ejpmr, 2025, 12(10), 348-365

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

SJIF Impact Factor 7.065

Research Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF BILASTINE AND MONTELUKAST IN BULK AND DOSAGE FORM USING QUALITY BY DESIGN (QBD) APPROACH

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DOI: https://doi.org/10.5281/zenodo.17312350

Article Received on 27/08/2025 **Article Revised on 16/09/2025** **Article Accepted on 06/10/2025**

ABSTRACT

A simple, precise, rapid and accurate stability indicating RP-HPLC method for estimation of Bilastine and Montelukast in bulk and dosage form using quality by design (QBD) approach. An Kromasil C18, 250 mm X 4.6 mm dimension with 5µm particle size, the mobile phase, consisting of 0.05 % OPA and Methanol in the ratio of 50:50 v/v, prepare mixture of 0.1% OPA: Acetonitrile: methanol in the ratio of 20:40:40 % v/v/v used as a diluent for preparation of solution. The flow rate was 1.0 ml/min and the effluents were monitored at 225 nm. The retention time was 2.66 min for Bilastine and 8.97 min for Montelukast. The RP-HPLC method optimization study and statistical analysis were performed using Design Expert® software. The mobile phase composition (X1) and flow rate (X2) were selected as independent variables and retention time, asymmetry and Theoretical plates were selected as dependent variables. The linearity for this method was found to be in the range of 80-120 µg/ml and 40-60µg/ml for Bilastine and Montelukast. This Robust method was capable to recover accurately and precisely from 80 %, 100 % and 120 % level of target concentration. Forced degradation studies were performed under different conditions like Acidic, Basic, oxidation, Photo and Thermal degradation. Considerable Degradation was found in Acidic and basic degradation. The results of the study showed that the proposed stability indicating RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Bilastine and Montelukast in pharmaceutical dosage form.

KEYWORDS: Bilastine and Montelukast, RP-HPLC, Drug, Stability indicating, quality by design (QBD).

INTRODUCTION

Antihistamines are the agent which treat allergic rhinitis, common cold, influenza, and other allergies. Nonsedating antihistamines are the first treatment option for the allergic rhino conjunctivitis including urticaria, according to the existing recommendations.[1] Bilastine (BLS) is not structurally relevant to many other antihistamines. The CDSCO approved Drug Combination Bilastine and Montelukast Sodium were used as allergic rhinitis and mild to moderate asthma and it was approved by CDSCO on 11th of March, 2020.[2]

Bilastine is also an inverse agonist for the H1 receptor antihistamine. Chemically it is, 2- [4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-ethylpropanoic acid Clinical studies have shown that Bilastine is as efficacious as other nonsedating antihistamines in allergic rhino conjunctivitis and chronic urticaria in individuals from 12 and 18 years of age, respectively.[3,4]

The Merck (MSD) developed the drug Montelukast, Mont in Montelukast stands for Montreal. Montelukast chemically it is (*R*)-1-[(1-(3-(2-(7-chloro-2-quinolinyl)-ethenyl) phenyl)-3-(2-(2-hydroxy-2-propyl) phenyl) propyl) thiomethyl] cyclopropane acetic acid, is a potent and selective antagonist of the cysteinyl leukotriene receptor 1 subtype (CysLT1).[5] Montelukast is a leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies.[6] It is mainly used to control and prevent symptoms caused by asthma (such as wheezing and shortness of breath) and in allergic rhinitis.[7] Montelukast is a CysLT1antagonist; it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it.[8] This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. Because of its mechanism of action, it is not useful in the treatment of acute asthma attacks.[9]

Literature review revealed that several methods for analysis of Bilastine and Montelukast Sodium either alone or with other drugs by RP-HPLC, UPLC and HPTLC have been reported.[10]. Only one Method for simultaneous estimation of this combination has been reported by UV Spectroscopic method.[11] But there was no method for simultaneous estimation of this combination by RP-HPLC reported till now. According to literature survey there is no official method for the estimation of Bilastine and Montelukast by high performance liquid chromatography (HPLC) in pharmaceutical dosage forms.[12] Hence, an attempt has been made to develop new method stability indicating for the simultaneous estimation and validation of Bilastine and Montelukast in pharmaceutical formulation in accordance with the ICH guidelines.[13,14]

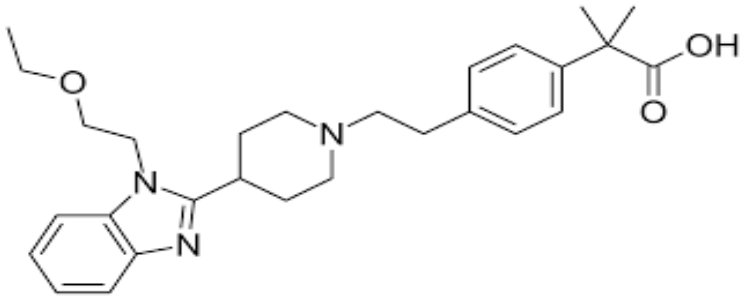


Fig. 1. Chemical structure of Bilastine.

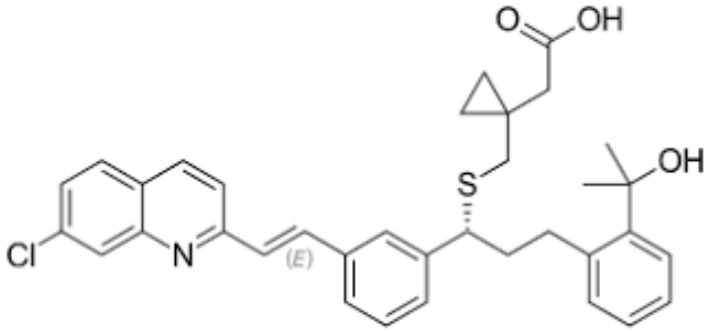


Fig. 2. Chemical structure of Montelukast.

MATERIALS AND METHODS

MATERIALS

Bilastine and Montelukast tablet was purchased from a local pharmacy. HPLC grade Phosphoric acid, Acetonitrile and Methanol were purchased from merck. High purity water was prepared using Milli Q purification system.

Instrumentation

For HPLC, the chromatographic system consists of Agilent HPLC, 1260 infinity II, Quaternary Gradient with Openlab EZ Chrome workstation Software. Kromasil C18 column, UV -Vis detector was used for higher data quality for more confidence. For weighing of the materials and sample, Jasco made analytical balance with CY224C model with weighing capacity 2 mg to 200 gm was used. For measuring the pH of the solutions, Thermo Scientific pH meter, with Orian Star A211 model was used.

METHOD

UV spectroscopic method

Preparation of Diluent

Measure and transfer water: methanol in the ratio of 30:70 %v/v/v respectively, mix well and degas. Used as solvent for dissolving API

Selection of wavelength

Montelukast: Weighed accurately 20 mg Montelukast and transferred into 100 ml volumetric flask, added 70 ml of diluent and sonicated to dissolve the standard completely and diluted up to the mark with diluent (200 PPM). Further diluted 3 mL to 50 mL with diluent.

Bilastine: Weighed accurately 20 mg Bilastine and transferred into 100 ml volumetric flask, added 70 ml of diluent and sonicated to dissolve the standard completely and diluted up to the mark with diluent (200 PPM). Further diluted 2 mL to 50 mL with diluent.

Determination of λ Max

The standard solutions were scanned separately between 400nm to 200nm. From the spectrum show high absorbance.

