Chemical Science International Journal

Volume 32, Issue 3, Page 52-61, 2023; Article no.CSIJ.99455
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

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

Narmada Vallakeerthi [a*], Rachala Swetha [a], T. Tejaswi [a], A. Ravinder Nath [a], K. Bhavyasri [b], N. Naresh Reddy [c] and P. Muralidhar Reddy [c*]

[a] Department of Pharmacy, University College of Technology, Osmania University, Hyderabad-500007, Telangana, India.
[b] Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad-500027, India.
[c] Department of Chemistry, University College of Science, Osmania University, Hyderabad-500007, Telangana, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2023/v32i3848

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99455

Original Research Article

Received: 07/03/2023
Accepted: 09/05/2023
Published: 16/06/2023

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 (AI) and commercial formulations.

**Corresponding author: E-mail: rishikaravi@gmail.com;*

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 1-5µg 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 (Ki = 64Nm) [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 H1 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 $C_{28}H_{37}N_3O_3$. 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.

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 (Cysteinyl Leukotrienes) 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 name 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].

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.

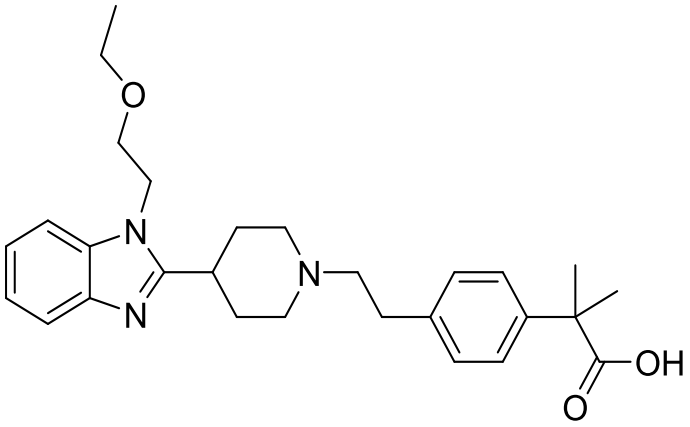

Fig. 1. Bilastine structure

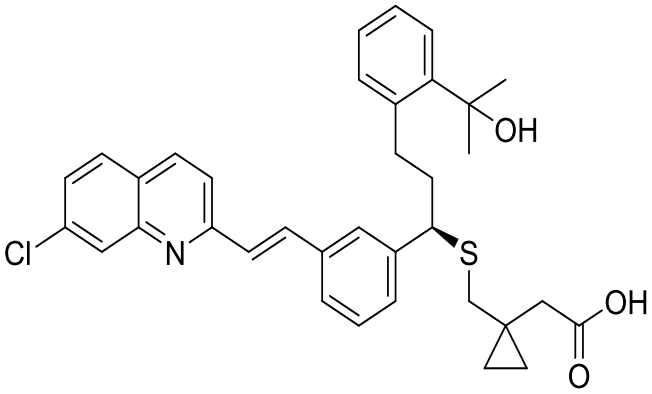

Fig. 2. Montelukast structure

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Bilastine and Montelukast samples were gifted from the JRS Labs. Whereas an analytical grade KH_2PO_4 was procured from Finer chem limited, 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 µm particle size, dimensions at 25^0C 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 KH_2PO_4 was dissolved in a volume beaker, then in 1000 ml volumetric flask it was made up-to the mark 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 10 mg of Bilastine according to the prescribed standard was added into a 100 ml clean volumetric flask along with 20 ml DMF (Dimethyl Formamide). Later, it was dissolved in using mobile phase i.e., diluent by using ultra sonication. This solution was prepared up to the mark and taken as stock solution (1000µg/ml). Further from the stock solution an ml was pipetted to 10 ml volumetric flask and the volume using diluent was made to the mark (100µg/ml).

2.5 Preparation of the Individual Montelukast Standard (Stock Solution)

The weighed 10 mg 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 in using mobile phase i.e., diluent by using ultra sonication. This solution was prepared up to the mark and taken as stock solution (1000µg/ml). Further from the stock solution an ml was pipetted to 10 ml volumetric flask and the volume using diluent was made to the mark (100µg/ml).

