

Original Research Article

**Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Bilastine and Montelukast in Tablet Dosage Form**

**ABSTRACT**

**Place of study:** Department of Pharmacy, University College of Technology, Osmania University, Hyderabad, Telangana

**Aim:** The present study is describing about the development of a new RP-HPLC method for the simultaneous estimation of Bilastine and Montelukast in Active Pharmaceutical Ingredient (API) and commercial formulations.

**Methodology:** In the present investigation, an Inertsil ODS C18 column dimensions of 250 mm length x 4.6 Internal diameter x 5-micron particle size has been chosen. Phosphate buffer and acetonitrile were opted as isocratic mobile phase at a ratio of 30:70, with a flow rate of 1 ml/min. The pH of the developed buffer was maintained at 4.6 and the temperature was set at room temperature. The wavelength of Bilastine and Montelukast was observed at 260 nm. For both Bilastine and Montelukast the retention time has been observed at 2.319 and 4.299 minute correspondingly. The percentage purity of both the drugs was found to be 100.6 % and 100.3 % correspondingly. The developed method satisfied all the system suitability parameters for Bilastine and Montelukast and the observed values for theoretical plates were found to be 1.3 and 1.4 respectively, tailing factor were found to be 5117.5 and 3877.8 respectively with a resolution of 9.0.

**Results:** Finally, the method was validated by parameters such as precision, accuracy, robustness and ruggedness. The linearity and range were from 1-5 µg and 100-500 µg concentrations series, the correlation coefficient for both the drugs was noted to be 0.999 while the mean percentage recovery was observed at 100.1 percent & 100.4 percent. The % RSD for repeatability was found to be 0.31 and 0.38. The % RSD of intermediate precision was found to be 0.12 & 0.15 correspondingly. The LOD values were found as 2.94, 3.03 and for the LOQ values were found as 9.87, 10.1.

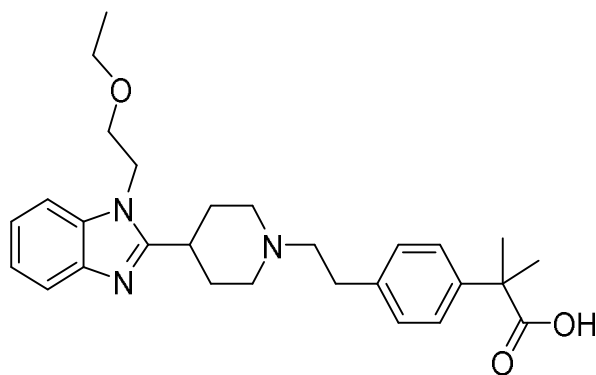
**Conclusion:** The developed method which was validated was observed to be novel, accurate, simple, robust, precise, repeatable for the present study with a suitable RP-HPLC technique to concurrently determine Bilastine and Montelukast present in the commercial formulation. This developed method is useful on daily basis due to their accurate results, reproducibility, robustness for the estimation of samples in routine quality control departments.

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

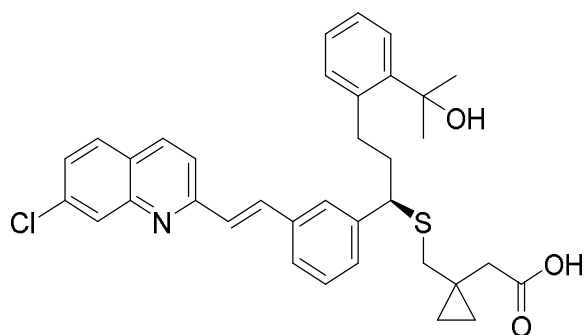
## 1. INTRODUCTION

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 ( $K_i = 64\text{Nm}$ ) [1]. At the time of sensitive feedback pole, cells release histamine and other substances by undergoing degranulation. Bilastine, on adhering to and protecting against the H 1 receptor activation, can slow the progression of acute symptoms by releasing histamine from mast cells[2]. The IUPAC name for the Bilastine was “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 for the Bilastine drug was  $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_3$ . Bilastine has a Molecular Weight of  $463.622\text{ g}\cdot\text{mol}^{-1}$ . Bilastine is soluble in the natural solvent chloroform at a concentration of about 30 mg/ml.