Selection of Wavelength

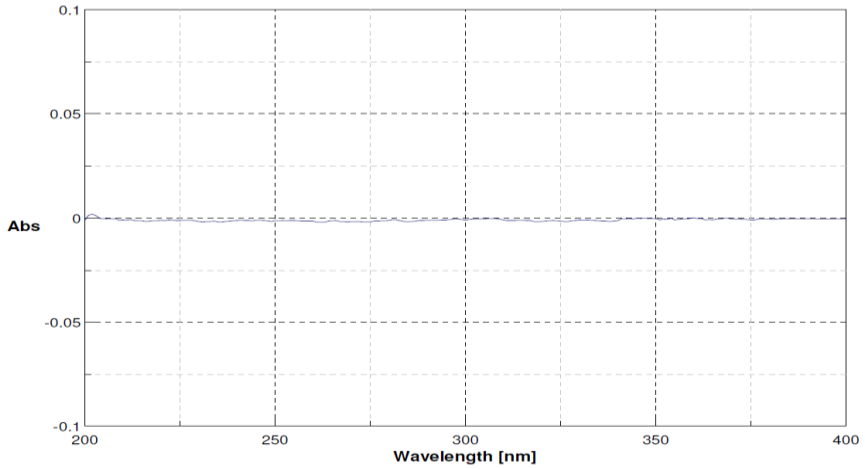


Fig 3. UV Spectra of Blank.

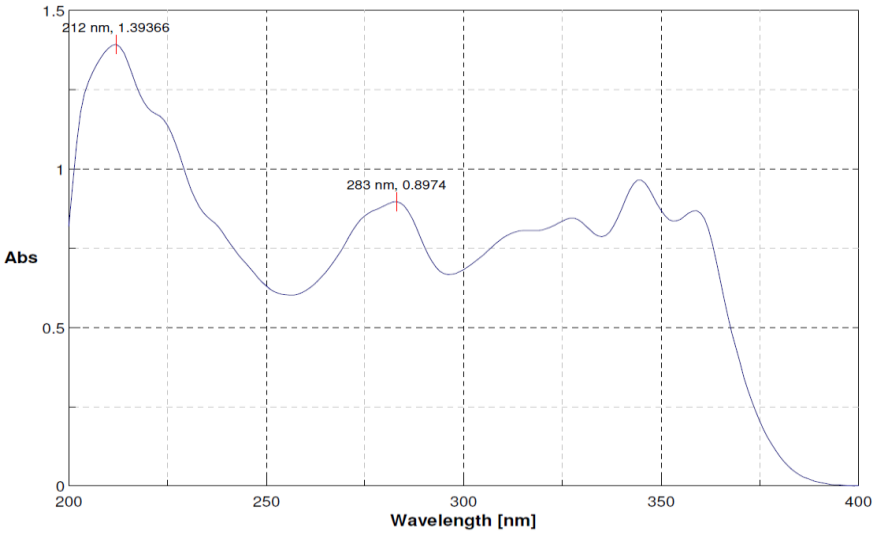


Fig 4. UV Spectra showing λ max of Montelukast

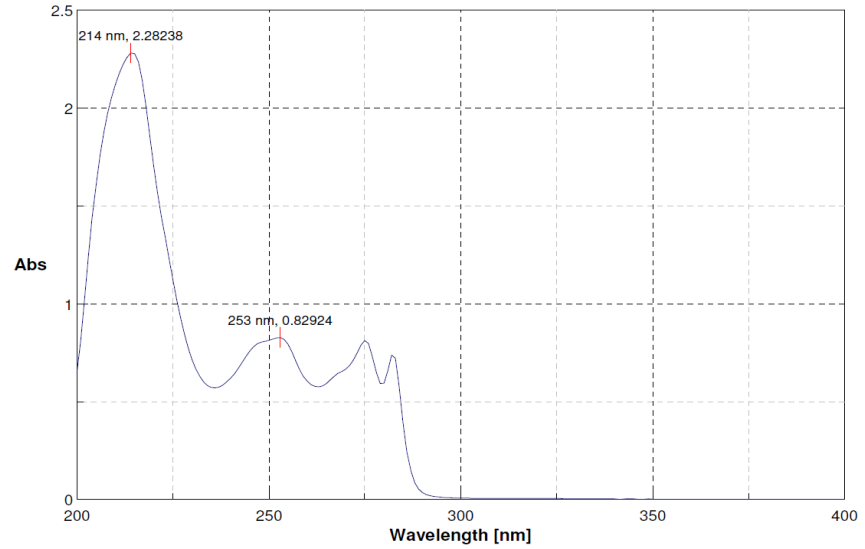


Fig 5. Spectra showing λ max of Bilastine.

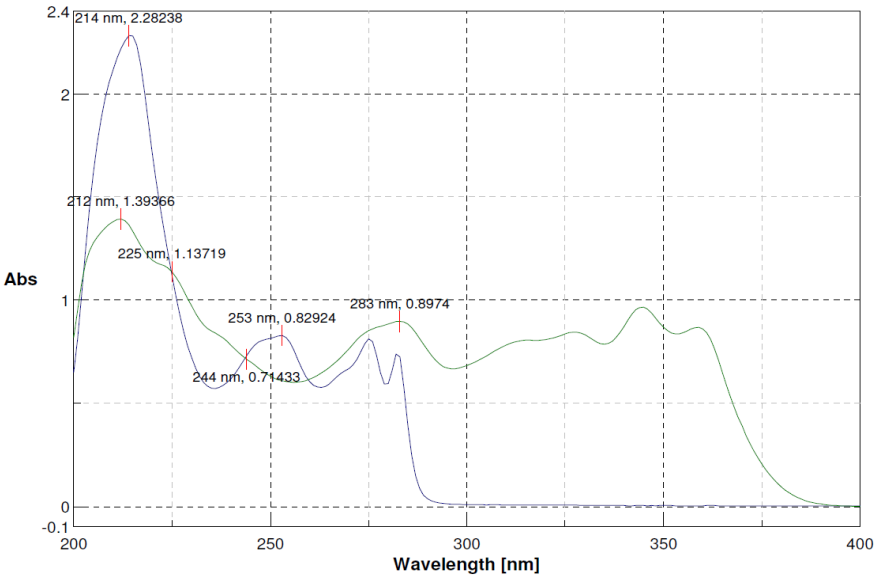


Fig 6. Spectra showing λ max of Bilastine and Montelukast.

Observation: Both standard solutions were scanned between 200 nm to 400 nm. Q-absorption point was determined for both drugs.
Absorption maxima of Montelukast: 283 nm, 212 nm
Absorption maxima of Bilastine: 253 nm, 214 nm
Overlay Q point: 244 nm, 225 nm.
Both drugs show significant absorption at 225 nm wavelength. Hence 225 nm wavelength will used for chromatography development.

RP-HPLC method
Preparation of solution
Preparation of 0.05 % Orthophosphoric acid: Measure and transfer accurately about 0.5 ml of orthophosphoric acid in 1000 of water Mixed well. filter through 0.45µ nylon membrane disc filter and degas.

Preparation of Mobile phase: Prepare mixture of 0.05 % orthophosphoric acid and Methanol in the ratio of 50:50 v/v respectively, mix well.

Preparation of Diluent: Prepare mixture of 0.1% OPA in Water, Acetonitrile and Methanol in the ratio of 20:40:40 v/v respectively, mix well.

Preparation of Blank: Use diluent as blank.

