A New RP-HPLC Assay Method Development and Validation for Simultaneous Quantitation of Bilastine and Montelukast in Bulk and Tablet Dosage Form

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ABSTRACT

Introduction: Estimation of individual drug components from the multidrug combination tablet is considered a quite tedious task. Moreover, the combination of Bilastine and Montelukast in the oral tablet dosage form is recently launched in the market for the treatment of allergic rhinitis associated with asthma in adults. Further, no reliable method with precise and accurate quantification is available for simultaneous estimation of both drugs.

Aim: Development and validation of a rapid, simple, precise yet reliable method for simultaneous estimation of Bilastine and Montelukast from the bulk mixture and a tablet formulation.

Methodology: The analysis was performed on a high-performance liquid chromatographic system using Hypersil BDS C-18 column as a stationary phase and triethylamine buffer: Acetonitrile as a mobile phase operated in a gradient mode. The flow rate of the mobile phase was 1.0 ml/min with 40°C column oven temperature and 10°C autosampler temperature. Injection volume for all samples was fixed at 10 µl and the spectrum was recorded at 220nm.

Result: The retention time of Bilastine and Montelukast was 1.67 and 7.43 min respectively and the run time for 1 sample analysis was 13 min. The calibration standards show a good linear relationship with R² value of 0.99966 for Bilastine and 0.99929 for Montelukast.

Conclusion: The observations recorded proved that the proposed analytical method is reliable for accurate, precise and rapid quantification of both drugs from the bulk drug as well as pharmaceutical dosage forms.

Key Words: Bilastine, Force degradation, ICH Guidelines, Montelukast, RP-HPLC, Validation

INTRODUCTION

Asthma and allergic rhinitis (AR) are the two commonest diseases that affect millions of people around the world. Seasonal allergic rhinoconjunctivitis (SARC) and asthma trigger the histamine and cysteinyl leukotrienes (CysLTs) which are causative agents of inflammation.¹ To minimize the morbidity and mortality associated with asthma, safe and appropriate therapies are needed. The cells responsible for inflammation such as mast cells and basophils develop leukotrienes towards the early phase, and eosinophils and macrophages produce them in the next phase. Leukotriene C₄, D₄, and E₄ are cysteinyl leukotrienes that contribute towards the production of mediators of inflammation in the oesophageal airways.^{2, 3}

Montelukast is a leukotriene receptor antagonist used to treat asthma and recommended to prevent and treat asthma in patients with age group 2 and more.⁴ Montelukast can control asthma in adults if symptoms continue by using sporadic short-acting β -agonists and the patient is unable to use an inhaled corticosteroid. It's also reported to relieve seasonal AR problems in a patient's age group of 15 and up to where most therapies aren't functioning or aren't accepted.^{3, 5, 6} Bilastine is the latest H₁-antihistaminic drug that is recently got approval to treat AR and chronic urticaria as a symptomatic treatment.⁷ Literature survey reveals that Montelukast API and the commercial dosage form are official in Indian Pharmacopoeia.⁸ Bilastine exemplifies the success of antihistamine studies in terms of effectiveness and safety. Many diseases related to allergies, such as allergic rhino-conjunctivitis

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and urticaria, can be treated with H1-antihistamines because of histamine's involvement in allergic responses.⁹ Bilastine is antihistamine and anti-allergic, having larger tolerance for H1-receptors and negligible susceptibility to other receptor types. The drug has a quick rapid action with an extended half-life.^{9, 10}

An extensive literature review showed that many spectroscopic methods including UV spectrophotometer and high-performance liquid chromatography are available for the estimation of Bilastine and Montelukast in pharmaceutical dosage forms either alone or with other drug combinations reported are UV spectrophotometry¹¹, HPLC¹²⁻¹³, HPLC/PDA,¹⁴ LC-MS,¹⁵ and HPTLC^{16, 17} methods. However, no method for simultaneously estimating Bilastine and Montelukast has been documented so far.

Bilastine and Montelukast Sodium (**Figure 1**), a novel formulation, is recently launched in the market under the brand names of MONTEK BL Tablet 10'S, BILAZEST M Tablet 10's by Sun Pharmaceuticals Pvt Ltd, and Abbott Healthcare Ltd respectively. Each of these combinations contains Bilastine 20 mg and Montelukast 10 mg.¹⁸

The present investigation presents a successful attempt to develop a short, simple, precise, yet repeatable simultaneous estimation of Bilastine and Montelukast drugs using HPLC.