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  $\text{C}_{35}\text{H}_{36}\text{ClNO}_3\text{S}$ . Molecular Weight is  $586.183\text{ g}\cdot\text{mol}^{-1}$ . 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: Bilastine Structure**



**Figure 2: Montelukast Structure**

In the estimation of the Bilastine and Montelukast drug, both individual and other drug combination 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, Comparative to literature survey the developed method runtime is less and Retention times for both the drugs were very less when compare to reported works. 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 samples gifted from the Chandra Labs. Whereas an analytical grade  $\text{KH}_2\text{PO}_4$  was procured from Finerchem limited, then Merck supplied Orthophosphoric acid & water, Methanol required for HPLC analysis were bought from 'Lichrosolv- Merck.'

### 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 C18 250 mm length x 4.6 Internal Diameter x 5  $\mu\text{m}$  particle size, dimensions at 25<sup>0</sup>C temperature level. The isocratic mobile phase contains Phosphate buffer with 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. Phosphate buffer preparation with pH of 4.6**

About 6.8 gm of  $\text{KH}_2\text{PO}_4$  was dissolved in a 1000 ml volume beaker, then till 1000 ml it was diluted with HPLC water, and with orthophosphoric acid, the pH was 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 was taken, and by using an ultrasonic water bath, it was degassed at around 5 mins. Then this above prepared mobile phase solution was filtrated by vacuum purification using a 0.45-micron filter paper.

### **2.3.3. Preparation of Diluent (Blank)**

Mobile phase was used as diluent.

## **2.4. Preparation of the individual Bilastine Standard (Stock Solution)**

The weighed 10mg of Bilastine according to the prescribed standard was added into a 100 ml clean volumetric flask along with 20ml DMF (Dimethyl Formamide). Later, it was dissolved by using ultra sonication by using mobile phase i.e., diluent, it was prepared up to the mark (stock solution). Further 1 ml from the above stock solution was pipetted into a 10 ml volumetric flask and the volume was made up to the mark with the diluent.

## **2.5. Preparation of the individual Montelukast Standard**

The weighed 10mg of Montelukast according to the prescribed standard was added into a 100 ml clean volumetric flask along with 20ml DMF (Dimethyl Formamide). Later, it was dissolved by using ultra sonication by using mobile phase i.e., diluent, it was prepared up to the mark (stock solution). Further 1 ml from the above stock solution was pipetted into a 10 ml volumetric flask and the volume was made up to the make with diluent.

## **2.6. Preparation of Sample Solution:(Tablet)**

About ten tablets were taken into mortar and pestle to obtain fine powder, with weight equivalent to 20 mg of Bilastine& 10 mg Montelukast was taken in a 100mlcompletely dry and clean volumetric flask along with the addition of 70mldiluent and is dissolved usingultra sonication by using mobile phase i.e., diluent it was prepared up to the mark. Further 1 ml from the above stock solution was pipetted into a 10 ml volumetric flask and the volume was made up to the make with diluent.

## 2.7. Procedure

20 $\mu$ L of the sample was infused into the HPLC instrument, and the peak areas for Bilastine and Montelukast were measured. The formulae are then used to calculate the percent Assay.

## 3. RESULTS AND DISCUSSION

The proposed work involves the development and validation of the selected bilastine and montelukast drug determination concurrently with the aid of RP-HPLC method that is in both bulk and combined formulations according to ICH guidelines Q<sub>2</sub> (R1).

### 3.1. Analytical Method Validation

The method was developed and validated by parameters such as precision, accuracy, LOD, LOQ, ruggedness, robustness, system suitability as per the ICH standards.