Preparation of Standard solution
Preparation of Bilastine Standard stock solution: Weigh accurately about 50 mg of Bilastine and transfer it into 50 mL amber colored volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent. Mixed well.

Preparation of Montelukast Standard stock solution: Weigh accurately about 26 mg of Montelukast sodium (Equivalent 25 mg of Montelukast) working standard and transfer it into 50 mL volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent.

Further dilute 5 mL each of Bilastine stock solution and 5 mL of Montelukast Standard stock solution to 50 mL with diluent. (100 ppm Bilastine and 50 ppm of Montelukast).

Preparation of Sample solution
Weigh 20 tablet and take average of tablet. Crush the 10 tablets into fine powder in dry and clean mortar pestle. Weigh the tablet powder equivalent to 100 mg of Bilastine and 50 mg of Montelukast Transfer it in a clean and dry 250 mL of volumetric flask, add 150 ml of diluent sonicate it for 15 minutes with intermittent shaking. Make the volume up to the mark with diluent. Filter the solution through suitable 0.45 µ syringe filter dis-carding 3-5 mL of filtrate.

Further dilute 5 mL of sample stock solution to 20 mL clean volumetric flask make upto the mark with diluent. Mixed well.

Method development optimization
The optimized HPLC conditions of several mobile phases with different compositions were tested to develop an optimization of chromatographic conditions like tailing factor, peak shape, and the number of theoretical plates. For the selection of the mobile phase, primarily 0.05 % Orthophosphoric acid and Methanol (50:50 v/v) has been tested for different compositions. The mobile phase containing a mixture of 0.05 % Orthophosphoric acid and Methanol (50:50 v/v) at a flow rate of 1 mL/ minute, Injection volume was 20 µl, with 225 nm wavelength. Sample temperature was ambient and column temperature was 40 ºC. Sample run for 15 min resulting chromatogram was found to be satisfactory and proper system suitability parameters obtained. Optimized chromatographic conditions, system

suitability parameters for estimation of Bilastine and Montelukast by proposed RP-HPLC method.

Optimization of Developed RP-HPLC Method with Design Space and Control

All the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert version 7.0.0; State-Ease Inc., Minneapolis, MN, USA).

Application of design of experiments for method optimization

Design of experiments (DOE-1): Thus, 3^2 factorial design used with 9 trial runs to study the impact of two factors on the three key response variables. In this design 2 factors were evaluated, each at 3 levels and experimental trials were performed at all possible combinations. The mobile phase composition (X1) and flow rate (X2) were selected as independent variables and retention time, asymmetry and Theoretical plates were selected as dependent variables. The resulting data were fitted into Design Expert 7.0.0. software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to determine the influence of mobile phase composition and flow rate on dependent variables. The probable trial runs using 3^2designs.0 center point per block considered while designing the experiment

Preparation of standard stock solutions to inject in DOE runs

Preparation of Standard solution

Preparation of Bilastine Standard stock solution: Weigh accurately about 50 mg of Bilastine and transfer it into 50 mL amber colored volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent. Mixed well.

Preparation of Montelukast Standard stock solution: Weigh accurately about 26 mg of Montelukast sodium (Equivalent 25 mg of Montelukast) working standard and transfer it into 50 mL volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent.

Standard mixture for DOE: Further dilute 5 mL each of Bilastine stock solution and 10 mL of Montelukast Standard stock solution to 50 mL with diluent. (100 ppm Bilastine and 50 ppm of Montelukast).

2 independent factors with codes and their ranges as follows

Developed parameters considered as center points and ranges design as follows:

1) % Methanol in mobile phase (X1): ± 10% v/v
2) Flow rate (X2): ± 0.2 ml/Min

3 dependent factors with codes as follows

1) R.T. of Bilastine: A
2) R.T. of Montelukast: B
3) Resolution between Bilastine & Montelukast: C

Table 1. Translation of coded levels in actual values.

Level of Variable	Range of Factors	
	Methanol (%v/v) (A)	Flow Rate (mL/min) (B)
Low Level (-1)	40	0.8
Medium Level (0)	50	1.0
High Level (1)	60	1.2
Variable level in actual form		
Run	X1	X2
1	50	0.8
2	60	0.8
3	50	1.0
4	60	1.2
5	40	0.8
6	40	1.0
7	60	1.0
8	50	1.2
9	40	1.2

Method Validation
The developed method was validated as per ICH guidelines for following parameters.

System Suitability
System suitability test ensure that the method can generate results of acceptable accuracy and precision. System suitability test were carried out repeatability of the proposed method, other parameters like retention time, no. of theoretical plates, asymmetry factor were studied and found satisfactory.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the method for determination of Bilastine and Montelukast in tablet was determined by comparing the retention time and spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the impurity and excipients.

Linearity
The linearity is the ability of the method to give the test results which are directly related to the concentration of the analyte in sample. Aliquots of standard solution of the drugs were taken to prepare sample solutions in the concentration range of 48-72 µg/ml. Each of the solution was analyzed. Linear fit was illustrated graphically by plotting peak area versus concentration of pazopanib. and demonstrated using Linear Regression analysis

Accuracy (Recovery) Accuracy was determined over the range 80% to 120% of the sample concentration.

Preparation of Accuracy Solution
An accurately weighed amount of the Bilastine and Montelukast API and Placebo as per above mentioned amount of in to 50 mL volumetric flask. Add 30 mL of diluent, sonicate this solution for 20 minutes, with intermittent shaking cool and make volume up to the mark with diluent.

Transfer 5.0 mL of the stock solution to 25 mL volumetric flask and make volume up to the mark with diluent.

Precision: Method precision and Intermediate precision studies of Bilastine and Montelukast in tablet were performed on the same day and 2nd days same concentrations of Bilastine and Montelukast. Reproducibility studies were performed by evaluating the responses of same concentrations of Bilastine and Montelukast and the results are expressed in relative standard deviation (%RSD).

Robustness: The robustness of the developed method evaluated by its capacity to remain unaffected by small but deliberately change in method parameter. The robustness was studied by varying the flow rate at ±0.1 ml/min and wavelength at ±2.0 nm. the result expressed in % Assay, the acceptance criteria for robustness is absolute difference of Area is not greater than 2%.

FORCED DEGRADATION STUDIES
Specificity of the method was determined by calculating percent amount of possible degradation products produced during the forced degradation study. The stress conditions applied for degradation study involved acid hydrolysis, base hydrolysis, thermal, photo and oxidative degradation for finding out the stability nature of the drug. The degradation samples were prepared by taking suitable aliquots of the drug and drug product solutions, and then undertaking the respective stress testing procedures for each solution. After the fixed time period the treated drug solutions were diluted with solvent. The specific stress conditions are described as follows.

Table 2. Force degradation and condition.

Degradation	Conditions
Acid Trial-1	2 mL, 5 N HCl for 3 Hrs
Acid Trial-2	2 mL, 5 N HCl for 10 Hrs
Base Trial-1	2 mL, 5 N NaOH for 3 Hrs
Base Trial-2	2 mL, 5 N NaOH for 10 Hrs
Oxidative Trial-1	2 mL of 30% hydrogen peroxide with 3 Hr bench top
Oxidative Trial-1	2 mL of 30% hydrogen peroxide with 24 Hr bench top
Thermal	105ºC for 24 Hrs
Photo-Open	1.2 million lux Hrs and 200 watt/square

Preparation of Force degradation Solution
For Hydrolytic and Oxidative degradation, weigh 20 tablet and take average of tablet. Crush the 10 tablets into fine powder in dry and clean mortar pestle. Fine tablet powder used for the sample preparation.

