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Development of RP-HPLC Method for Simultaneous Determination of Bilastine and Montelukast by Qbd Approach and Its Validation

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Abstract: This study proposes to develop and validate the RP-HPLC method for Bilastine (BIL) and Montelukast (MKT) by QbD to substantiate the RP-HPLC analysis as per ICH validation guidelines. Quality by Design (QbD) allows the accomplishment of specific unsurprising quality with a predetermined and wanted determination. The simultaneous estimation of BIL and MKT was performed with C18 (4.6×250 mm, 5-µm particle size) with an LC-10AD pump and PDA detector. The mobile phase employed methanol and ammonium acetate buffer pH-3.6 at 85:15 v/v. The flow rate was maintained at 1.0 ml/min, and BIL and MKT were detected at 249nm and 293 nm by UV detector, respectively. The HPLC method provided linear responses found in the 200–600 µg/ml range. The correlation coefficient was 0.9995 for BIL and 0.9991 for MKT. The LOD and LOQ for BIL and MKT were found to be 0.493, 1.495 µg/ml, and 0.693, 2.100, respectively. The percentage recovery for BIL was 95.33 to 102.06, and for MKT was 96.31 to 104.05, respectively. Calculated information acquired for both the preliminaries roughly coordinates with the information given by Design expert programming, showing the chromatographic condition's genuineness. Design-Expert version 10 ("DX10") software has calculated this calculation, setting a composite design of significant parameters. A new selective, rapid, accurate, precise, and sensitive RP-HPLC method was developed and evaluated for the simultaneous determination of Bilastine (BIL) and Montelukast sodium (MKT) in a bulk and pharmaceutical dosage form. This method is useful in the routine quality analysis of combinations of BIL and MKT in bulk and its tablet formulations.

Keywords: Bilastine, Montelukast sodium, RP-HPLC, UV-Spectroscopy, QbD, validation.

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1. INTRODUCTION

Bilastine (2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl) ethyl) phenyl]-2-methyl propionic acid) is chemically used as a non-sedative antihistamine. It is a selective histamine H1 receptor antagonist. Bilastine is indicated for the symptomatic treatment of allergic rhinitis and rhino-conjunctivitis (seasonal and perennial), and urticaria in adults.[1] It meets the current European Academy of Allergy and Clinical Immunology (EAACI) and Allergic Rhinitis and its Impact of Asthma (ARIA) criteria for medication used to treat allergic rhinitis. It got approved in India by the DCGI in February 2019. Bilastine is a selective histamine H1 receptor antagonist, and it has less or no affinity for different other receptors such as serotonin, bradykinin, leukotriene D4, calcium, muscarinicM3-receptor, α1-adrenoceptors, β2-adrenoceptor and H2, and H3 receptor. During hypersensitive reactions, mast cells undergo degranulation, discharging different substances like histamine. By restricting and forestalling the enactment of the H1 receptor, Bilastine diminishes the improvement of hypersensitive side effects because of the arrival of the receptor from the mast cell.

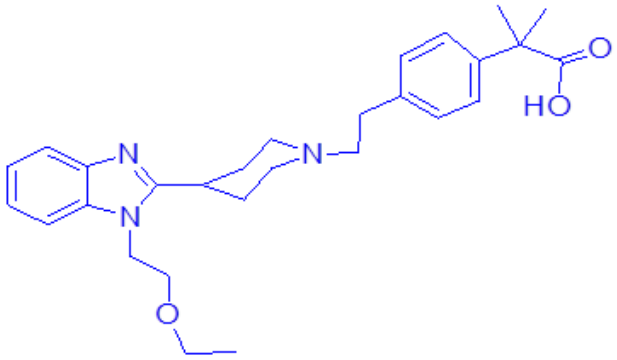


Fig 1: Chemical Structure of Bilastine

Montelukast Sodium (MKT) 1-[[[(1R)-1-[3-[(1E)-2-(7-Choloro-2-quinolinyi) ethyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl] thio] methyl] cyclopropane acetic acid [2] is chemically used to treat asthma (Anti-asthmatics) and allergic rhinitis. It is a Leukotriene receptor antagonist (LTRA). It is generally indicated for Asthma and seasonal or year-round allergies. It was first approved by the US FDA in 1998 as Merck's brand name singular for clinical use.[3- 4] The medication is a member of the leukotriene receptor antagonist (LTRA) category of drugs. Although efficacy can be demonstrated, using LTRAs such as Montelukast is usually in addition to or complementary to inhaled corticosteroids or other agents in sequential asthma management. Regardless, in 2008 there was FDA research into the possibility of Montelukast causing neuropsychiatric effects such as agitation and hallucinations and others in individuals who took the medication.[4-5] When such CysLT binds to the corresponding CysLT receptors, such as the CysLT receptor type 1 located on airway smooth muscle cells, airway macrophages, and on various pro-inflammatory cells such as eosinophils and some specific activities of myeloid stem cells that facilitate the pathophysiology of asthma and allergic rhinitis stimulated. Subsequently, Montelukast is a leukotriene receptor antagonist that binds with high affinity and selectivity to the CysLT type 1 receptor, which in turn helps in inhibiting any physiological effect of CysLT, such as LTC4, LTD4, and LET4 on the receptor that may facilitate asthma or allergic rhinitis.

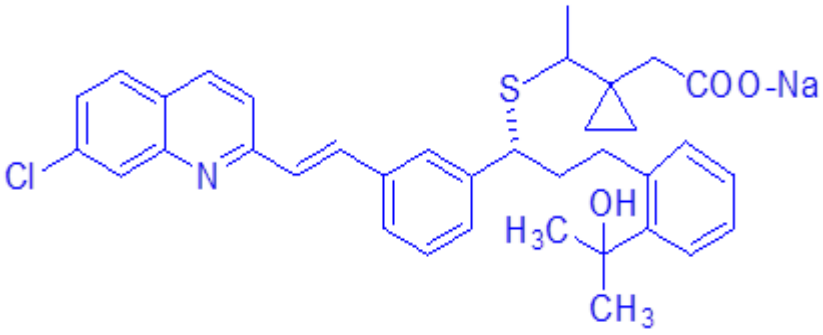


Fig 2: Chemical Structure of Montelukast Sodium

V. Amrendra Chaudhary, Anusha Kota, and Syed Muneer (mention citation) developed only the RP-HPLC method to estimate a single Bilastine drug in the pharmaceutical dosage form. There is no reported RP-HPLC method for Bilastine and Montelukast combination.[6] Andressa T.D.S, Gabriela R. b, Isadora D.H develop UV- spectroscopic method for only determination of Bilastine using experimental design for robustness by using 0.1N HCl. They concluded that the method is specific, linear, accurate, precise, and robust at 210 nm, confirming that it is rapid and useful for routine quality control determination of Bilastine in tablets.[7] Peethalaprathyusa, Raja, and Sundarajan develop UV-spectroscopic determination of Bilastine in bulk and pharmaceutical formulations. Methanol and phosphate buffer (pH-2) was used as a solvent. They concluded that the proposed method was successfully applied for the marketed formulation of a single Bilastine tablet.[8] Shaista firdous, S.H Rizwan developed a new UPLC method for evaluating