MATERIAL AND METHODS

Chemical and reagent

Bilastine and Montelukast Sodium working standards were generously gifted from M/s. Synokem Pharmaceuticals Limited, Haridwar. Combination tablets of Bilastine and Montelukast Sodium were prepared with a label claim of 20 mg and 10 mg respectively. The components of the mobile phase, i.e., Acetonitrile and Milli-Q water were of HPLC grade. The other reagents like Triethylamine, Orthophosphoric acid, NaOH, Hydrochloric Acid, Hydrogen Peroxide used were of analytical grade from the analytical laboratory of M/s. Kusum Healthcare Pvt. Ltd., Rajasthan, India.

Instrumentation

Calibrated HPLC (Make: Shimadzu LC-2010 CHT), analytical weighing balance, sonicator, oven, and pH meter was used during the experiment.

Chromatographic Conditions

Shimadzu LC-2010 HPLC with Hypersil BDS C-18 Column (100×4.6 mm, 3µm particle size) as a stationary phase and 0.1% v/v Triethylamine buffer of pH-3.00: Acetonitrile as a mobile phase in gradient mode. The buffer was filtered through a 0.45 µm membrane filter and degassed by sonication. The mobile phase solvent was pumped at 1ml/min, the

column oven temperature was kept at 40°C and λ max was set at 220 nm. Injection volume for all samples was fixed at 10 µl and under these conditions; the run time was 13 min.

Preparation of solutions

Preparation of Diluent

Acetonitrile: Water, 1:1, and same used as blank solution

Preparation of Buffer Solution

Accurately added 3 ml of Triethylamine into 3000 ml of HPLC water, mixed well and pH was adjusted to 3.00 with orthophosphoric acid. Filtered the buffer through a 0.45 µm membrane filter and degassed.

Preparation of Stock solution and Standard Solution

The stock solution of Bilastine and Montelukast was prepared by transferring the 101.02 mg of Bilastine and 52.67 mg of Montelukast Sodium (equivalent to 50.77 mg of Montelukast) into a 100 ml volumetric flask. About 50 ml of acetonitrile was added to the flask and sonicated at a temperature below 20°C, with occasional shaking and the final volume was made up to 100 ml. Further, 5 ml of this stock solution was transferred into 25 ml of volumetric flask and the final volume was made up with diluent to get a final concentration of 200.14 µg/ml of Bilastine and 100.75 µg/ml of Montelukast (after potency correction, 99.06% for Bilastine and 99.23% for Montelukast) and used as a standard solution after filtration through 0.45 µm nylon syringe filter.

Preparation of placebo solution

Accurately weighed and transferred the placebo powder equivalent to 200 mg of Bilastine (about 2000 mg placebo, the average weight of 1 tablet is 230 mg) into a 200 ml volumetric flask. About 100 ml of acetonitrile was poured and sonicated for 20 minutes below 20°C and the final volume was made up with diluent. A 5 ml of aliquot was transferred in a 25 ml flask and the volume was made up with diluent, mixed and filtered through a 0.45 µm nylon syringe filter and used as a placebo solution.

Preparation of sample solution

Ten intact tablets of Bilastine and Montelukast Sodium was put in a 200 ml volumetric flask, about 100 ml of acetonitrile was added and sonicated for 20 minutes below 20°C, with occasional shaking. The final volume was made up after cooling the solution. A 5 ml solution was transferred into a 25 ml volumetric flask and volume made up with diluent and filtered through a 0.45 µm nylon syringe filter and used as a sample solution.

Analytical validation of the method

The developed method was validated according to ICH Guidelines (ICH Q2 R1).¹⁹ The parameters assessed were

specificity, linearity, precision (system/method/intermediate), accuracy (recovery), stability in analytical solution, robustness, the limit of detection (LOD) and limit of quantitation (LOQ) and forced degradation study.

System suitability acceptance criteria

This test confirms the resolution and reproducibility of the chromatographic system used for analysis.^[19] Six replicate injections of standard solution were injected and the chromatogram was recorded. The number of theoretical plates should be greater than 2000, the tailing factor should be less than 2, the resolution should be more than 2 and the % RSD of six injections should be less than 2.