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 100 ml completely dry and clean volumetric flask, in addition 70 ml diluent was added to dissolve using ultra sonication. Later, it was prepared up to the mark. Further from the stock solution an ml was pipetted to 10 ml volumetric flask and the volume using diluent was made to the mark (100µg/ml).

2.7 Procedure

20 µL 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_2 (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.

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 µl has been injected to the column by using Inertsil ODS C18 column with the given dimensions of 250 mm length × 4.6 mm internal diameter × 5 µm 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

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

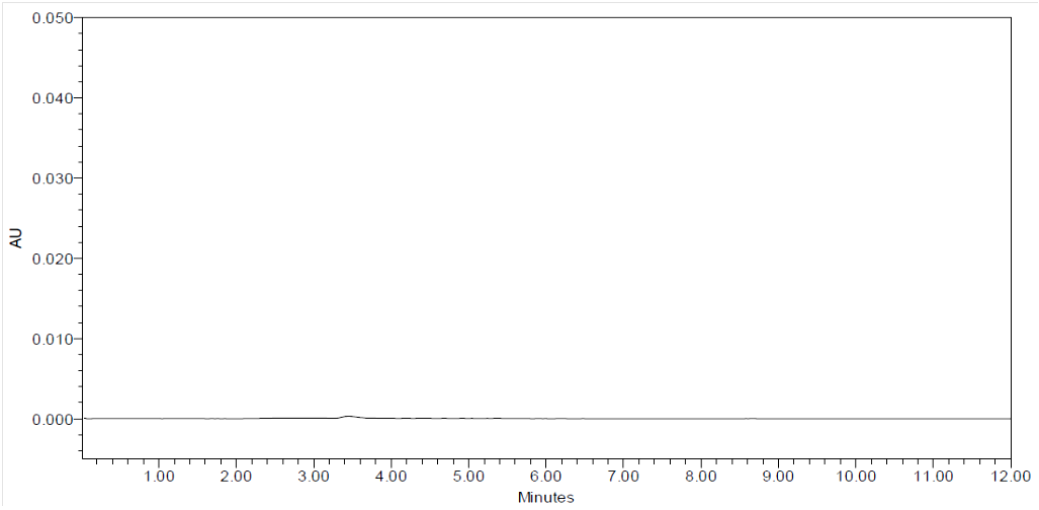

Fig. 3. Chromatogram of blank

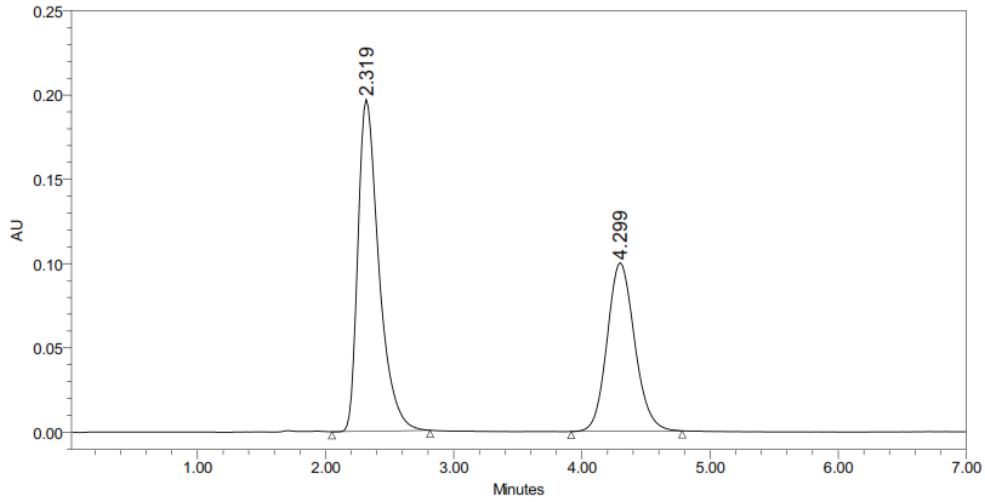

Fig. 4. Chromatogram of standard

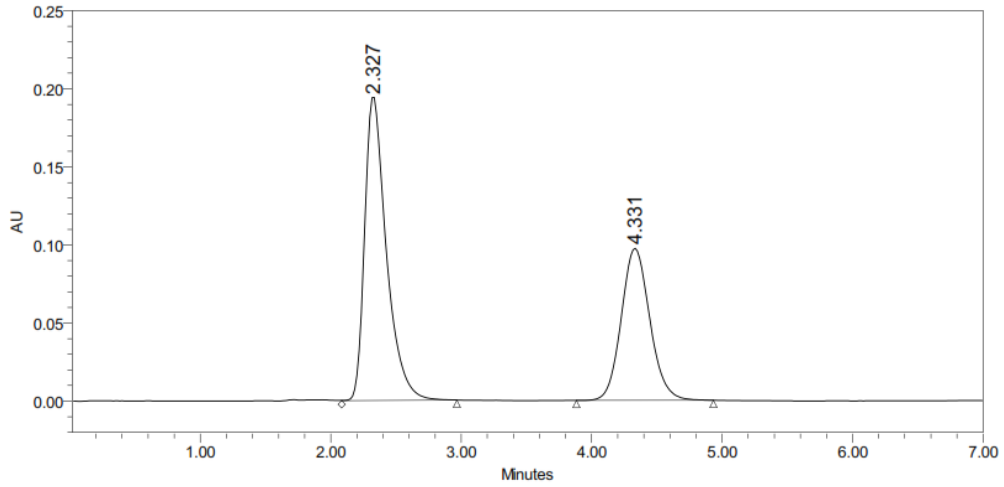

Fig. 5. Chromatogram of sample

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 Tables 3, 4 and graphs in Figs. 6 and 7.