Bilastine bulk and pharmaceutical dosage form. The advanced UPLC method achieved good precision and accuracy, suitable for routine Bilastine analysis.PH 3.5 Sodium Phosphate 10mM Buffer: Methanol: Acetonitrile (60:30: 10 (v/v/v)) used as a mobile phase. The flow rate should be kept at 0.5 ml/min, with a PDA detector at 248 nm.[9] Jelena Terzic, Igor Popavic, AnjaTumpa, et al., Introduced the QBD concept for Bilastine and its degradation impurities determination by hydrophilic interaction liquid chromatography (HILC). An interpretation was obtained from their work to identify conditions where adequate separation could be achieved in minimal analysis time in a robust region.[10] The origination of Quality by Design (QbD) was illustrated as a methodology that covers a superior logical comprehension of basic interaction and item characteristics, planning controls and tests given the logical furthest reaches of comprehension during the advancement stage and utilizing the information obtained during the life-pattern of the item to chip away at a consistent improvement climate. [11-13] QbD doesn't mean less insightful testing; rather, it implies that appropriate investigation with perfect timing depends on science and hazard evaluation. Execution of QbD assists with fostering a tough and powerful (solid) technique that assists with going with ICH accordingly. Thus, drug ventures are taking on the idea of QbD. Factors influencing heartiness are considered to improve the insightful technique in QbD climate. Quality by design has become a crucial concept for method development and validation in the pharmaceutical industry.[14,15] QbD cantered on the robustness of analytical techniques that recognize controlled variation. Quality is of great importance when it comes to drugs in particular. Pharmaceutical quality can be defined as a product that has predetermined quality attributes and regulatory specifications.[16,17] As indicated by the data removed from writing to information, there is little a solitary RP-HPLC strategy detailed for the concurrent assessment of Bilastine and Montelukast utilizing the Quality by Design (QbD) approach in the drug plan. According to the ICH rule, the strategy was approved for linearity, exactness, accuracy, LOD, LOQ, framework appropriateness, and selectivity. The main objective of this study was to implement a QbD approach to manage, develop, and approve the RP-HPLC strategy, to lay out and thoroughly understand the technique, and to work in quality during the strategy development to ensure ideal strategy execution throughout the product.

2. MATERIALS AND METHODS

Working standards active pharmaceutical ingredients Bilastine and Montelukast were obtained as a gift sample from Glenmark Pharmaceutical Ltd and Curex Pharma Pvt. Ltd. Maharashtra, India. The marketed formulation (Bilazap M®) label claims each tablet contains 20 mg Bilastine and 10 mg Montelukast sodium (30mg) used for the simultaneous estimation. Methanol, HPLC grade water, ammonium acetate, trimethylamine, glacial acetic acid, and other chemicals for the study were purchased from the thermopiles fine chem. Industry.

2.1. Instruments

The HPLC (Shimadzu, Japan) system is equipped with two pumps (model LC-20AD), a PDA detector (model SPD-M20A), and fixed loop (20µl) injection system, and a C18 column. The eluent was monitored using a photodiode array detector (PDA). The sonicator was generally used for the degassing of standard, sample, and mobile phase solutions. An ultrasonic bath was used to extract the drug from the tablets. A pH meter was used to maintain the pH of the buffer used in the study. UV-Visible spectrophotometer Shimadzu-1800 was also used for the selection of the wavelength. Other equipments used were an analytical balance Shimadzu, a pipette, and a 45-micron filter.

2.2. Methods

2.3. UV Spectroscopy Method

2.3.1. Preparation of standard stock solution

(a) Bilastine: 50 mg of Bilastine standard is accurately weighed, transferred into a 50 ml volumetric flask, and dissolved in diluents to obtain a solution containing 1000 µg/mL Bilastine. Transfer 10 ml of the stock solution to a 100 ml volumetric flask. The volume is made up to the mark with diluent to obtain a standard working solution of 100 µg/mL.

(b) Montelukast: 100 mg Montelukast standard is accurately weighed, transferred into a 100 ml volumetric flask, and dissolved in the diluent to give a solution containing 1000 µg/mL. Transfer 10 ml of the stock solution to a 100 ml volumetric flask. The volume is made up to the mark with diluent to obtain a standard working solution of 100 µg/mL.

2.4. Diluent

For Bilastine: Methanol+ Water (80:20 % v/v)

For Montelukast: water

2.5. Procedure for maximum wavelength (λ)

Pipette 1 mL of each stock solution in a different volumetric flask, then transfer it to a 10 mL flask. A working standard solution of 10µg/mL is obtained by adding diluent to the volume until it reaches the desired level.

2.6. RP-HPLC Method Development

2.7. Determination of Bilastine and Montelukast by HPLC

2.7.1. Preparation of Mobile phase

Mobile phase: 85 volumes of methanol: 15 volumes of ammonium acetate buffer pH-3.6 (buffer prepared by dissolving 3.85 g of ammonium acetate in 1000 mL of water, adding 1 mL of trimethylamine adjusted to pH 3.6 with glacial acetic acid)

2.7.2. Preparation of standard and sample solutions

(A) Bilastine standard stock solution (200µg/mL):20 mg of Bilastine standard solution is accurately weighed and transferred to a 100 mL volumetric flask. The volume was topped up to the mark with diluent.

(B) Montelukast standard stock solution (100µg/mL): 10 mg of Montelukast was accurately weighed and transferred into a 100 mL volumetric flask. The volume was topped up to the mark with diluent.

(C) Preparation of tablet solution: Standard stock solution was prepared by taking the weight of 10 tablets. The equivalent weight of the tablet was 1591.5 mg. Crush the 10 tablets and take the equivalent weight of the tablet and transfer them into 100 mL of the volumetric flask to make 1000 µg/mL solution.

2.8. Selection of wavelength

The sensitivity of an HPLC method that uses UV detection depends on the correct selection of the detection wavelength. The ideal wavelength provides a good response for the drugs to be detected. In this study, solutions of the drug Bilastine and Montelukast (10 ppm) in diluent (methanol: water) were prepared. These drug solutions were then scanned in the 200-400 nm UV region, and the spectrum was recorded.

2.9. Chromatographic conditions

- ✓ Column: C18
- ✓ Mobile phase: Methanol: Acetate buffer (pH3.6) (85:15)
- ✓ Flow Rate: 1.0 mL/min
- ✓ Detection Wavelength: Bilastine- 249nm, Montelukast -293
- ✓ Mobile phase Run time: 10 min
- ✓ The volume of Injection: 20.0 µL
- ✓ Software: LC solution
- ✓ Detector: PDA detector

Diluents: methanol: water (50:50)

3. EXPERIMENTAL AND RESULTS

3.1. UV-spectroscopy

3.2. Procedure for determination of maximum wavelength (λ)

Pipette 1 mL of each stock solution in a different volumetric flask, then transfer it to a 10 mL flask. A working standard solution of 10 µg /mL is obtained by adding diluent to the volume until it reaches the desired level.