Specificity

The capacity of the developed method was measured in terms to analyze response in presence of any interfering factor (like degradation product) is known as specificity.^[19] It was performed by injecting a single injection of a blank solution, common placebo, standard solution and sample solution.

Force degradation Study

Forced degradation study was carried out under conditions like acidic, basic, oxidation, UV light, thermal and humidity exposure. The drug product sample and placebo (control sample) were allowed to degrade. To perform acid hydrolysis, 10 ml samples of the drug product and placebo were treated with 1 ml of 0.1N HCl and stored under water bath at 80°C for 1 hr, then neutralize with 1 ml 0.1N NaOH. To conduct base hydrolysis, 10 ml samples of the drug product and placebo were treated with 1 ml of 0.1N NaOH and stored under a water bath at 80°C for 1 hr and then neutralize with 1 ml 0.1N HCL. The oxidation study was carried out by treating the 10 ml drug product sample and placebo with 1 ml of 0.3% H₂O₂ for 1 hr. For thermal degradation, powdered drug products and placebo were put into a hot air oven at 80°C for up to 8 hr. The humidity studies were carried out by exposing the powdered sample of the drug product and placebo for 8 hr above 75% RH. For photolytic degradation, powdered samples of the drug product and placebo were placed under UV light at 254nm in a UV chamber for 8 hr. Further, these samples were diluted with diluents to get a range equivalent to 200µg/ml of Bilastine and 100µg/ml of Montelukast. A single injection of each control sample and each degraded solution were injected and chromatograms were recorded and analyzed.

Precision

It is the measure of how close the agreement between observations obtained from practical samplings of the same sample under different conditions. The precision of an analytical method was further demonstrated by system precision, method precision, and intermediate precision.

System Precision

The blank solution was injected in a single followed by six replicates injection of standard solution and chromatograms were recorded. The % RSD of these six injections was calculated.

Method precision

After establishing system suitability as per method, the blank solution was injected in single, and six sample solutions that were prepared independently were injected in duplicate. The mean value of duplicate injection was used for the calculation of the result.

Intermediate Precision

A separate analyst completed the intermediate precision analysis on a different day, using a different instrument with the same chromatographic condition. For intermediate precision, a blank solution and six sample solutions from the same formulation batch (as used for process precision) were prepared. After assessing the suitability, a blank solution was injected once and six sample solutions, each prepared separately, were injected in duplicate. For six sample solutions, the % RSD should be within requirements.

Ruggedness

An analytical method can reproduce the previous results in different laboratories or different circumstances without the occurrence of unexpected differences in the obtained results. Results obtained from method precision and intermediate precision were used for the calculation of ruggedness.

Linearity and Range

Appropriate aliquots from a stock solution of Bilastine and Montelukast were taken in different ml of volumetric flasks and diluted up to the mark with diluent to obtained final concentrations of 100.07-300.21 µg/ml and 50.38-151.14 µg/ml of Bilastine and Montelukast respectively and injected in duplicate.

Limit of detection (LOD)/Limit of quantitation (LOQ)

LOD is the least amount of analyte which provides a recordable response, whereas the LOQ is the least quantity of analyte that can be quantified accurately and precisely. The LOD and LOQ were calculated using a signal: noise ratio of 3:1 for LOD and 10:1 for LOQ.

Accuracy (recovery)

A triplicate sample solution was prepared for 80, 100, and 120% levels for label claim of Bilastine 20mg and Montelukast 10mg strength. After establishing system suitability, Injections of accuracy samples were given in duplicate. Accu-

racy was determined against their respective standard weight taken while preparation of accuracy sample.

Robustness

Robustness study was executed by doing some intentional changes in the chromatographic conditions i.e., altering the flow rate of mobile phase ($\pm 10\%$), column oven temperature ($\pm 5^\circ\text{C}$), and Wavelength ($\pm 5\text{ nm}$). After establishing system suitability as per the chromatographic method, again system suitability was established with the altered method. Blank and three method precision samples were injected in a single for each robustness parameter studied.

Stability in analytical solution

The stability of the analytical solution was carried out by injecting sample solution (Method precision sample) initially and at specific time intervals to monitor change in response with time.