Hydrolytic and Oxidative degradation: Weigh the tablet fine powder equivalent to 100 mg of Bilastine and 50 mg of Montelukast Transfer it in a clean and dry 250 mL of volumetric flask, add 150 ml of diluent sonicate it for 15 minutes with intermittent shaking. Then subjected to the conditions specified in Table 2 for acid, base and oxidative condition. Make the volume up to the mark with diluent. Filter the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of filtrate. Further dilute 5 mL of sample stock solution to 20 mL clean volumetric flask make upto the mark with diluent. Mixed well.

Photo Degradation: Take 20 Tablets in a two separate Petri Plates and expose it under UV and white light for 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt/square. After exposure, Crush the 10 tablets into fine powder in dry and clean mortar pestle. Weigh the tablet powder equivalent to 100 mg of Bilastine and 50 mg of Montelukast Transfer it in a clean and dry 250 mL of volumetric flask, add 150 ml of diluent sonicate it for 15 minutes with intermittent shaking. Make the volume up to the mark with diluent. Filter the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of filtrate. Further dilute 5 mL of sample stock solution to 20 mL clean volumetric flask make upto the mark with diluent. Mixed well.

Heat degradation: Take 20 Tablets (10mg /160 mg) in Petri Plates and expose it to heat at 105°C for 24Hrs. Crush the 10 tablets into fine powder in dry and clean mortar pestle. Weigh the tablet powder equivalent to 100 mg of Bilastine and 50 mg of Montelukast Transfer it in a clean and dry 250 mL of volumetric flask, add 150 ml of diluent sonicate it for 15 minutes with intermittent shaking. Make the volume up to the mark with diluent. Filter the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of filtrate. Further dilute 5 mL of sample stock solution to 20 mL clean volumetric flask make upto the mark with diluent. Mixed well.

RESULT AND DISCUSSION

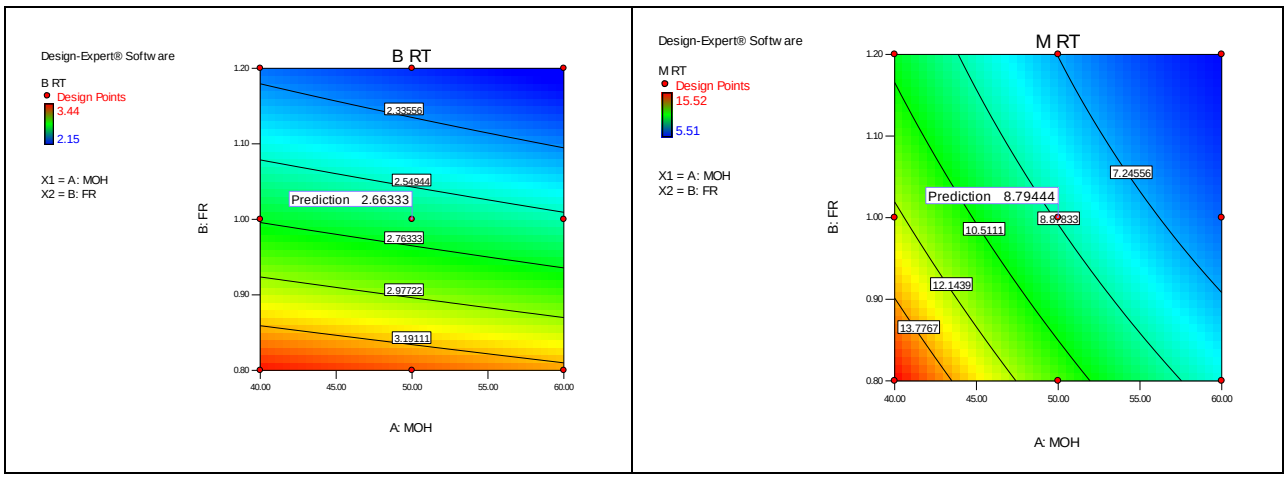
The method development consisted of two phases: the first is optimization and the second is Validation.

Method Development and Optimization: Optimum wavelength of 225 nm was selected to reduce the baseline noise at the absorption maximum of Bilastine and Montelukast. Based on Bilastine and Montelukast solubility, 0.05% OPA: methanol 50:50 % v/v was selected as the mobile phase. Initially, reversed-phase C18 analytical columns (Kromasil C18, 250 mm X 4.6 mm, 5 µm were tested with mobile phase composed of variable composition of water, acetonitrile, methanol and 0.05 % OPA in water- with a flow rate 1.0 mL per min.

Nine experiments each were conducted using the full factorial design (3 factors, 2 levels, 9 runs), in order to rationally examine the effects of flow rate and organic phase concentration in primary screening followed by optimization of an analytical method. It was observed that responses like theoretical plates, retention time, and tailing factor were optimum with a mobile phase composed of mixture 0.05 % OPA and Methanol in the ratio of 50:50 v/v eluted Bilastine and Montelukast through the C18 stationary phase Kromasil C18, 250 mm X 4.6 mm, 5 µm. In method optimization again, nine experiments were conducted using the full factorial design (3 factors, 2 levels, 9 runs), in order to examine the effects of flow rate and the final concentration of the organic phase. Experimental factors and levels used in the experimental design.

We have selected DOE trial no.3 which has following parameters.

Runs	Factor1	Factor 2	Response 1	Response 2	Response 2
	A: % Methanol	B: Flow rate	R.T. of Bilastine	R.T. of Montelukast	Resolution
3	50	1.0	2.64	8.81	26.28



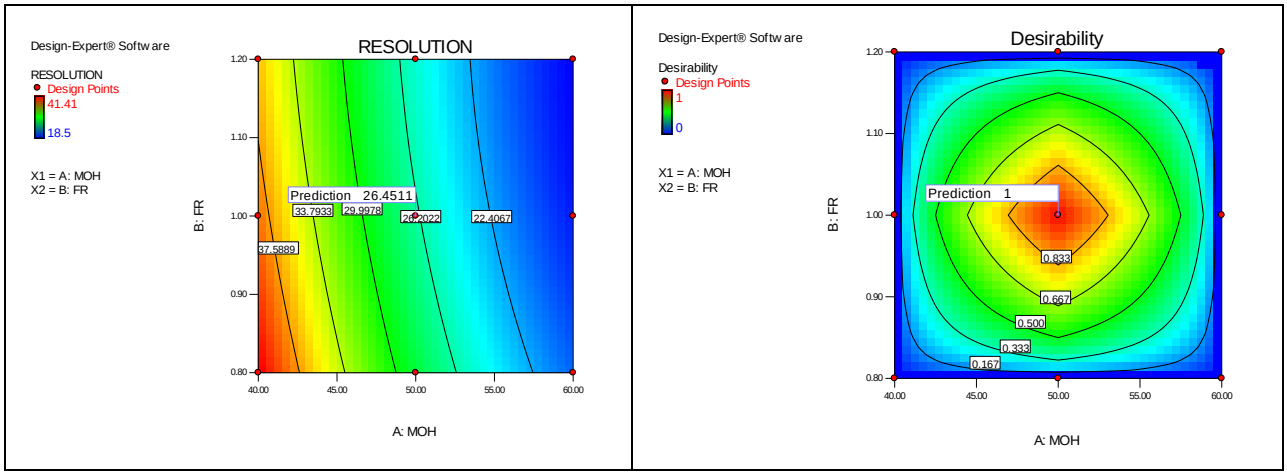


Fig 7: Design space.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH guidelines.