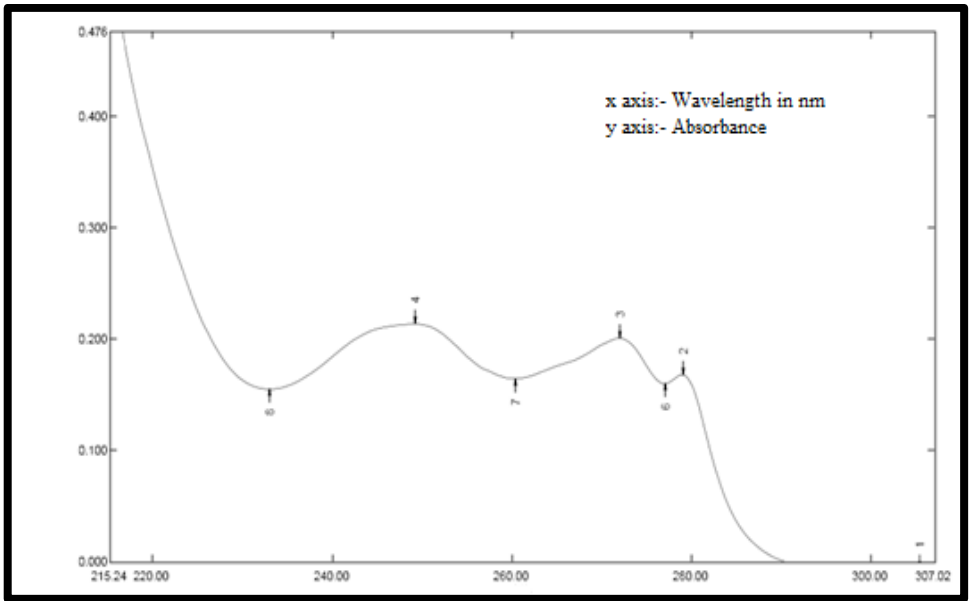


Fig 3: Maximum wavelength of Bilastine

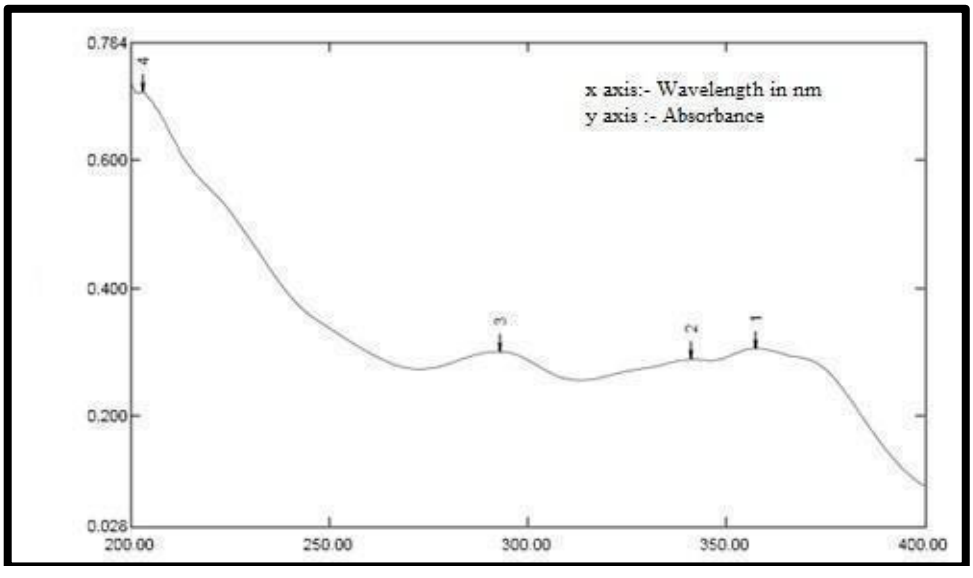


Fig 4: Maximum wavelength of Montelukast

3.1. Validation of UV- Spectrophotometric method

3.2. Bilastine

3.3. Linearity

The linearity for Bilastine was evaluated by setting up the arrangement in the scope of 5-30 µg/mL. The correlation coefficient for calibration for the alignment bend of Bilastine was viewed as 0. 999. The method was linear in a 5-30µg/mL concentration range. (Table1)

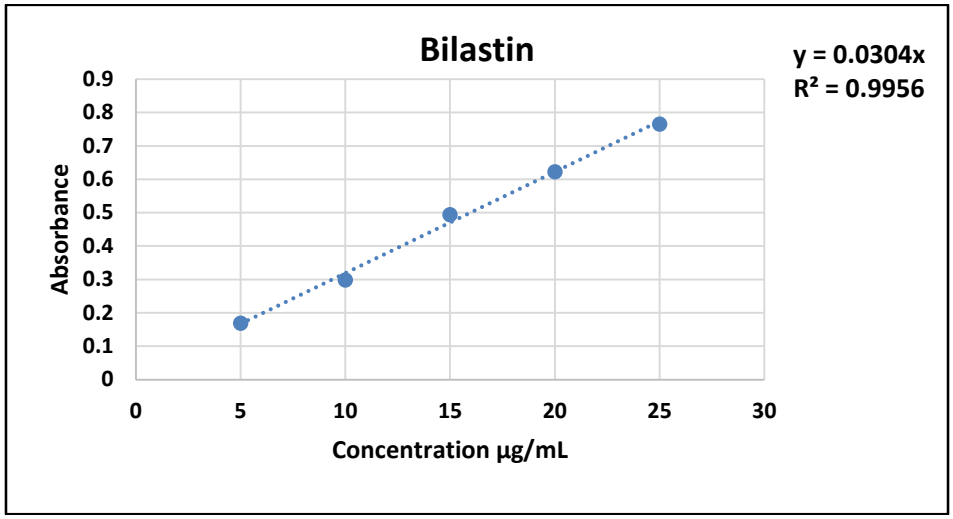


Fig 5: Calibration Curve of Bilastine

Table1: Linearity data of Bilastine

Sr.no.	Conc. µ g/mL	Absorbance-I	Absorbance-II	Mean	SD	%RSD
1	5	0.084	0.071	0.0775	0.009	0.11
2	10	0.149	0.133	0.141	0.011	0.08
3	15	0.218	0.227	0.2225	0.006	0.02
4	20	0.287	0.269	0.278	0.012	0.045
5	25	0.369	0.419	0.394	0.035	0.089
6	30	0.44	0.46	0.45	0.014	0.031

3.4. Precision

3.4.1. Interday Precision

The inter-day precision for the estimation of Bilastine was performed at three different concentration levels of 10, 20, and 30 µg/mL, and the absorbance was measured at 249 nm. The testing was done on three different days, and the % RSD was found to be 0.46, 0.015, and 0.020, respectively. Table 2 displays the study of Interday precision.

Table 2: Interday precision of Bilastine

Sr.no	Concentration µ g/mL	Absorbance I	Absorbance II	Mean	SD	% RSD
1	10	0.148	0.158	0.153	0.007	0.046
2	20	0.278	0.284	0.281	0.004	0.015
3	30	0.44	0.453	0.446	0.009	0.020

3.4.2. Intraday Precision

The intraday variation for the estimation of Bilastine was carried out at three different concentration levels of 10, 20, and 30 µg/mL, and absorbance was measured at 293 nm. During the study of intraday precision, the Bilastine was used in a concentration of 10µg/mL, 20µg/mL, and 30µg/mL were analyzed on the same day, and % RSD was found to be 0.53 and 1.08, respectively. (Table3)

Table 3: Intraday precision of Bilastine

Sr.No	Concentration	µ g/mL	Absorbance I	Absorbance II	Mean	SD	% RSD
1	10		0.168	0.156	0.162	0.008	0.052
2	20		0.294	0.274	0.284	0.014	0.049
3	30		0.447	0.459	0.453	0.008	0.018

3.5. *Repeatability*

Repeatability or framework reasonableness tests were done on the standard arrangement of Bilastine. Not entirely set in stone getting ready five repeats of 30µg/mL of Bilastine, and the absorbance was estimated at 249nm. The mean region, SD, and % RSD were viewed as 0.44, 0.0046, and 1.04 individually.