RESULT AND DISCUSSION

Method optimization

To develop an accurate yet fast method for simultaneous estimation of Bilastine and Montelukast from the bulk mixture and tablet formulation for anti-allergic treatment, we have made a successful method after attempting several hits and trials on different compositions of mobile phases, flow rate, column composition, this optimized method was found suitable for validation. The mobile phase comprising Acetonitrile: 0.1% v/v triethylamine buffer-pH 3.00, at 1.0 ml/min with gradient mode was found optimized. The flow rate was optimized to give sharp and distinguished peaks with the lowest tailing factor. The RT of Bilastine and Montelukast was observed at 1.68 and 7.38 min, respectively, with a resolution of 42.2. (Table 1 and Figure 2)

System suitability

The theoretical plate count, tailing factor, resolution and %RSD was calculated and obtained results were presented in Table 2.

Specificity

After reviewing the chromatograms, it can be concluded that no factorial interference was observed at the retention time of Bilastine and Montelukast peak in the blank chromatogram (Figure 3A) and in the placebo sample solution (Figure 3B).

Forced degradation

Forced degradation studies were executed as per the methodology mentioned earlier. The summary of the results obtained for degradation studies is presented in Table 3.

Figure 4, Figure 6 and Figure 8 show Montelukast is highly susceptible to acidic, peroxide and photolytic degradation, whereas Bilastine is susceptible only to acidic degradation. Degradation products were seen in peroxide and photolytic chromatograms and were well separated from main analytes.

System Precision

The %RSD of six replicates injection of the standard solution was 0.1% for Bilastine and 0.0% for Montelukast.

Method Precision

Mean area of two injections used for calculation of % of label claim. The value of % RSD of six method precision samples was presented in Table 4.

Intermediate precision

Similarly calculated as per method precision and %RSD was calculated and presented in Table 4.

Ruggedness

The overall relative standard deviation of twelve assay values; six method precision samples and six intermediate precision samples were calculated and presented in Table 4.

Linearity and Range

Calibration standards of various concentrations i.e., 50%, 70%, 90%, 100%, 120% and 150% were prepared from the standard stock solution and diluted with diluents to get the concentration of 100.07-300.21 $\mu\text{g/ml}$ and 50.38-151.14 $\mu\text{g/ml}$ of Bilastine and Montelukast respectively. The linear regression data of the calibration curve (Figure 10A and 10B) shows a good linear relationship between area vs concentration. The obtained results were presented in Table 5.

LOD and LOQ

The limit of detection and limit of quantitation were calculated by using signal to noise ratio and the obtained results were presented in Table 5.

Accuracy (recovery)

The accuracy of the assay method was calculated at three levels i.e., 80%, 100% and 120% of sample concentration and the obtained result were presented in Table 6.

Robustness

Robustness of the analytical method was carried out as per methodology and overall mean and overall SD was calculated by from % assay value of six method precision plus three % assay value of each robustness parameter. From the overall mean, overall SD, mean of overall % RSD was calculated and obtained result was presented in Table 7A and Table 7B.

Assay of Drug Product

Assay % of the sample solution was calculated from the average of % label claim of Bilastine and Montelukast content found in the six-method precision sample and obtained results are 100.6% for Bilastine and 101.5% for Montelukast.

Stability in analytical solution

Stability in analytical solution was carried out by injecting method precision sample at specific time interval and % difference in area count concerning the area count of first method precision sample. Also, we have observed no significant change in RT, TF, TP and resolution over 27 hours and obtained results were presented in **Table 8**.

CONCLUSION

As per the present investigations, a unique stability-indicating chromatographic method was developed to achieve a simultaneous estimation of Bilastine and Montelukast. The method was validated with accord to official guidelines and found to be accurate, simple yet precise with good reproducibility. The method showed good sensitivity towards estimating Bilastine and Montelukast in combination with no co-eluting peaks at the RT of Bilastine and Montelukast. Assay % was found to be 100.6% for Bilastine and 101.5% for Montelukast. Montelukast drug was found susceptible to oxidation and photolytic degradation. The prepared sample solution was stable in aqueous up to 27 hours and calibration standards show a good linear relationship. A smaller run time of 13 min for the estimation of both drugs will increase the acceptance of the method for routine analysis of quality control samples.

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Author Contributions

Chandra Umesh: Overall responsibility

Kumar Manish: Assist to perform some activity of the work

Sharma Shrestha, Gupta Pankaj: writing and review.

