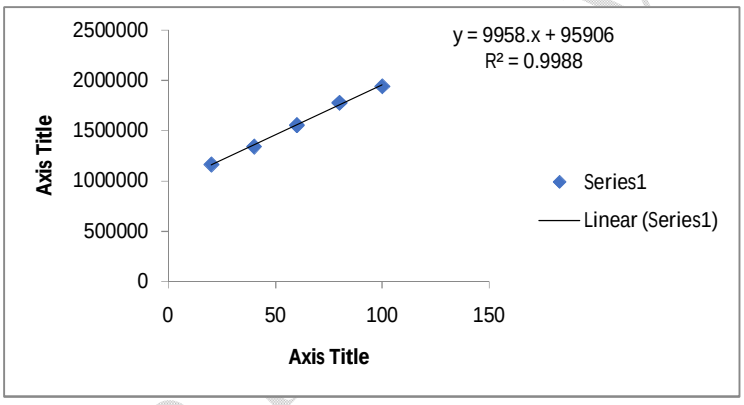


**Figure 7: Linearity graph for Montelukast.**

### 3.5. Accuracy study

The accuracy was determined withthe help of a recovery study. The recovery technique is executed at three levels such as 50%, 100%, 150%. Later, the standard solution is injected into the chromatographic system. Calculation of quantity required and the amount added for Bilastine and also Montelukasthas done as well as determine the individual recovery and mean recovery values. The outcomes as depicted in the table 5,6.

**Table 5: Showing accuracy results for Bilastine**

| % Concentration at a specific level | Bilastine Area | Amount added in mg | Amount retrieved in mg | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 353757 | 5 | 5.0 | 100.3% | 99.56% |
| 100% | 4735178 | 10 | 9.94 | 99.3% | |
| 150% | 5911698 | 15 | 14.8 | 99.1% | |

**Table 6: Montelukast Accuracy results**

| % Concentration at a specific level | Montelukast Area | Amount added in mg | Amount retrieved in mg | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 2332744 | 5 | 5.10 | 101.8% | |
| 100% | 3132697 | 10 | 9.99 | 99.9% | 100.4% |
| 150% | 3918997 | 15 | 14.9 | 99.1% | |

**3.6. Precision study**

Evaluation of precision was done with coefficient variance for six replicate injections depending on the requirement. For five times, the injection of the standard solution was done & gauged the location for all five Injections in HPLC. The %RSD for the area of five reproduce shots were noticed. The results are displayed in table7,8.

**Table 7: Bilastine Precision results**

| S.no. | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Bilastine | 2.320 | 2265419 | 196958 |
| 2 | Bilastine | 2.341 | 2204588 | 197584 |
| 3 | Bilastine | 2.356 | 2247569 | 195874 |
| 4 | Bilastine | 2.344 | 2258741 | 194583 |
| 5 | Bilastine | 2.325 | 2258967 | 194587 |
| Mean | | | 2255501 | |
| Std.dev | | | 6545.5 | |
| %RSD | | | 0.31 | |

**Table 8: Montelukast Precision results**

| S.no. | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Montelukast | 4.302 | 1401475 | 100274 |
| 2 | Montelukast | 4.305 | 1401345 | 100078 |
| 3 | Montelukast | 4.325 | 1402415 | 98425 |
| 4 | Montelukast | 4.315 | 1404775 | 98165 |
| 5 | Montelukast | 4.312 | 1408614 | 98154 |

| | | | | |
|---|---|---|---|---|
| Mean | | | 1491354 | |
| Std.dev | | | 5882.5 | |
| %RSD | | | 0.38 | |

**3.7. Ruggedness**

The precision was carried out on different days to review the approach's intermediate accuracy. The standard solution was injected five times, and the area for all five injections was measured in HPLC. The percentage RSD for the area of five replicate injections was discovered. Tables 9 and 10 show the results.