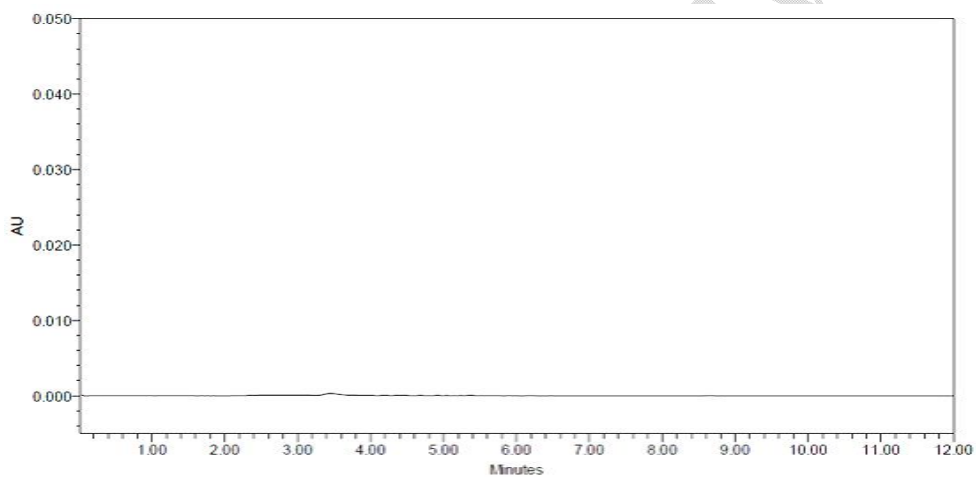


Figure 3: Chromatogram of Blank

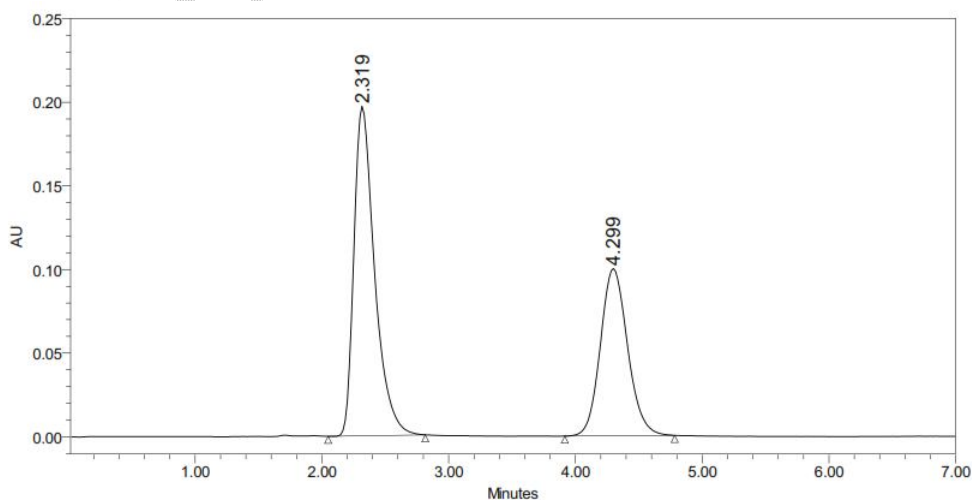
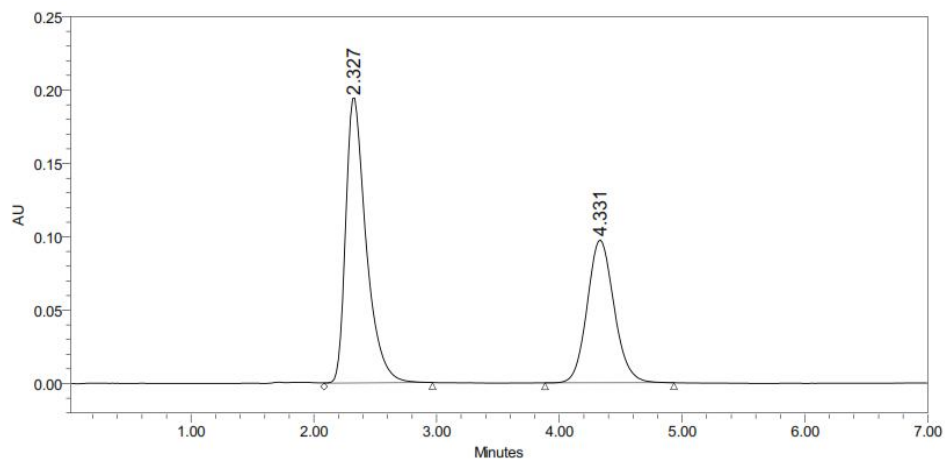


Figure 4: Chromatogram of Standard



**Figure 5: Chromatogram of Sample**

### 3.2. System Suitability Parameters

The system suitability parameters such as retention time, USP theoretical plate count, USP Tailing were assessed. Mobile phase has been sent via column at flow rate at 1ml / min to equilibrate the column at the desired temperature level. The solution of about 20  $\mu$ l has been injected to the column by using Inertsil ODS C18 column with the given dimensions of 250 mm length  $\times$  4.6 mm internal diameter  $\times$  5  $\mu$ m particle size. Sodium phosphate buffer and Acetonitrile at 30:70 ratio was taken as mobile phase at pH of 4.6 in order to reach chromatographic separation between two drugs. The system suitability parameters have been mentioned in the Table 1 [13, 14].