REFERENCES

- Lavorini F, Matucci A, Rossi O, Pistolesi M, SKY study investigators. Concomitant bilastine and montelukast as additive therapy for seasonal allergic rhinoconjunctivitis and mild-to-moderate asthma. The SKY study. *Allergy*. 2020 Mar;75(3):675.
- Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis*. 1990 Jul 1; 142(1):112-9.
- Neighbour H, McIvor A. Montelukast in the treatment of asthma and allergic rhinitis. *Clin Pract*. 2013 May 1;10(3):257.
- Matsuse H, Kohno S. Leukotriene receptor antagonists pranlukast and montelukast for treating asthma. *Exp Opin on pharmacothera*. 2014 Feb 1;15(3):353-63.
- Peters-Golden M, Henderson Jr WR. The role of leukotrienes in allergic rhinitis. *Annals of Allergy, Asthma & Immunology*. 2005 Jun 1;94(6):609-18.
- Rajashekar YR, Shobha SN. Randomized prospective double-blind comparative clinical study of ebastine and its combined preparation of montelukast in persistent allergic rhinitis. *Nat J Physio Pharma Pharmacol*. 2018;8(3):319-24.
- Bousquet J. World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001; 108:s147-334.
- Indian Pharmacopoeia, Vol. 2. New Delhi: Controller of Publication, Govt. of India, Ministry of Health and Family Welfare; 2010. p. 1704-6.
- Corcóstegui R, Labeaga L, Innerarity A, Berisa A, Orjales A. Preclinical pharmacology of bilastine, a new selective histamine H1 receptor antagonist. *Drugs in R & D*. 2005 Nov;6(6):371-84.
- Jauregizar N, de la Fuente L, Lucero ML, Sologuren A, Leal N, Rodríguez M. Pharmacokinetic-pharmacodynamic modelling of the antihistaminic (H1) Effect of Bilastine. *Clinpharmacokinet*. 2009 Aug; 48(8):543-54.
- Patel DJ, Patel SA, Patel SK. Simultaneous determination of montelukast sodium and bambuterol hydrochloride in tablet dosage form by ultraviolet spectrophotometry (Dual wavelength method). *Int J Pharm Biol Res*. 2010;1:71-5.
- Pawar V, Pai S, Rao GK. Development and validation of UV spectrophotometric method for simultaneous estimation of montelukast sodium and bambuterol hydrochloride in bulk and tablet dosage formulation. *Jordan J Pharm Sci*. 2008; 1:152-8.
- Patil S, Pore YV, Kuchekar BS, Mane A, Khire VG. Determination of montelukast sodium and bambuterol hydrochloride in tablets using RP-HPLC. *Indian J Pharm Sci*. 2009;71:58-61.
- Rathore AS, Sathiyarayanan L, Mahadik KR. Development of validated HPLC and HPTLC methods for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in bulk drug and pharmaceutical dosage form. *Pharm Anal Acta*. 2010;1:106-11.
- Rote A, Niphade V. Determination of montelukast sodium and levocetirizine dihydrochloride in combined tablet dosage form by HPTLC and first derivative spectrophotometry. *J LiqChromatogrRelatTechnol* 2011;34:155-67.
- Lasseter KC, Sologuren A, La Noce A, Dilzer SC. Evaluation of the single-dose pharmacokinetics of Bilastine in subjects with various degrees of renal insufficiency. *Clin drug invest*. 2013; 33: 665-673.
- Berrueta LA, Ferná'ndez-Armentia M, Bakkali M, Gonzalo A, Lucero ML. Matrix solid-phase dispersion technique for the determination of a new antiallergic drug, Bilastine, in rat faeces. *J Chromato B*. 2001; 760: 185-190.
- Berrueta LA, Ferná'ndez-Armentia M, Bakkali M, Gonzalo A, Lucero ML. Matrix solid-phase dispersion technique for the determination of a new anti-allergic drug, Bilastine, in rat faeces. *J Chromato B*. 2001; 760: 185-190.
- ICH Harmonised Tripartite Guideline on validation of Analytical Procedure: Text and Methodology, Q2R1, Current Step 4 version, amended on November, 2005; <https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf> (Last accessed: March 11, 2021)

20. Emanuelli J, Schapoval EE. Stability-indicating HPLC method for estimation of Omarigliptin in tablets—oxidative and photolytic kinetics and degradation products formed under oxidative conditions. *Microchemical J.* 2020 Sep 1;157:105084.