Table 4: Repeatability study of Bilastine

Sr. no	Concentration µ g/mL	Absorbance at 249 nm	
1	30	0.449	Mean=0.4458 SD=0.0046 %RSD= 1.04
2	30	0.447	
3	30	0.451	
4	30	0.440	
5	30	0.442	

3.6. *Accuracy*

1 mL (300 µg/mL) of the binary mixture of the standard drug solution was taken into three different flasks labeled A, B, and C. Next, add 0.5, 1, and 1.5 mL of the sample solution to flasks A, B, and C. Add spike 50%, 100%, and 150% standard solution and dilute to 10mL. The area of each solution peak was measured at 249 nm and 293 nm. Bilastine and Montelukast were calculated at each level, and the % recovery was calculated. During the recovery study, Bilastine and Montelukast were used in a concentration of 50%, 100%, and 150%. The % amount recovered was 100.29%, 96.39%, and 95.09%, respectively, for Bilastine and 96.28%, 97.42%, and 99.23% for Montelukast. The recovery study is shown in Table 5 and Table 6.

Table 5: Recovery Study of Bilastine

Sr.no	%level	Absorbance I	Absorbance II	Mean	% RSD	Amount Added	Amount Found	% Amount
1	50	0.121	0.146	0.133	0.132	10	10.02	100.29
2	100	0.259	0.267	0.263	0.021	20	19.27	96.39
3	150	0.396	0.389	0.392	0.012	30	28.52	95.09

Table 6: Recovery study of Montelukast

%level	Absorbance I	Absorbance II	Mean	% RSD	Amount Added	Amount Found	% Amount
50	0.057	0.064	0.060	0.08	5	4.81	96.28
100	0.086	0.173	0.129	0.47	10	9.74	97.42
150	0.195	0.208	0.201	0.04	15	14.88	99.23

3.7. *Montelukast*

3.8. *Linearity*

The linearity of Montelukast was evaluated by setting up the arrangement in the scope of 5-30 µg/mL. The Correlation coefficient for calibration for the alignment bend of Montelukast was viewed as 0.999. This method was designed to be linear over a 5-30 µg/mL concentration range. (Table7)

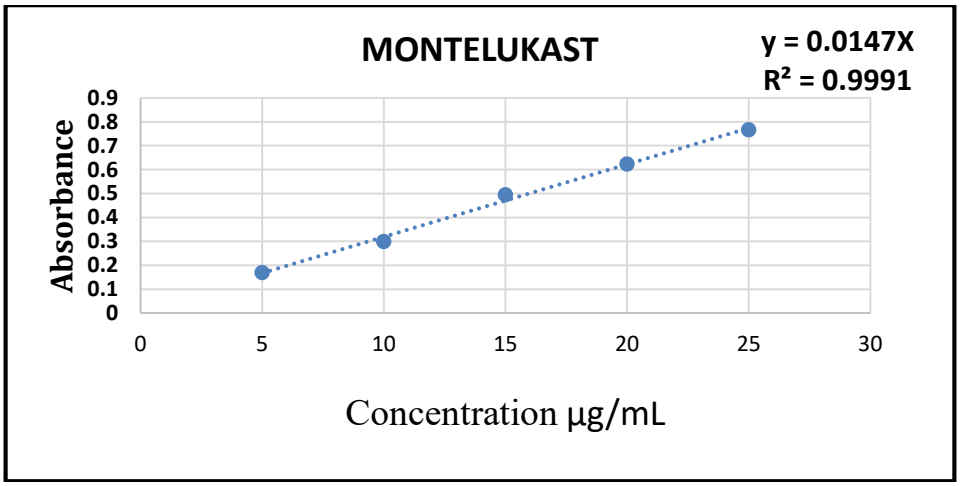


Fig 6: Calibration Curve of Montelukast

Table 7: Linearity data of Montelukast						
Sr.no	Concentration µ g/mL	Absorbance I	Absorbance II	Mean	SD	%RSD
1	5	0.169	0.072	0.120	0.068	0.56
2	10	0.299	0.139	0.219	0.113	0.51
3	15	0.494	0.237	0.365	0.181	0.49
4	20	0.623	0.269	0.446	0.250	0.56
5	25	0.766	0.423	0.594	0.242	0.40
6	30	0.915	0.459	0.687	0.322	0.46

3.9. Interday Precision

The Interday variation for the estimation of Montelukast was carried out at three different concentration levels of 10, 20, and 30 µg/mL, and absorbance was measured at 293 nm. The testing was done on three different days, and the % RSD was found to be 0.013, 0.001, and 0.006, respectively. Table 8 displays the study of Interday precision.

Table 8: Interday Precision of Montelukast						
Sr. no	Concentration µ g/mL	Absorbance I	Absorbance II	Mean	SD	% RSD
1	10	0.316	0.322	0.319	0.004	0.013
2	20	0.609	0.608	0.608	0.0007	0.001
3	30	0.897	0.889	0.893	0.0056	0.006

3.10. Intraday Precision

The intraday variation for the estimation of Montelukast was carried out at three different concentration levels of 10, 20, and 30 µg/mL, and absorbance was measured at 293 nm. During the study of intraday precision, the drug was used in a concentration of 10 µg/mL, 20 µg/mL, and 30 µg/mL were analyzed on the same day, and % RSD was found to be 0.50, 0.524, and 0.441respectively. (Table9)

Table 9: Intraday Precision of Montelukast						
Sr. no	Concentration µ g/mL	Absorbance I	Absorbance II	Mean	SD	% RSD
1	10	0.327	0.156	0.241	0.120	0.500
2	20	0.597	0.274	0.435	0.228	0.524
3	30	0.876	0.459	0.667	0.294	0.441

3.11. Repeatability

Repeatability or framework reasonableness tests were done on the standard arrangement of Montelukast. Not entirely set in stone getting ready five repeats of 30µg/mL of Montelukast, and the absorbance was estimated at 293 nm. The mean region, SD, and % RSD were viewed as 0.886, 0.00886, and 1.00 individually. (Table 10)

Table 10: Repeatability study of Montelukast			
Sr.no	Concentration µ g/mL	Absorbance at 249 nm	
1	30	0.888	Mean= 0.886 SD= 0.00886 %RSD=1.00
2	30	0.882	
3	30	0.896	
4	30	0.891	
5	30	0.873	