**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. Pharmaceutical formulation assay:

The development of the technique was used to determine the estimation of Bilastine and Montelukast tablet dosage form. The resulting data of the drugs were compared with that of the label claim of the drugs and was represented in the Table 2.

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

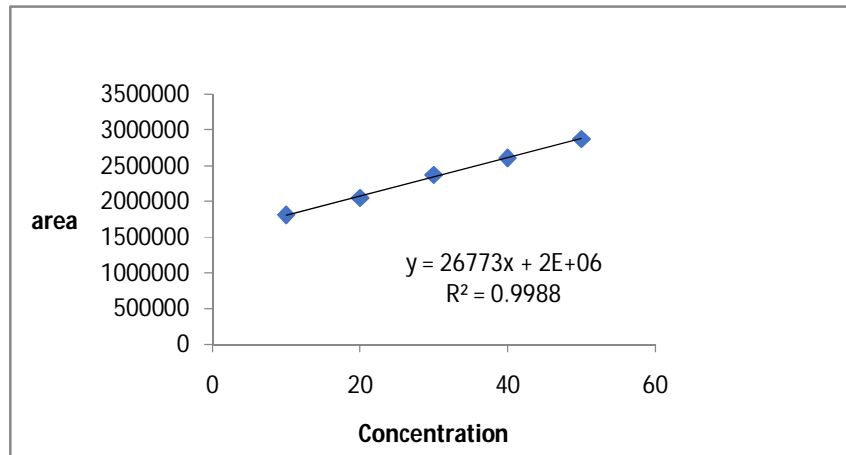
	<b>Label Claim (mg)</b>	<b>% Assay</b>
<b>Bilastine</b>	20	100.6
<b>Montelukast</b>	10	100.3

### 3.4. Linearity and Range

The present work includes the concentration levels from 100 ppm-500 ppm and 1 ppm-5ppm. The concentration the drug is directly proportional to the response of the analyte and at each concentration level, the area of the analyte has been taken to calculate the co-relation co-efficient. The chromatographic system was injected with the drug concentrations mentioned above. The peak areas were calculated for all the concentration levels. The plotting of graph against the concentration vs peak area. The calculated co-relation co-efficient values has been mentioned in Table 3, 4 and graphs in Figure 6 and 7.

**Table 3: Bilastine Linearity Results**

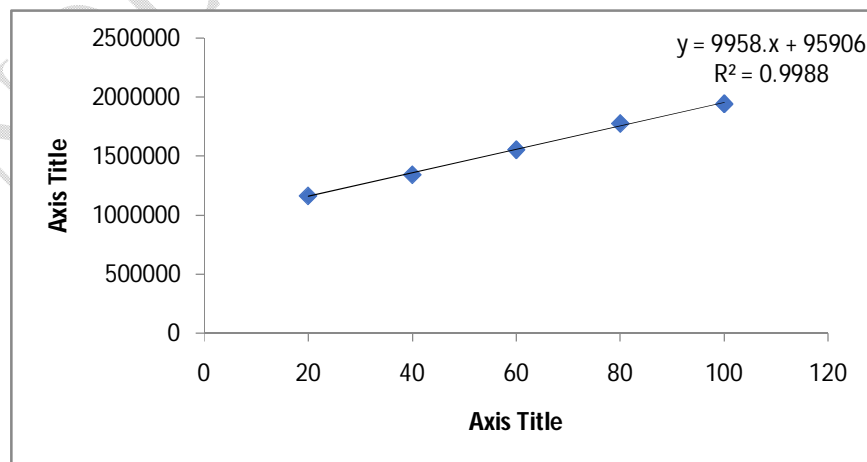
<b>S. No</b>	<b>Sample Name</b>	<b>RT</b>	<b>Area</b>	<b>Height</b>
1	Injected Concentration-1	2.309	1810101	145867
2	Injected Concentration-2	2.322	2044873	176895
3	Injected Concentration-3	2.324	2367122	206674
4	Injected Concentration-4	2.336	2602248	228475
5	Injected Concentration-5	2.340	2869772	259345



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

**Table 4: Montelukast Linearity Results**

S. No	Sample Name	RT	Area	Height
1	Injected Concentration-1	4.304	1164173	74586
2	Injected Concentration-2	4.323	1342555	87689
3	Injected Concentration-3	4.214	1556824	101999
4	Injected Concentration-4	4.524	1774565	117084
5	Injected Concentration-5	4.218	1956421	129409



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

### 3.5. Accuracy study

With the aid of recovery study, the accuracy has to be determined. The recovery study was performed at 3 concentration levels as 50%, 100%, and 150%. The standard solution is further injected into chromatographic instrument. The recovery of Bilastine and Montelukast has been calculated from the known concentrations of spiked amounts. The values are mentioned in table 5,6.