System suitability

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The system suitability parameters like theoretical plates, retention time, tailing factor, were studied and found satisfactory. The results show the system suitability parameters in Table 1a.

Table 3. System suitability parameters.

	Bilastine	**Montelukast**
Symmetry factor	**1.15**	**1.05**
Theoretical plates	**9963**	**18069**
S. No.	**Area**	
1	60132059	33508573
2	60105234	33560259
3	60101586	33510261
4	60125867	33519545
5	60210532	33490568
Mean	**60135056**	33517841
%RSD	**0.07**	**0.08**

The result is well within the acceptance criteria, and the study concludes the suitability of the analytical system for the analysis.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include degradants, matrix, etc. In the case of assay, demonstration of specificity requires that it can be shown that the procedure was unaffected by the presence of excipients. Specificity of an analytical method was its ability to measure accurately and specifically the analyte of interest without interference from blank and placebo.

Table 4. Specificity.

Component	**Retention time (min)**	**Symmetry Factor**	**Purity angle**	**Purity threshold**
Blank	-	-	-	-
Placebo	-	-	-	-
For Bilastine				
Standard solution	2.66	1.15	1.65	2.71
Sample solution	2.66	1.14	2.36	3.42
For Montelukast				
Standard solution	8.97	1.05	2.13	3.40
Sample solution	8.97	1.06	2.81	3.96

Sample Name: BLANK

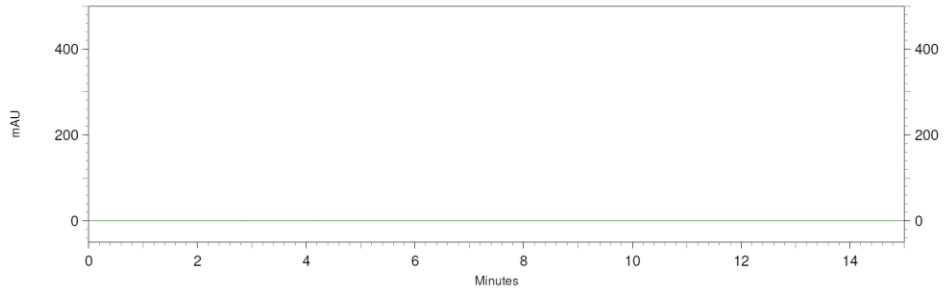


Fig. 8. Chromatogram of Blank.

Sample Name: STANDARD SOLUTION

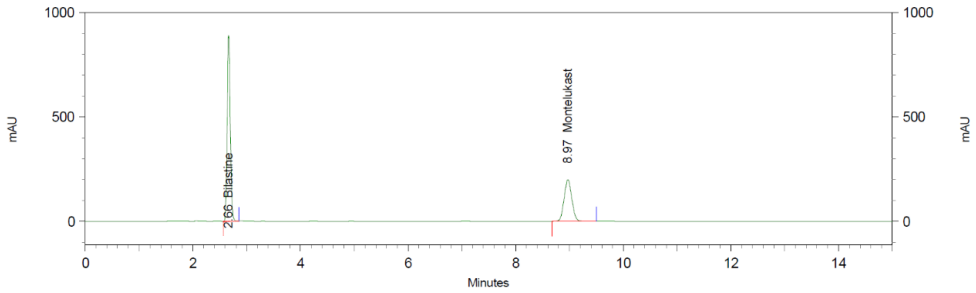


Fig. 9. Chromatogram of Standard.

Sample Name: TEST SAMPLE

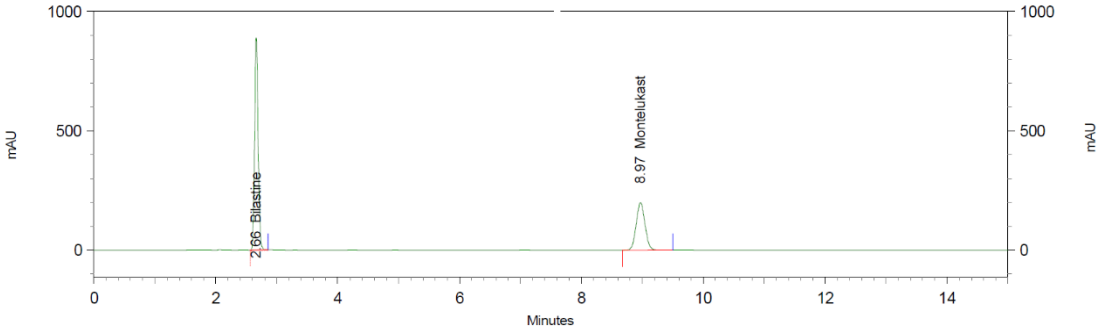


Fig. 10. Chromatogram of Test Sample.

Sample Name: PLACEBO

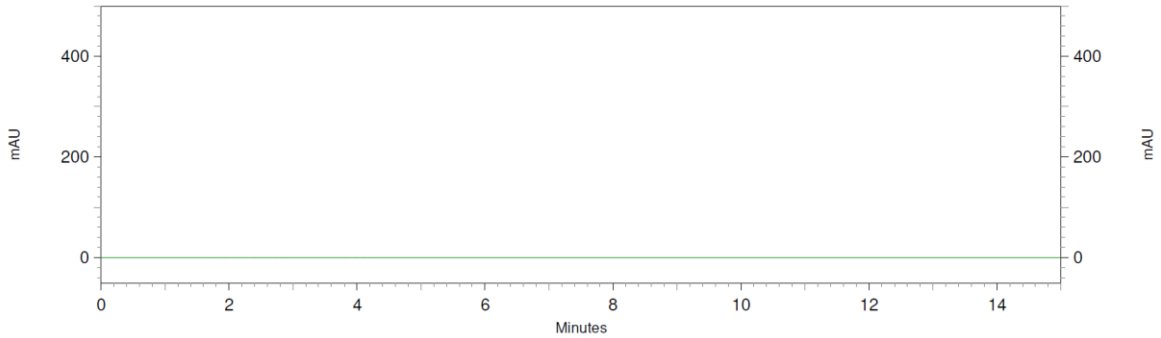


Fig. 11. Chromatogram of Placebo Sample.

Linearity

The linearity of Bilastine and Montelukast was determined in the concentration range of 80 to 120 µg/mL and 40 to 60 µg/mL respectively. The calibration graph of Bilastine and Montelukast is shown in Fig The linearity data is presented in Table 5.

Table 5: Linearity of Bilastine.