3.12. Simultaneous estimation

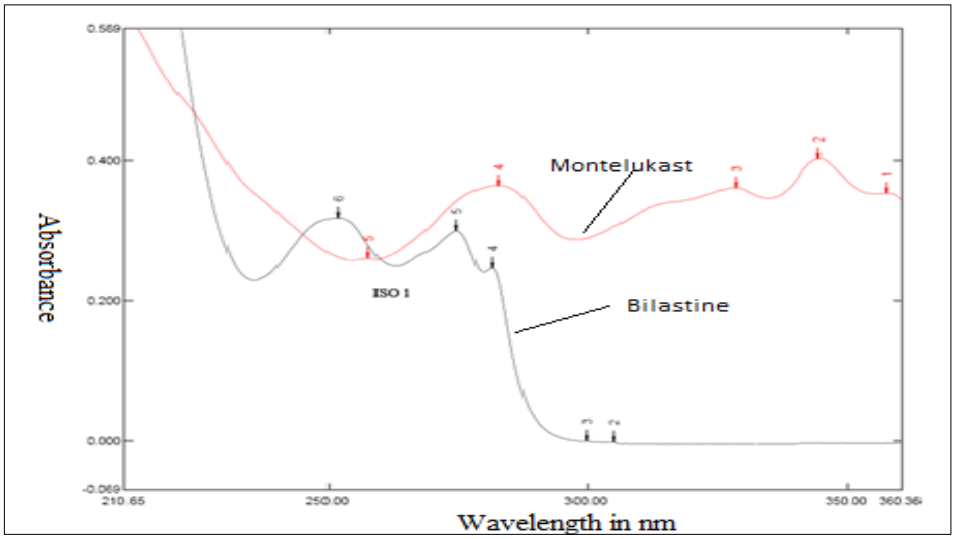


Fig 7: Overlay spectra of Bilastine 10mcg and Montelukast 10 µ g/mL

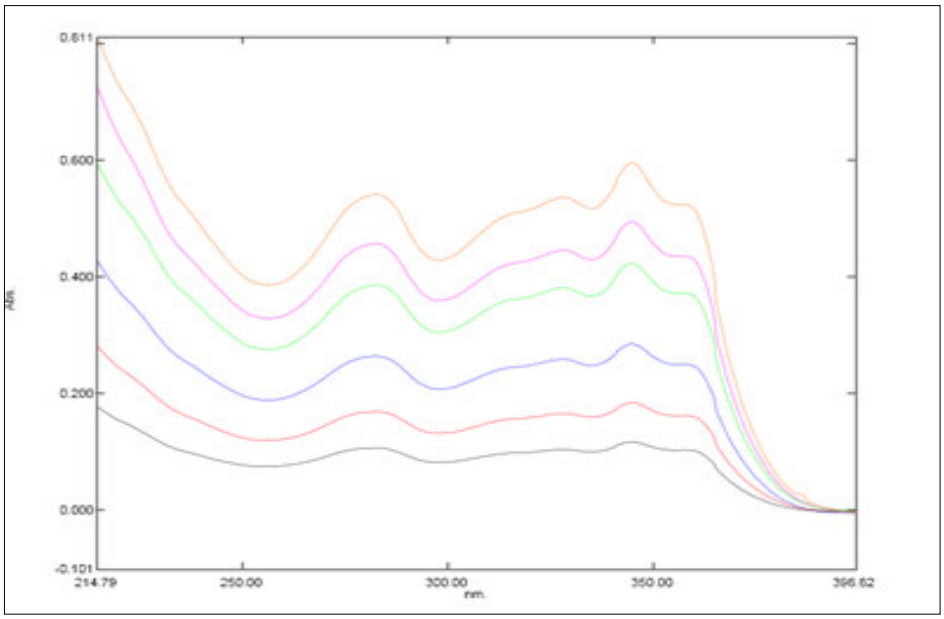


Fig 8: Overlay spectra of Montelukast at concentrations 2, 4, 6, 8, 10, 12 µg/mL

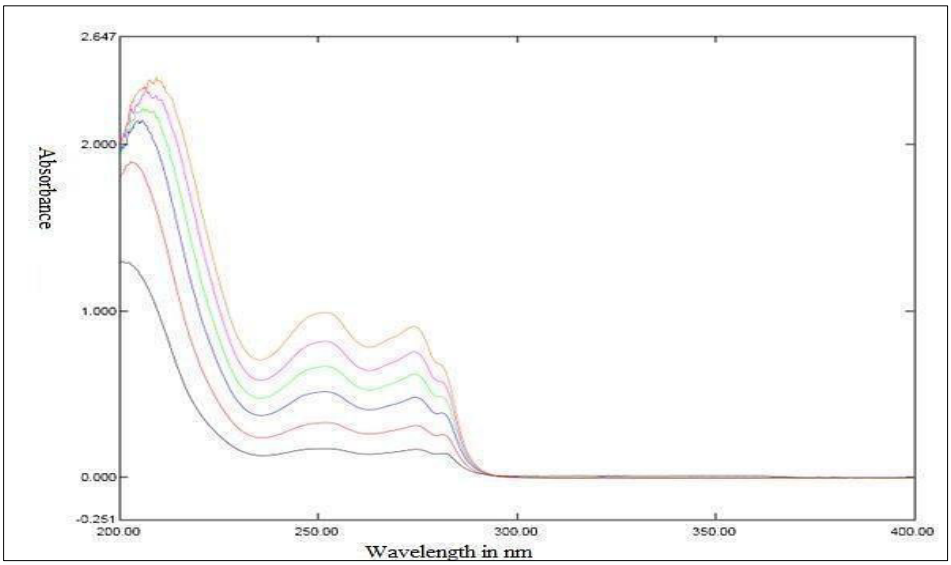


Fig 9: Overlay spectra of Bilastine at concentrations 5, 10, 15, 20, 25, and 30 µg/mL

3.13. HPLC Results

3.14. Mobile Phase Optimization

For the HPLC method, the study development of Bilastine and Montelukast API contains different mobile phases, from methanol and buffer, in different ratios and volumes at different flow rates. Based on various experiments, a mixture of methanol and buffer (85:15) at a flow rate of 1.0 mL/min and a detection wavelength of 249 nm was superior to another mixture in peak shape, theoretical plate, and asymmetry.