**Table 5: Accuracy Results for Bilastine**

<b>% Concentration at specification level</b>	<b>Bilastine Area</b>	<b>Amount added in mg</b>	<b>Amount retrieved in mg</b>	<b>% Recovery</b>	<b>Mean Recovery</b>
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**

<b>% Concentration at specification level</b>	<b>Bilastine Area</b>	<b>Amount added in mg</b>	<b>Amount retrieved in mg</b>	<b>% Recovery</b>	<b>Mean Recovery</b>
50%	2332744	5	5.10	101.8%	100.4%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

### 3.6. Precision study

Precision was determined with co-efficient variance of six replicate injection based on requirement. The standard solution has been injected five times and gauged in HPLC. From this %RSD of the area for 5 shots has been observed. The outcome has been represented in Table 7 and 8.

**Table 7: Bilastine Precision results**

<b>S. No</b>	<b>Sample Name</b>	<b>RT</b>	<b>Area</b>	<b>Height</b>
1	Bilastine	2.321	2265426	196946
2	Bilastine	2.342	2204572	197589
3	Bilastine	2.354	2247558	195867
4	Bilastine	2.343	2258772	194575
5	Bilastine	2.327	2258956	194576
Mean			2247057	
Std. dev.			24604.24	
% RSD			1.09	

**Table 8: Montelukast Precision results**

<b>S. No</b>	<b>Sample Name</b>	<b>RT</b>	<b>Area</b>	<b>Height</b>
1	Montelukast	4.306	1401345	100264
2	Montelukast	4.304	1401325	100074
3	Montelukast	4.324	1402426	98432
4	Montelukast	4.314	1404725	98151
5	Montelukast	4.316	1408632	98145
Mean			1403691	
Std. dev.			3089.682	
% RSD			0.22	

### 3.7. Ruggedness

On various days, different analysts using various instruments the precision was performed to estimate the ruggedness. The area was determined for the standard solutions that was injected for 5 times and the % RSD was calculated. The result has been depicted in table 9, 10.

**Table 9: Bilastine Ruggedness results**

S. No	Sample Name	RT	Area	Height
1	Bilastine	2.323	2165443	186946
2	Bilastine	2.318	2104572	187572
3	Bilastine	2.346	2147553	185865
4	Bilastine	2.353	2158725	184573
5	Bilastine	2.334	2189641	184576
Mean			2153187	
Std. dev			31244.73	
% RSD			1.45	

**Table 10: Montelukast Ruggedness results**

S. No	Sample Name	RT	Area	Height
1	Montelukast	4.312	1401467	
2	Montelukast	4.316	1401354	
3	Montelukast	4.324	1402424	
4	Montelukast	4.325	1404765	

5	Montelukast	4.316	1408611	
Mean			1403724	
Std. dev			3056.444	
% RSD			0.21	

### 3.8. Robustness

The robustness has been calculated by modifying the flow rate, composition of the mobile phase, pH according to the optimized conditions. Flow rate has been lowered to 0.8 ml / min and elevated to 1.2 ml / min. Results were mentioned in table 11, 12,13& 14.

**Table 11: System Suitability Results of Bilastine (Flow Rate)**

S. No	The Flow Rate in ml/min	Result of system suitability	
		USP theoretical plate count	USP Tailing Factor
1.	0.8	883.3	1.54
2.	1.0	1234.0	1.2
3.	1.2	969.2	1.5

**Table 12: System Suitability Results of Montelukast**

S.No.	The flow rate in ml/min	System Suitability results	
		USP theoretical Plate count	USP Tailing Factor
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 values (Mobile phase)**

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

**Table14: System Suitability values for Montelukast Mobile phase)**

S.No.	Organic Composition changes in Mobile Phase	Result of system suitability	
		USP theoretical Platecount	USP Tailing Factor
1.	10% less	1747.6	1.11
2.	Actual	1547.3	1.1
3.	10% More	1947.9	1.1