Level	Conc (µg/mL)	Area	Mean
80%	80.0	48098152	48096772
		48105631	
		48086532	
90%	90.0	54171464	54178860
		54163259	
		54201857	
100%	100.0	60120813	60142889
		60123596	
		60184258	
110%	110.0	66087591	66067865
		66023421	
		66092584	
120%	120.0	72018178	72024903
		72036964	
		72019568	
Corr. Coefficient			1.0000
Intercept			47768
Slope			599730
% Y-co-ordinate			1.34

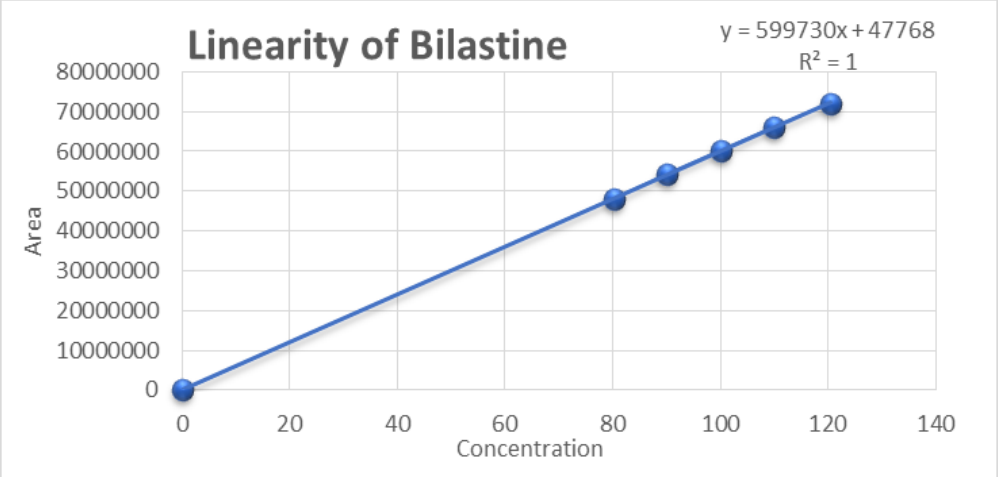


Fig.12. Linearity plot.

Table 6: Linearity of Montelukast.

Level	Conc (µg/mL)	Area	Mean
80%	40.0	26847575	26837098
		26853129	
		26810591	
90%	45.0	30237261	30224489
		30210569	
		30225638	
100%	50.0	33507718	33523479
		33512591	
		33550129	
110%	55.0	36904899	36905983
		36896521	
		36916528	
120%	60.0	40244635	40232110

		40216589	
		40235107	
Corr. Coefficient			0.9999
Intercept			50954
Slope			668600
% Y-co-ordinate			1.66

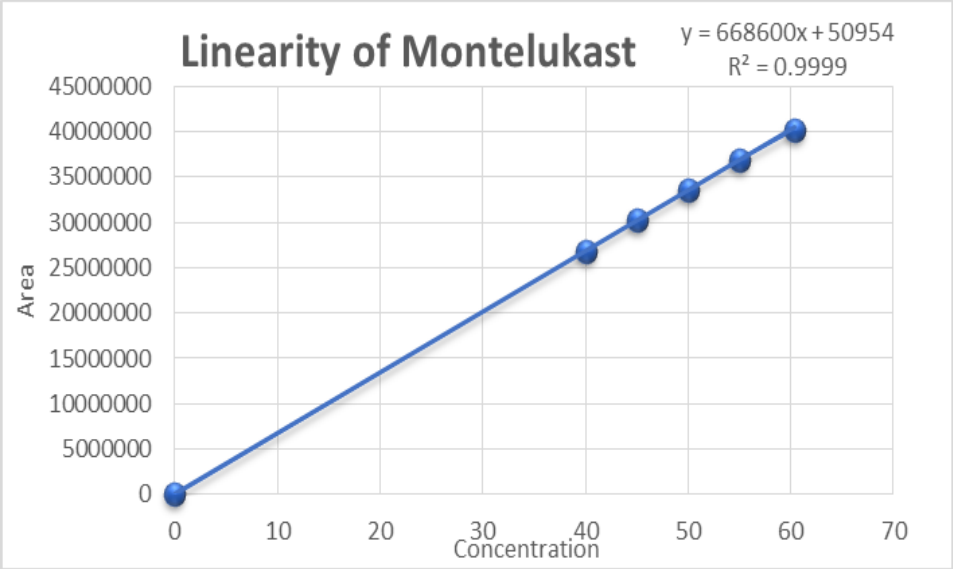


Fig. 13. Linearity plot.

Accuracy (Recovery)

Accuracy was determined over the range 80% to 120% of the sample concentration.

Table 7: Accuracy data of Bilastine.

Level (%)	Area	Bilastine Recovered conc	Bilastine Added Conc (µg/mL)	% Recovery	Mean % Recovery
80	48069532	79.94	81.00	98.69	99.79
	48520183	80.69	80.50	100.23	
	48325584	80.36	80.00	100.45	
100	60326591	100.32	100.50	99.82	99.65
	60542185	100.68	100.00	100.68	
	60085426	99.92	101.50	98.44	
120	72152634	119.98	121.00	99.16	99.65
	72359861	120.33	121.50	99.04	
	73012591	121.41	120.50	100.76	

Table 8: Accuracy data of Montelukast.

Level (%)	Area	Montelukast Recovered conc	Montelukast Added Conc (µg/mL)	% Recovery	Mean % Recovery
80	26850421	40.04	40.01	100.09	99.94
	26830596	40.01	40.49	98.82	
	26748651	39.89	39.52	100.92	
100	33526861	50.00	49.65	100.71	100.72
	33659864	50.20	50.13	100.13	
	33400489	49.81	49.16	101.31	
120	40259867	60.04	59.29	101.27	100.59
	40659765	60.63	60.25	100.64	
	39059568	58.25	58.32	99.87	

Precision

Precision of the method was established for Method precision and Intermediate precision studies was studied.

Table 9: Method precision of Bilastine.

Sample	Area	% Assay
Sample 1	59356201	98.87
Sample 2	59652482	99.20
Sample 3	59623051	99.19
Sample 4	59241857	98.22
Sample 5	58825669	97.91
Sample 6	59126875	98.20
Mean		**98.60**
STD DEV		**0.5594**
% RSD		**0.567**

Table 10: Method precision of Montelukast.

Sample	Area	% Assay
Sample 1	33026591	98.67
Sample 2	32956814	98.29
Sample 3	32523659	97.04
Sample 4	32962381	98.02
Sample 5	33025690	98.58
Sample 6	33142572	98.72
Mean		**98.22**
STD DEV		**0.635019**
% RSD		**0.647**

Table 11: Intermediate Precision pool data of Bilastine.

Parameter	Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC Instrument No.	**HPLC-02**	**HPLC-05**
Sample No.	**% Assay**	
1	98.87	99.56
2	99.20	98.94
3	99.19	98.87
4	98.22	97.16
5	97.91	97.80
6	98.20	98.03
Mean	**98.49**	
% RSD	**0.72**	

Table 12. Intermediate Precision pool data of Montelukast.

Parameter	Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC Instrument No.	**HPLC-02**	**HPLC-05**
Sample No.	**% Assay**	
1	98.67	97.45
2	98.29	98.25
3	97.04	99.02
4	98.02	97.29
5	98.58	99.31
6	98.72	97.87
Mean	**98.21**	
% RSD	**0.71**	

All of these solutions were injected in predetermined chromatographic conditions and observed for various parameters and found within limit. Mean area was subjected to statistical analysis to determine % RSD and found within limit as per ICH guideline Q2R1.

Robustness

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 13: Robustness data of Bilastine.

Changes in parameters	Values	Area	Absolute difference
Control	As per method	59356201	
Flow rate (± 0.1 mL/min)	+0.1 mL/min	59506324	0.3
	-0.1 mL/min	59283641	-0.1
Change in Wavelength (± 2 nm)	+2 nm	59201634	-0.3
	-2 nm	59452013	0.2

Table 14: Robustness data of Montelukast.

Changes in parameters	Values	Area	Absolute difference
Control	As per method	33026591	0.2
Flow rate (± 0.1 mL/min)	+0.1 mL/min	33076252	-1.5
	-0.1 mL/min	32546233	-0.8
Change in Wavelength (± 2 nm)	+2 nm	32762320	1.8
	-2 nm	33612314	0.2

There was not much variation in the results obtained with respective to area after the deliberate changes done in wavelength and flow rate. Which demonstrates that the developed method is robust. development and validation of RP-HPLC for analytical assay method of Bilastine and Montelukast in pharmaceutical ophthalmic solution.