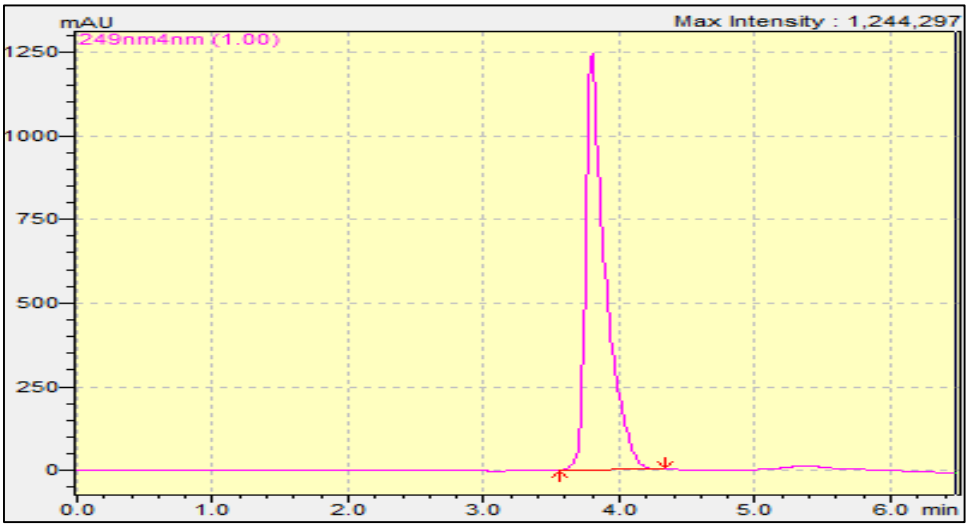


Fig 10: Optimized chromatogram of Bilastine API

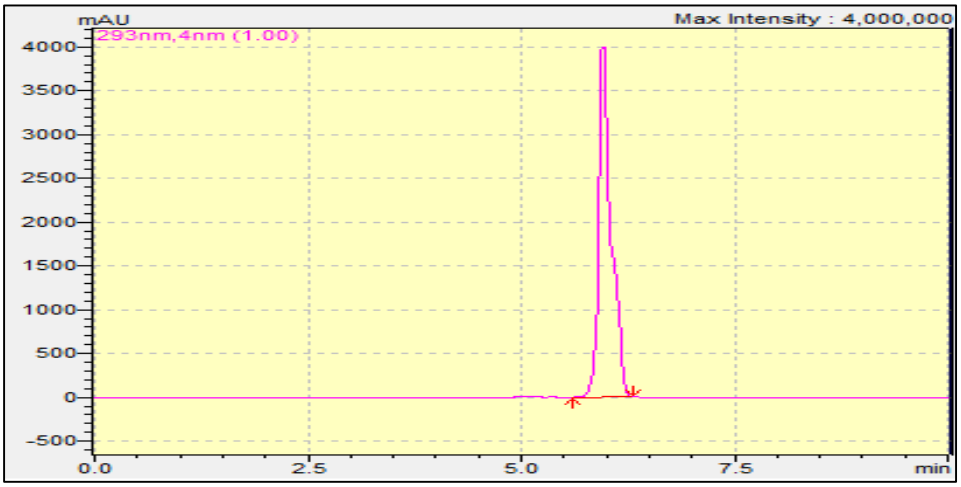


Fig 11: Optimized chromatogram of Montelukast API

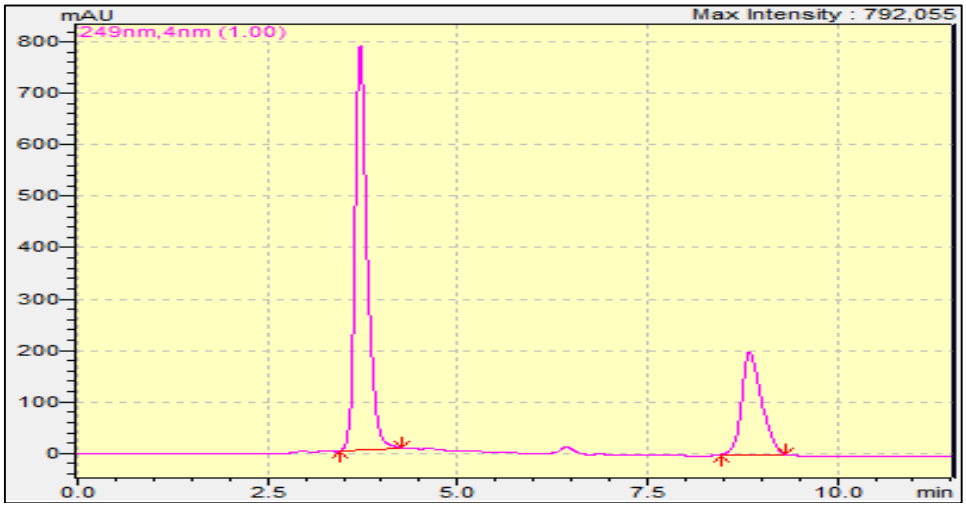


Fig 12: Chromatogram of BIL and MKT formulation in methanol: buffer (85:15), flow rate (1.0 mL/min)

3.15. System suitability testing

The identical standard solution of Bilastine 200µg/mL and Montelukast 100µg/mL in the blend was injected in the RP-HPLC column with succeeding optimized chromatographic parameters, and the chromatogram was recorded. The chromatogram was analyzed to estimate retention time, peak area, number of theoretical plates, tailing factor, etc. The obtained results were compared with the limits given in ICH guidelines Q2R1. The appropriate process was used, added five times, and reported results for each seen chromatogram. Accordingly, the mean retention time and mean area were computed.[18]

Table 10.1: Optimized Chromatographic Parameters

Sr. No.	Particular	Optimized Chromatographic Parameters
1	Column	C18
2	Mobile Phase	Methanol: Acetate buffer (pH3.5) (85:15)
3	Flow Rate	1.0 mL/min
4	Detection Wavelength:	Bilastine- 249nm, Montelukast -293
5	Run time	10 min
6	Injection Volume	20.0 µL
7	Software	LC solution
8	Detector	PDA detector
9	Diluents	Methanol: water (50:50)

3.16. HPLC Method Validation

Different validation parameters are studied here by using optimized chromatographic conditions.

3.17. Linearity

Linearity for Bilastine and Montelukast was assessed by preparing a solution in the range of 200-600 µg/mL. Take 2, 3, 4, 5, and 6 mL of the sample stock solution, transfer it to a 10 mL volumetric flask, and make up the mark with diluent. The correlation coefficient for the Bilastine and Montelukast calibration curves was 0.999.

Table 11: Linearity data of Bilastine by HPLC

Concentration µ g/mL	Retention time	Area I	Area II	Mean	SD	%RSD
200	3.555	3407.913	3401.23	3404.5715	4.72	0.0013
300	3.439	4697.002	4711.02	4704.011	9.91	0.0021
400	3.422	6156.756	6141.62	6149.188	10.70	0.0017
500	3.495	7963.865	7958.56	7961.2125	3.75	0.0004
600	3.456	9667.614	9659.29	9663.452	5.88	0.0006

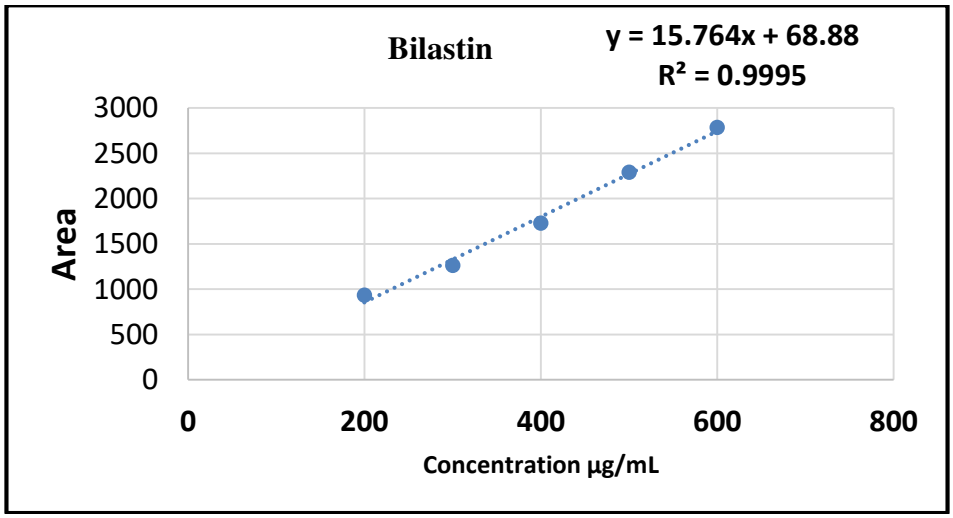


Fig 13: Calibration Curve of Bilastine by HPLC

Table 12: Linearity data of Montelukast HPLC						
Concentrationμ g/mL	Retention time	Area I	Area II	Mean	SD	%RSD
200	6.433	932.29	918.32	925.331	9.84	0.01064
300	6.934	1262.34	1327.14	1294.74	45.82	0.03539
400	7.003	1729.6	1892.7	1810.65	114.62	0.0633
500	7.292	2289.54	2387.09	2338.32	68.98	0.0295
600	7.392	2783.75	2907.57	2845.16	86.84	0.03053