**3.9. Limit of Detection and Limit of Quantification:** The lowest concentration of analyte has been injected for calculating the detection level and quantification level. The LOD and LOQ levels were calculated from the calibration curves by using the below mentioned formulas based on ICH standards. The results are shown in the table 15.

$$\text{LOD} = 3.3 \times \text{SD/slope},$$

$$\text{LOQ} = 10 \times \text{SD/slope}$$

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

Drug	LOD	LOQ
<b>Bilastine</b>	2.94	9.87
<b>Montelukast</b>	3.03	10.1

## CONCLUSION

As per the ICH standards the RP-HPLC method has been developed and validated and the method has been rapid, sensitive, simple, specific, accurate, economic, ethical, cost effective, precise, robust for concurrent estimation and quantification of Bilastine and Montelukast in marketed dosage form. By using this method analysis can be carried out for the regular batch analysis of pure and pharmaceutical dosage forms and quality monitoring. The final results of the recovery studies were found to be accurate indicating no interferences from the excipients.

## REFERENCES

1. Wang XY, Lim-Jurado M, Prepageran N, Tantilipikorn P, Wang DY. Treatment of allergic rhinitis and urticaria: a review of the newest antihistamine drug bilastine. *Therapeutics and clinical risk management*. 2016;12:585-597.
2. Lucero ML, Artech JK, Sommer EW, Casadesus A. Preclinical toxicity profile of oral bilastine. *Drug and Chemical Toxicology*. 2012;35(sup1): 25-33.
3. Narasimhan K. Difficult-to-Treat and Severe Asthma: Management Strategies. *American Family Physician*. 2021;103(5):286-290.
4. Lu CY, Zhang F, Lakoma MD, Butler MG, Fung V, Larkin, EK, Kharbanda EO, Vollmer WM, Lieu T, Soumerai SB. Asthma treatments and mental health visits after a Food and Drug Administration label change for leukotriene inhibitors. *Clinical therapeutics*. 2015; 37(6):1280-1291.
5. Rana N, Rajesh K, Patel N, Patel P, Limbachiya U, Pasha Y. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Montelukast Sodium and Ebastine in Tablet Dosage Form. *Indian journal of pharmaceutical sciences*. 2013;75:599-602.
6. Pardeshi PJ, Gaware V, Dhamak K. Development and Validation of RP-HPLC Method for the Estimation of Bilastine from bulk and Formulation. *Asian Journal of Pharmaceutical Analysis*. 2020;10(2):109-111.
7. Shrikrishna B, Nisharani R. Analytical method development and validation for simultaneous estimation of Montelukast and Ebastine by HPLC. *Research Journal of Pharmacy and Technology*. 2015; 8 (1):1-5.
8. Tamilselvi N, Sruthi K. Development of validated HPLC method for simultaneous estimation of Fexofenadine hydrochloride and Montelukast sodium in tablet dosage form. *International Journal of Pharmaceutical Sciences and Research*. 2012;3(12):48-76.

9. Umesh C, Manish K, Shrestha S, Pankaj G. Development and validation of RP-HPLC method for In-Vitro Dissolution Testing of Bilastine and Montelukast Sodium Tablets. International Journal of Pharmaceutical Sciences and Drug Research. 2021;13(3).
10. Saloni K, Nachiketa P, Neetu D. Advancement and Validation of Analytical Methods for Synchronised Estimation of Bilastine as well as Montelukast Salt in Consolidated Tablet Dose Kind. International Journal of All Research Study Education as well as Scientific Approaches.2021;9(4):28-30.
11. Amaresha S, RakeshK. RP-UPLC approach growth and also recognition for the synchronised estimate of montelukast and ebastine in bulk as well as pharmaceutical dose kind. Int. J. of Pharmacy and also Analytical Research Study .2018;7(1):96-105.
12. Pallavi K, BabuS. Validated UV Spectroscopic method for estimation of Montelukast sodium from bulk and tablet formulations. International Journal of Advances in Pharmacy, Biology and Chemistry. 2012; vol 1(2) 104-111.
13. Sanjeev A, Bhaskar S. Development and Validation of RP-HPLC Method in Simultaneous Estimation of Lamivudine and Abacavir as Tablet Dosage Form. JOURNAL OF CRITICAL REVIEWS. 2020; vol 7(2) 1358-1370.
14. Sanjeev A, Narmada V. Stability Indicating Method Development And Validation, Stress Degradation Studies For Dacarbazine By Using rp-Hplc. Neuro Quantology. 2022; Vol 20(9) 6270-6278.