Table 3. Bilastine linearity results

S. No	Sample Name	RT	Area	Height
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

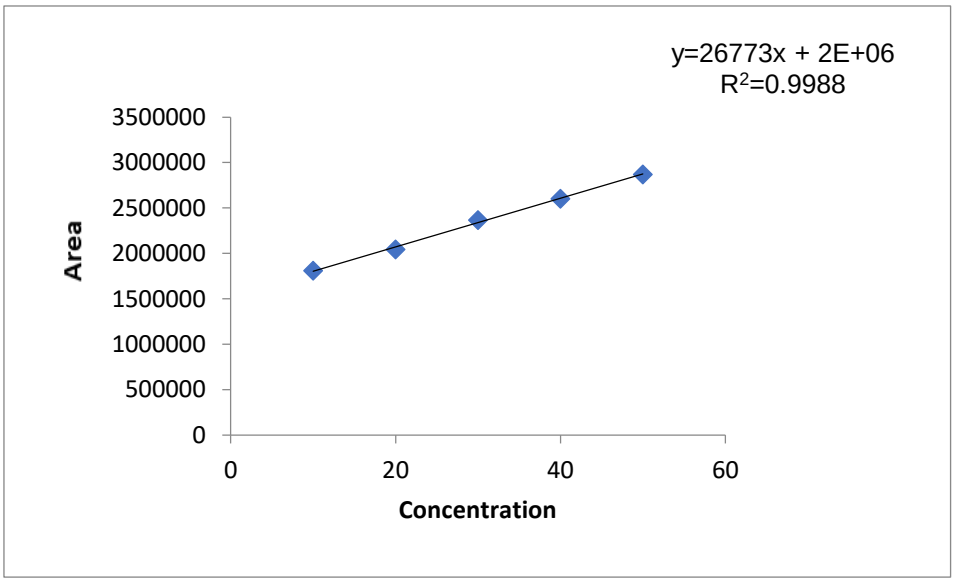

Fig. 6. Linearity graph for Bilastine

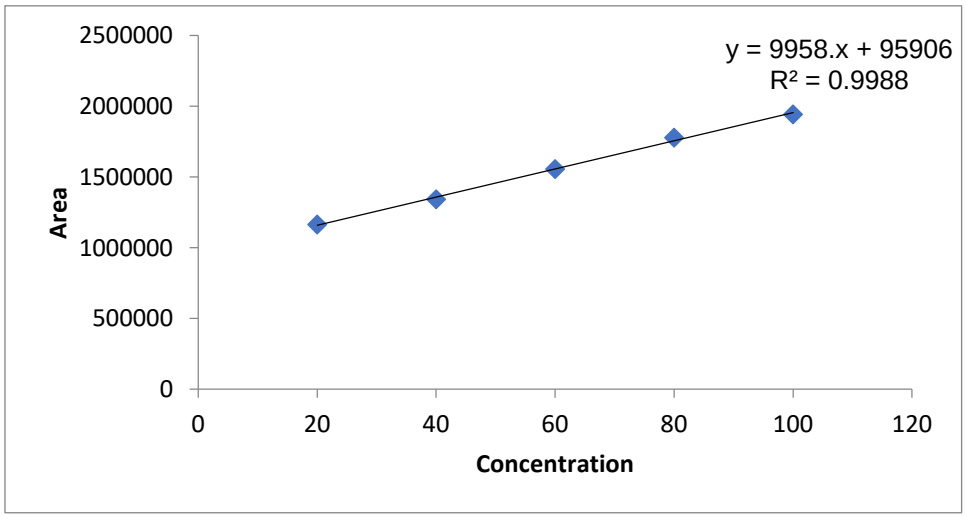

Fig. 7. Linearity graph for Montelukast

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

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 Tables 5 & 6.

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 times has been observed. The outcome has been represented in Tables 7 and 8.

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 5. Accuracy results for Bilastine

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

Table 6. Accuracy results for Montelukast

% Concentration at specification 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%	

Table 7. Bilastine precision results

S. No	Sample Name	RT	Area	Height
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

S. No	Sample Name	RT	Area	Height
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	

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	95634
2	Montelukast	4.316	1401354	95251
3	Montelukast	4.324	1402424	95182
4	Montelukast	4.325	1404765	95172
5	Montelukast	4.316	1408611	95163
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, according to the optimized conditions. Flow rate has been lowered to 0.8 ml / min and elevated to 1.2 ml/ min. Mobile phase has been reduced to 10% and increased to 10 %. Results were mentioned in Tables 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 (Flow Rate)

S. No.	The Flow Rate in ml/min	Result of system suitability	
		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

Table 13. System Suitability Results for Bilastine (Mobile phase)