Table 1: optimized conditions of method development.

Parameter	Condition
Mobile Phase	0.1% v/v Triethylamine buffer: pH-3.00: Acetonitrile
Flow rate	1ml/min
Wavelength	220 nm
Volume of injection	10µl
Column	Hypersil BDS C-18 Column (100×4.6 mm, 3 µm)
Run time	13 min

Table 2: Result of System suitability parameters

Sr. No	Parameters	Bilastine	Montelukast
1	Retention time	1.671	7.432
2	Resolution	42.3	-
3	Theoretical Plate	2354	48595
4	Tailing factor	1.4	1.1
5	% RSD	0.1	0.0

RSD: Relative standard deviation

Table 3: A brief overview of degradation of Bilastine and montelukast in different conditions.

Degradation Condition	% Assay		% Degradation	
	Bilastine	Montelukast	Bilastine	Montelukast
Acidic hydrolysis	98.9	99.6	1.7	1.9
Basic hydrolysis	99.8	100.9	0.8	0.6
Oxidation	100.2	95.9	0.4	5.6
Humidity	101.0	101.8	-0.4	-0.3
Photolytic	101.0	96.8	-0.4	4.7
Thermal	101.7	101.7	-1.1	-0.2

Table 4: Result of Method Precision, Intermediate Precision and Ruggedness

Sample no. MP/IP	Ruggedness of Bilastine		Ruggedness of Montelukast	
	MP % Assay	IP % Assay	MP % Assay	IP % Assay
1	100.1	98.2	100.9	98.4
2	100.4	99.4	101.1	99.5
3	100.4	99.2	101.6	98.4
4	101.0	101.2	102.1	101.4
5	100.7	101.9	101.5	101.8
6	100.9	101.9	101.8	101.5
Mean	100.6	100.3	101.5	100.2
SD	0.34	1.57	0.44	1.59
%RSD	0.3	1.6	0.4	1.6
Overall Mean±SD	100.4±1.09		100.8±1.31	
Overall RSD (%)	1.1		1.3	

Table 5: Result of Linearity, LOD and LOQ

% level of Calibration Standards	Stock vol. taken(ml)	Dilution (mL)	Bilastine		Montelukast	
			Conc. (µg/ml)	Mean Area	Conc. (µg/ml)	Mean Area
Linearity 50%	2.0	20	100.07	958365	50.38	747861
Linearity 70%	3.5	25	140.10	1320912	70.53	1051012
Linearity 90%	4.5	25	180.13	1688638	90.68	1356839
Linearity 100%	4.0	20	200.14	1846238	100.76	1491488
Linearity 120%	6.0	25	240.17	2215541	120.91	1818571
Linearity 150%	6.0	20	300.21	2786982	151.14	2311953
Intercept			43187		-44076	
Slope			9094.9		15473	
Regression Coefficient (R ²)			0.99966		0.99929	
LOD(µg/ml)			4.83		3.53	
LOQ(µg/ml)			14.64		10.70	

Table 6: Result of Accuracy (Recovery)

Recovery Sample Name	% Recovery Bilastine (BLS)				% Recovery Montelukast (MTK)			
	BLS added (mg)	BLS Recovered (mg)	BLS % Recovery	Mean±SD	MTK added (mg)	MTK Recovered (mg)	MTK % Recovery	Mean±SD
80% -1	79.496	80.336	101.1		40.168	40.091	99.8	
80% -2	80.357	79.720	99.2	100.6±1.23	39.980	40.859	102.2	100.6±1.36
80% -3	79.981	81.199	101.5		40.535	40.490	99.9	
100% -1	101.101	100.425	99.3		50.726	50.585	99.7	
100% -2	102.141	100.709	98.6	98.9±0.35	51.242	50.967	99.5	99.6±0.12
100% -3	101.596	100.514	98.9		50.855	50.721	99.7	
120% -1	120.209	121.809	101.3		60.759	60.464	99.5	
120% -2	121.448	121.151	99.8	100.3±0.87	60.133	61.356	102.0	100.7±1.25
120% -3	120.873	120.658	99.8		60.421	60.845	100.7	
Overall Mean±SD			99.9±1.09		Overall Mean±SD		100.3±1.06	
Overall %RSD			1.1		Overall %RSD		1.1	