7.2.6. FORCED DEGRADATION

Table 15: Force Degradation for Bilastine.

Reagents	Conditions	Area	% Assay	% Degradation
Control	As such sample	60139652	NA	NA
Acid-1	2 mL, 5 N HCl for 3 Hrs	59564769	99.04	0.96
Acid-2	2 mL, 5 N HCl for 10 Hrs	60419076	100.46	-0.46
Base-1	2 mL, 5 N NaOH for 3 Hrs	39980400	66.48	33.52
Base-2	2 mL, 5 N NaOH for 10 Hrs	52219949	86.83	13.17
Oxidative-1	2 mL of 30% hydrogen peroxide with 3 Hr bench top	59968496	99.72	0.28
Oxidative-2	2 mL of 30% hydrogen peroxide with 24 Hr bench top	60053267	99.86	0.14
Thermal	105ºC for 24 Hrs	60259381	100.20	-0.20
Photo	1.2 million lux Hrs and 200 watt/square	59879864	99.57	0.43

Table 16. Force Degradation for Montelukast.

Reagents	Conditions	Area	% Assay	% Degradation
Control	As such sample	33519682	NA	NA
Acid-1	2 mL, 5 N HCl for 3 Hrs	30348072	90.54	9.46
Acid-2	2 mL, 5 N HCl for 10 Hrs	22743533	67.85	32.15
Base-1	2 mL, 5 N NaOH for 3 Hrs	20447569	61.00	39.00
Base-2	2 mL, 5 N NaOH for 10 Hrs	27828859	83.02	16.98
Oxidative-1	2 mL of 30% hydrogen peroxide with 3 Hr bench top	33176670	98.98	1.02
Oxidative-2	2 mL of 30% hydrogen peroxide with 24 Hr bench top	33201482	99.05	0.95
Thermal	105ºC for 24 Hrs	33096867	98.74	1.26
Photo	1.2 million lux Hrs and 200 watt/square	32958429	98.33	1.67

Observation

The stress condition applies on Bilastine and Montelukast drug molecules. The 5-20 % degradation achieved in the Acid and Base stress condition. Drug molecules stable in oxidative, thermal and photolytic stress condition.

1) As such sample

Sample Name: SAMPLE AS SUCH

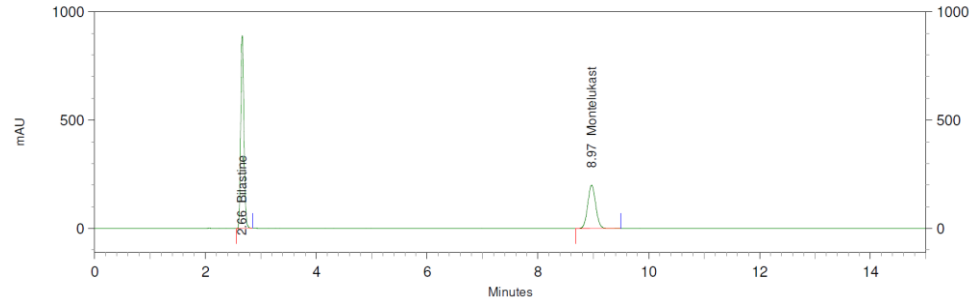


Fig. 14. As such sample of Bilastine and Montelukast.

2) Photo Degradation

Sample Name: PHOTOLYTIC SAMPLE

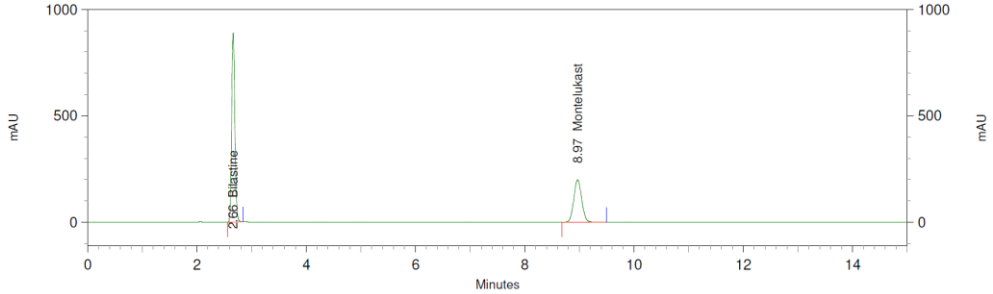


Fig. 15. Photo Degradation of Bilastine and Montelukast.

3) Thermal degradation

Sample Name: THERMAL SAMPLE

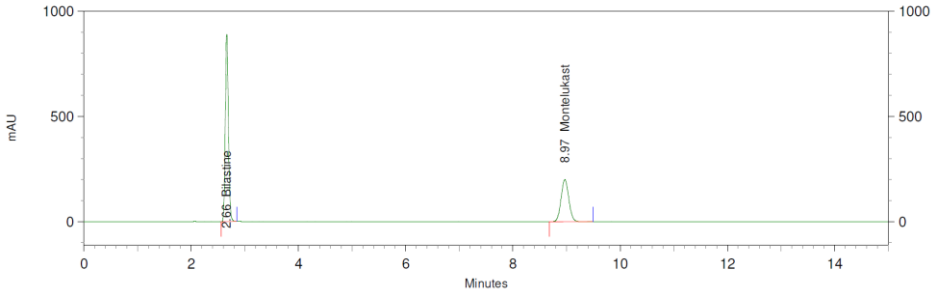


Fig. 16. Thermal degradation of Bilastine and Montelukast.

4) Acid Degradation

Sample Name: SAMPLE ACID TRIAL 1

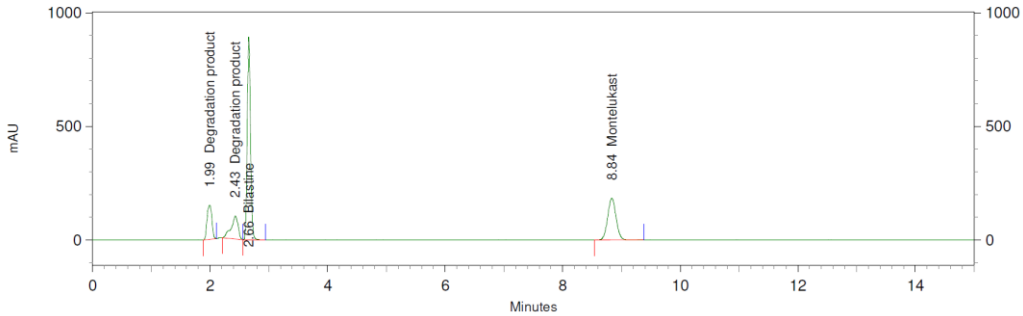


Fig. 17. Acidic Degradation of Bilastine and Montelukast Trial-1.