**Table 9: Bilastine Ruggedness results**

| S.NO | Drug Name | Retention Time | Area | Height |
|---|---|---|---|---|
| 1 | Bilastine | 2.325 | 2165419 | 186958 |
| 2 | Bilastine | 2.315 | 2104588 | 187584 |
| 3 | Bilastine | 2.356 | 2147569 | 185874 |
| 4 | Bilastine | 2.325 | 2158741 | 184583 |
| 5 | Bilastine | 2.331 | 218967 | 184587 |
| Mean | | | 219546 | |
| Std.dev | | | 2569 | |
| %RSD | | | 0.12 | |

**Table10:Montelukast Ruggedness results**

| S.NO | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Montelukast | 4.302 | 1401475 | 95623 |
| 2 | Montelukast | 4.305 | 1401342 | 95152 |
| 3 | Montelukast | 4.325 | 1402412 | 95168 |
| 4 | Montelukast | 4.315 | 1404773 | 95163 |
| 5 | Montelukast | 4.312 | 1408612 | 95153 |
| Mean | | | 1455258 | |
| Std.dev | | | 2345.5 | |
| %RSD | | | 0.15 | |

**3.8. Robustness**

Because of the robustness, deliberate changes in the circulation rate, mobile phase composition, and temperature variation were made to assess the impact on the method. The flow rate ranged from 0.8 ml/min to 1.2 ml/min. Tables 11,12,13, and 14 show the results.

**Table 11: Bilastine System suitability results (Flow rate)**

| S.no. | The flow rate in ml/min | Result of system suitability | |
|---|---|---|---|
| | | USPPlatecount | USPTailing |
| 1 | 0.8 | 883.3 | 1.54 |
| 2 | 1.0 | 1234.0 | 1.2 |
| 3 | 1.2 | 969.2 | 1.5 |

**Table 12: Montelukast system suitability results**

| S.no. | The flow rate in ml/min | Result of system suitability | |
|---|---|---|---|
| | | USP Plate count | USP Tailing |
| 1 | 0.8 | 1747.5 | 1.11 |
| 2 | 1.0 | 1546.2 | 1.1 |
| 3 | 1.2 | 1947.0 | 1.1 |

**Table13:Bilastine System suitability results(Mobilephase)**

| S.no. | Organic composition changes in Mobile Phase | Result of system suitability | |
|---|---|---|---|
| | | USP Platecount | USP Tailing |
| 1 | 10%Less | 883.3 | 1.54 |
| 2 | Actual | 1234.0 | 1.2 |
| 3 | 10%More | 969.2 | 1.5 |

**Table14: System suitability results for Montelukast Mobile phase)**

| S.no. | Organic composition changes in Mobile Phase | Result of system suitability | |
|---|---|---|---|
| | | USP Platecount | USP Tailing |
| 1 | 10%Less | 1747.6 | 1.11 |
| 2 | Actual | 1547.3 | 1.1 |
| 3 | 10%More | 1947.9 | 1.1 |

**3.9. LOD and LOQ:**In the order to estimate the RP-HPLC sensitivity LOD as well as LOQ has to be considered, which were calculated from the calibration curves by the formulas below based on ICH standards. The results are as depicted in table15.

LOD = 3.3σ/S and, LOQ = 10 σ/S, where

σ= Standard deviation of the y-intercept of the regression line,

S = Slope of the calibration curve

**Table 15: LOD, LOQ of Bilastine and Montelukast**

| Drug | LOD | LOQ |
|---|---|---|
| **Bilastine** | 2.94 | 9.87 |
| **Montelukast** | 3.03 | 10.1 |

## CONCLUSION

The HPLC technique recommended in this technique is considered clear-cut, exact, precise, and even sensitive for the estimation of Bilastine&Montelukast in pharmaceutical dose types. For this reason, this approach can comfortably be taken for regular quality assurance evaluation of pure as well as in their pharmaceutical dosage forms.

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**Comment [u4]:** The manuscript Needs more references and advanced


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