Table 7A: Result of Robustness of Bilastine

% Assay							
S. No.	MP ^a	FP ^b	FM ^b	CTP ^b	CTM ^{b,b,*}	WP ^b	WM ^b
1	100.1	101.7	101.1	101.1	101.1	101.6	101.1
2	100.4	103.3	103.2	103.0	103.3	103.7	103.4
3	100.4	102.8	102.6	102.4	102.5	102.5	102.5
4	101.0						
5	100.7						
6	100.9						
Mean	100.6 [#]	101.3 [*]	101.2 [*]	101.1 [*]	101.2 [*]	101.3 [*]	101.2 [*]
SD	0.3 [#]	1.1 [*]	1.1 [*]	1.0 [*]	1.1 [*]	1.2 [*]	1.1 [*]
Overall % RSD	0.3	1.1	1.0	1.0	1.0	1.2	1.1

Mean of overall % RSD

1.0

^a=(n=6), ^b=(n=3), ([#]=^a), (^{*}=^{a+b}), MP: Method Precision, FP: Flow Plus, FM: Flow Minus, CTM: Column Temperature Minus, CTP: Column Temperature Plus, WM: Wavelength Minus, WP: Wavelength Plus

Table 7B: Result of Robustness of Montelukast

% Assay							
S. No.	MP ^a	FP ^b	FM ^b	CTP ^b	CTM ^{b b, *}	WP ^b	WM ^b
1	100.9	101.5	101.5	101.1	101.3	101.7	101.4
2	101.1	102.0	104.1	102.8	103.6	102.8	103.8
3	101.6	100.8	102.8	101.2	102.1	101.6	102.4
4	102.1						
5	101.5						
6	101.8						
Mean	101.5 [#]	101.5 [*]	101.9 [*]	101.6 [*]	101.8 [*]	101.7 [*]	101.8 [*]
SD	0.4 [#]	0.5 [*]	1.0 [*]	0.6 [*]	0.8 [*]	0.6 [*]	0.9 [*]
Overall % RSD	0.4	0.5	1.0	0.6	0.8	0.5	0.9
Mean of overall % RSD				0.7			

^a=(n=6), ^b=(n=3), ([#]=^a), (^{*}=^{a+b}), MP: Method Precision, FP: Flow Plus, FM: Flow Minus, CTM: Column Temperature Minus, CTP: Column Temperature Plus, WM: Wavelength Minus, WP: Wavelength Plus

Table 8: Result of Stability in an aqueous solution

Stability In Aqueous Solution up to 27 hours											
Montelukast						Bilastine					
Time (Hr)	TP	RT	TF	Area	%DA	TP	RT	TF	Area	RS	%DA
Initial	48627	7.424	1.1	1498851	-	2361	1.676	1.4	1862986	42.2	-
7.5	49046	7.382	1.1	1513266	1.0	2402	1.684	1.4	1875222	42.1	0.7
20	48455	7.203	1.0	1499658	0.1	2415	1.685	1.4	1870292	42.1	0.4
27	47046	7.255	1.0	1502009	0.2	2381	1.697	1.4	1868972	42.1	0.3

TP: Theoretical plates; RT: Retention time; TF: tailing factor; DA: Difference in Area from initial; RS: Resolution

Figure 1: Structure of Bilastine (A) and Montelukast Sodium (B). **Figure 2:** A clear and sharp peaks of Bilastine and montelukast from (A): Standard solution; (B): tablet dosage form, under optimized conditions.

Figure 3: Specificity of the (A): Blank solution; (B) Placebo sample solution showing no interference.

Figure 4: Chromatograms showing acid degradation of (A): Placebo sample; and (B): Sample of Bilastine and Montelukast

Figure 5: Base mediated degradation of (A): Placebo and (B): sample containing Bilastine and Montelukast.

Figure 6: Chromatogram showing peroxide mediated degradation of (A): Placebo; (B): sample solution.

Figure 7: Chromatogram showing humidity degradation of (A): Placebo; and (B): Sample solution.

Figure 8: Chromatogram showing photolytic degradation of (A): Placebo; and (B): Sample solution.

Figure 9: Chromatogram showing thermal degradation of (A): Placebo; and (B): Sample solution.

Figure 10 (B): Graphical representation of linearity curve of Montelukast.

Figure 10 (A): Graphical representation of linearity curve of Bilastine.