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

Table 14. System Suitability Results for Montelukast (Mobile phase)

S. No.	Organic Composition changes in Mobile Phase	Result of system suitability	
		USP theoretical Plate count	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 LOD and LOQ Results

The analyte's 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.

$$LOD = 3.3 \times SD/slope,$$

$$LOQ = 10 \times SD/slope$$

Table 15. LOD, LOQ Results of Bilastine and Montelukast

Drug	LOD	LOQ
Bilastine	2.94	9.87
Montelukast	3.03	10.1

4. 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.

ACKNOWLEDGEMENTS

The authors are grateful to JRS labs in Hyderabad for providing pure drug samples, as well as the Head, Dean, and Principal of the Department of Pharmacy, University College of Technology, Osmania University, for allowing them to conduct this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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, Arteche 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, LieuT, 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, LimbachiyaU, 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. PardeshiPJ, GawareV, 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. TamilselviN, 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; 1(2):104-111.
13. Sanjeev A, Bhaskar S. Development and Validation of RP-HPLC Method in Simultaneous Estimationof Lamivudine and Abacavir as Tablet Dosage Form. JOURNAL OF CRITICAL REVIEWS. 2020;7(2):1358-1370.
14. Sanjeev A, Narmada V. Stability Indicating Method Development And Validation, Stress Degradation Studies For Dacarbazine By Usingrp-Hplc. Neuro Quantology. 2022;20(9):6270-6278.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/99455