Sample Name: SAMPLE ACID TRIAL 2

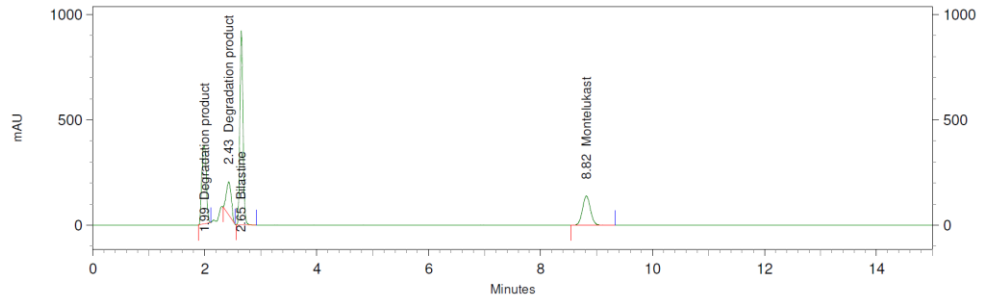


Fig. 18. Acidic Degradation of Bilastine and Montelukast Trial-2

5) Base Degradation

Sample Name: SAMPLE BASE TRIAL 1

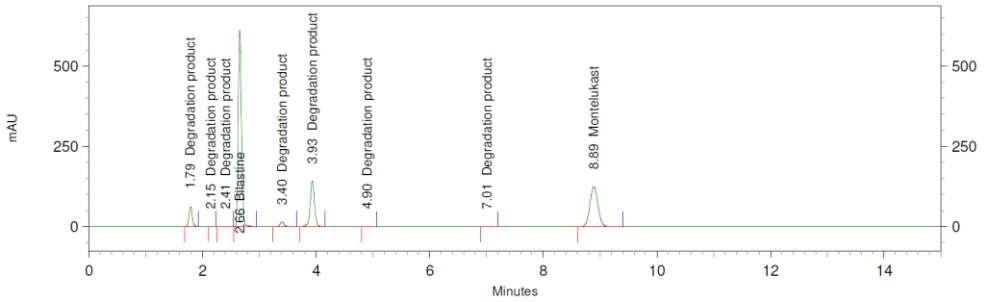


Fig. 19. Base Degradation of Bilastine and Montelukast Trial- 1.

Sample Name: SAMPLE BASE TRIAL 2

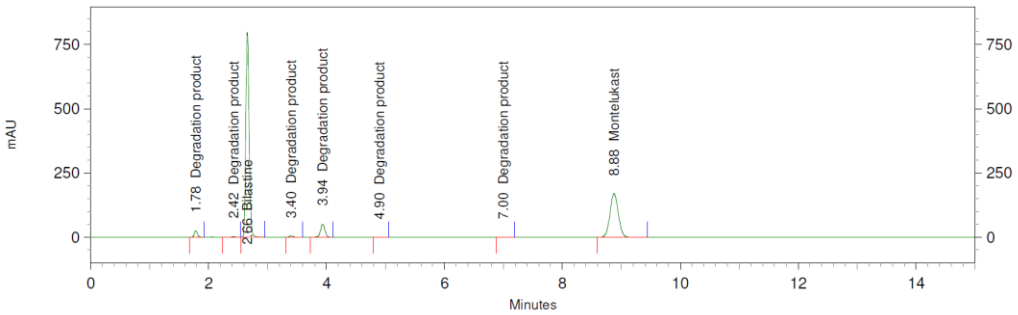


Fig. 20. Base Degradation of Bilastine and Montelukast Trial- 2.

6) Oxidative Degradation

Sample Name: SAMPLE PEROXIDE TRIAL 1

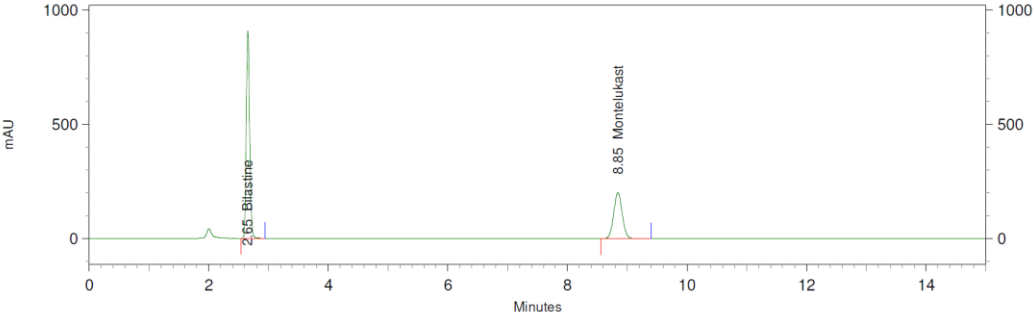


Fig. 21. Oxidative Degradation of Bilastine and Montelukast Trial- 1.

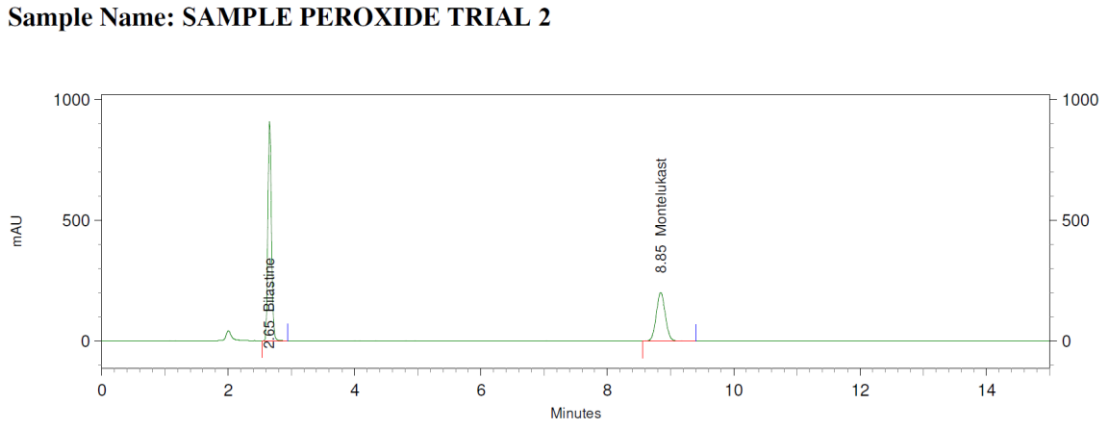


Fig. 22. Oxidative Degradation of Bilastine and Montelukast Trial- 2.

CONCLUSION

A simple, precise, rapid and accurate stability indicating RP-HPLC method was developed for estimation of Bilastine and Montelukast in bulk and pharmaceutical dosage form using quality by design (QBD) approach. A quality-by-design approach to HPLC method development has been performed. The method goals are clarified based on the analytical target product profile. The experimental design describes the scouting of the key HPLC method components including mobile phase and Flow rate. The % oraganic phase (X1) is the main variable (10%v/v). The flow rate (X2) is also an important factor (±0.2 ml/min). The interaction of flow rate and % organic phase was less influence on the response of the chromatographic system. The optimized for DOE trial no 2 was found that 50% Methanol and 1ml/min flow rate shows optimium results with 26.45 resolution and retention 2.66 min and 8.79 for Bilastine and Montelukast. Hence proposed Box behnken surface methodology model found fit for developed chromatographic method and it can be used to predict dependent variable within a design space. The developed method provides simple, accurate, and reproducible quantitative analysis for the determination of Bilastine and Montelukast in the presence of its degradants. It was found that Bilastine and Montelukast was rapidly degraded under Acidic and basic conditions. The proposed method can be used for the routine analysis and quality control assays of Bilastine and Montelukast in bulk and pharmaceutical dosage form. This method is recommended for future bioanalytical analyses because it can be easily modified to estimate Bilastine and Montelukast in various biological samples.

Conflict of Interest. None.

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