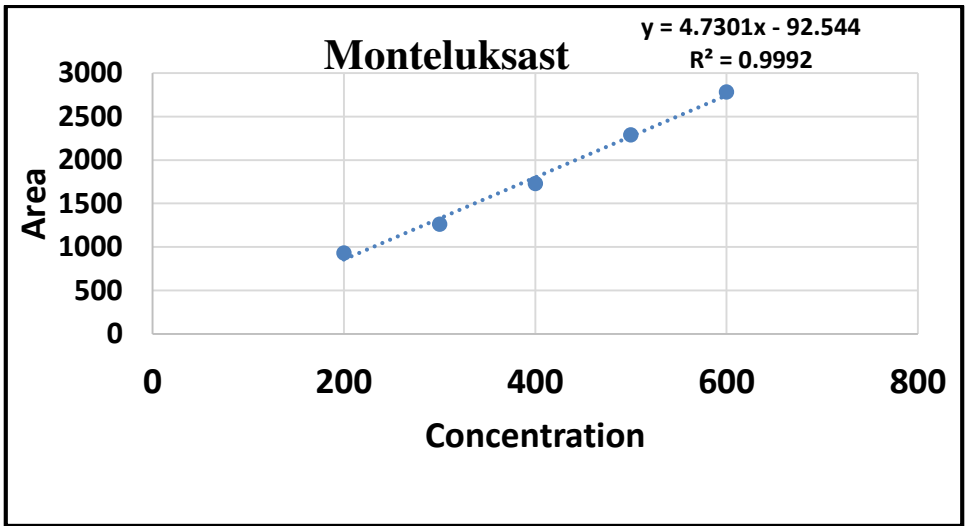


Fig 14: Calibration Curve of Montelukast by HPLC

3.18. Accuracy

Tables 13 and 14 display the recovery study (Accuracy) of Bilastine and Montelukast. Bilastine and Montelukast were used in a concentration of 50%, 100%, and 150%. For Bilastine, the % amount recovered was 100.23%, 102.06%, and 95.33%, respectively. For Montelukast, the % amount recovered was 97.07%, 104.05%, and 96.31%, respectively.

Table 13: Recovery study of Bilastine (Accuracy) by HPLC							
Sr.no	Concentration level %	Retention time	Area	Mean	Amount added	Amount found	% Amount
1	50	3.374	969.87	965.04	10	10.02	100.23
2	50	3.374	960.21				
3	100	3.364	1655.18	1651.64	20	20.41	102.06
4	100	3.364	1648.11				
5	150	3.364	2198.01	2192.64	30	28.60	95.33
6	150	3.364	2187.27				

Table 14: Recovery study of Montelukast (Accuracy) by HPLC							
Sr. no	Concentration level %	Retention time	Area	Mean	Amount added	Amount found	% Amount
1	50	7.23	617.69	623.44	5	4.85	97.07
2	50	7.23	629.19				
3	100	7.17	984.22	990.3	10	10.40	104.05
4	100	7.17	996.38				
5	150	7.13	1262.51	1257.43	15	14.44	96.31
6	150	7.13	1252.34				

3.19. Intraday Precision

Table 15 displays the study of the intraday precision of Bilastine and Montelukast. Both the drugs were used in a concentration of 200 μg/mL, 400 μg/mL, and 600 μgm/mL were analyzed on the same day, and % RSD was found to be 0.53 and 1.08, respectively.

Table 15: Intraday Precision					
Sr.no	Concentrations µ g/mL	Bilastine		Montelukast	
		Retention time	Area	Retention time	Area
1	200	3.751	4217.356	8.012	1445.514
	200	3.748	4201.249	8.012	1451.458
2	400	3.464	6697.395	7.112	2081.374
	400	3.464	6710.362	7.112	2098.320
3	600	3.447	7421.892	7.460	2860.677
	600	3.447	7408.857	7.460	2851.643
		%RSD	0.53*		1.08*

**** Sum of %RSD of all the readings.***

3.20. Interday Precision

Table 16 displays the study of the Interday precision of Bilastine and Montelukast. Both the drugs were used in a concentration of 200 µg/mL, 400 µg/mL, and 600 µg/mL were analyzed on different days, and % RSD was found to be 0.47 and 1.11, respectively.

Table 16: Interday Precision					
Sr.no	Concentrations µ g/mL	Bilastine		Montelukast	
		Retention time	Area	Retention time	Area
1	200	3.430	1749.29	7.505	957.059
2	200	3.428	1749.2	7.501	951.028
3	400	3.444	2882.71	7.563	1005.45
4	400	3.440	2870.64	7.559	1010.39
5	600	3.471	6209.92	7.618	2384.79
6	600	3.473	6224.86	7.619	2373.89
		%RSD	0.47*		1.11*

**** Sum of %RSD of All the readings.***

3.21. Repeatability

Repeatability or system suitability tests were carried out by 5 repeated injections of Bilastine and Montelukast sample solution. The % RSD was 0.094 and 1.22 for Bilastine and Montelukast, respectively. (Table 17)

Table17: Repeatability by HPLC			
Sr.no	Concentrations µ g/mL	Bilastine	Montelukast
		Area	Area
1	600	7890.32	2310.83
2	600	7805.63	2354.79
3	600	7859.82	2359.83
4	600	7839.94	2339.87
5	600	7699.86	2369.84
	Mean	**7819.114**	**2349.032**
	S.D	7.3499	28.6581
	% RSD	0.094	1.22

3.22. Limit of detection (LOD)

The limit of detection (LOD) of Bilastine and Montelukast was found to be 0.493 and 0.693, respectively (Table 18).

Table 18: Limit of Detection (LOD) By HPLC	
Bilastine	Montelukast
Formula LOD = 3.3×avg S.D/Slope	Formula LOD = 3.3×avg S.D/Slope
Avg.SD = 6.99	Avg.SD = 9.68
Slope = 46.73	Slope = 46.08
LOD = 3.3x 6.99/46.73 = 0.493	LOD = 3.3x9.68/46.08 =0.693

3.23. Limit of Quantitation (LOQ)

The limit of Quantitation (LOQ) of Bilastine and Montelukast was found to be 1.49 and 2.10, respectively (Table 19).

Table 19: Limit of Quantitation (LOQ) by HPLC	
Bilastine	**Montelukast**
Formula LOQ = 10×average S.D/Slope Avg.SD = 6.99 Slope = 46.73 LOD = 10x 6.99/46.73 = 1.495	Formula LOQ = 10 ×average S.D/Slope Avg.SD = 9.68 Slope = 46.08 LOD = 10x9.68/46.08 =2.100

3.24. Assay of Marketed Formulation

Table 20 displays the assay of Bilastine and Montelukast. Bilastine's mean area was 1651.64, and the % of drugs found was 102.06. Montelukast's mean area was 990.30, and the % of drugs found was 104.05.

Table 20: Assay of Marketed Formulation of Bilastine and Montelukast by HPLC			
Bilastine			
Conc.	**Area**	**Amount Found**	**% Label Claim**
20.00	1655.18	20.41	**102.06**
20.00	1648.11		
Mean	1651.64	-	-
SD	4.99		
%RSD	0.003		
Montelukast			
10.00	984.22	10.40	**104.05**
10.00	996.38		
Mean	990.3	-	-
SD	8.59		
%RSD	0.008		

4. QBD ASSISTED RESULTS

Table 21: Actual Design of Set as per Influential Factors (Bilastine in combination)						
Std	**Run**	**Block 1**	**Factor 1 Mobile Phase A**	**Factor 2 Mobile Phase B**	**Factor 3 Flow Rate**	**Area V**
z	1	Block 1	80	20	1.5	17214004
11	2	Block 1	80	15	1	0
13	3	Block 1	80	20	0.5	23187756
5	4	Block 1	75	15	1.5	0
7	5	Block 1	75	25	1.5	17328774
19	6	Block 1	80	20	1	25711736
3	7	Block 1	75	25	0.5	31811137
2	8	Block 1	85	15	0.5	26544289
12	9	Block 1	80	25	1	0
17	10	Block 1	80	20	1	25711700
4	11	Block 1	85	25	0.5	0
20	12	Block 1	80	20	1	25711736
16	13	Block 1	80	20	1	25711736
1	14	Block 1	75	15	0.5	0
10	15	Block 1	85	20	1	0
8	16	Block 1	85	25	1.5	0
15	17	Block 1	80	20	1	25711736
6	18	Block 1	85	15	1.5	14685872
9	19	Block 1	75	20	1	0
18	20	Block 1	80	20	1	25711736

Table 22: Design Summary for Bilastine			
Design Summary			
Study type	Response surface		
Initial design	Central composite	**Runs**	20

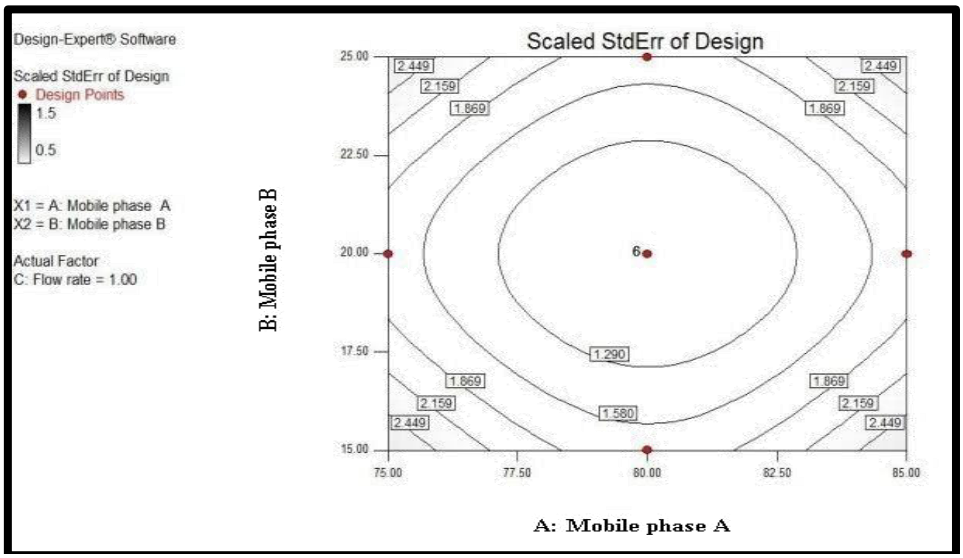
Design model	Quadratic			Blocks	No blocks						
Factor	Name	Unit	type	Low actual	High actual	Low coded	High coded	Mean	SD		
A	Mobile phase A	v/v %	Numerical	75	85	-1	1	80	3.535534		
B	Mobile phase B	v/v %		15	25	-1	1	20	3.535534		
C	Flow rate	mL/min		0.5	15	-1	1	1	0.353553		
Response	Name	Unit	Obs	Analysis	Min	Max	Mean	SD	Ratio	Trans	Model
Y1	Area	Volts	20	Polynomial	0	31311137	142521106	12187305	N/A	None	Quadratic

Table 23: ANOVA for response surface quadratic model for Bilastine

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	Df	Mean Square	F Value	p-value Probe> F	
Model	2.09172E+15	9	2.32413E+14	2.644402069	0.0729	not significant
A-Mobile phase A	6.25641E+12	1	6.25641E+12	0.071185567	0.7950	
B-Mobile phase B	6.25641E+12	1	6.25641E+12	0.071185567	0.7950	
C-Flow rate	1.04423E+14	1	1.04423E+14	1.188125106	0.3013	
AB	1.02084E+15	1	1.02084E+15	11.6151735	0.0067	Significant
AC	8.60637E+11	1	8.60637E+11	0.009792334	0.9231	
BC	8.60637E+11	1	8.60637E+11	0.009792334	0.9231	
A^2	2.88941E+14	1	2.88941E+14	3.28757114	0.0999	
B^2	2.88941E+14	1	2.88941E+14	3.28757114	0.0999	
C^2	2.72287E+14	1	2.72287E+14	3.09808229	0.1089	
Residual	8.78888E+14	10	8.78888E+13			
Lack of Fit	8.78888E+14	5	1.75778E+14			
Pure Error	0	5	0			
Cor Total	2.97061E+15	19				

- The Model F-value of 2.64 implies a 7.29% chance that a "Model F-value "this large could occur due to noise.
- The "Probe> F" values less than 0.0500 indicate that model terms are significant. In this case, AB is an effective model term. Values greater than 0.1000 indicate the model terms are not significant.
- If there are many insignificant model terms (not counting those required to support hierarchy), Model reduction may improve your model.



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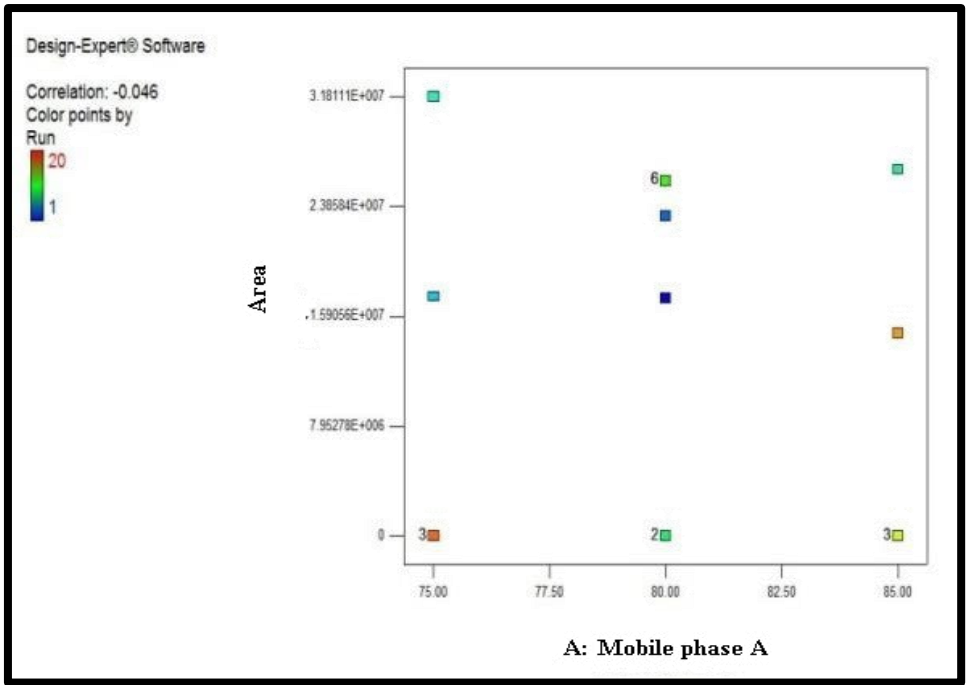
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6
3
2
3
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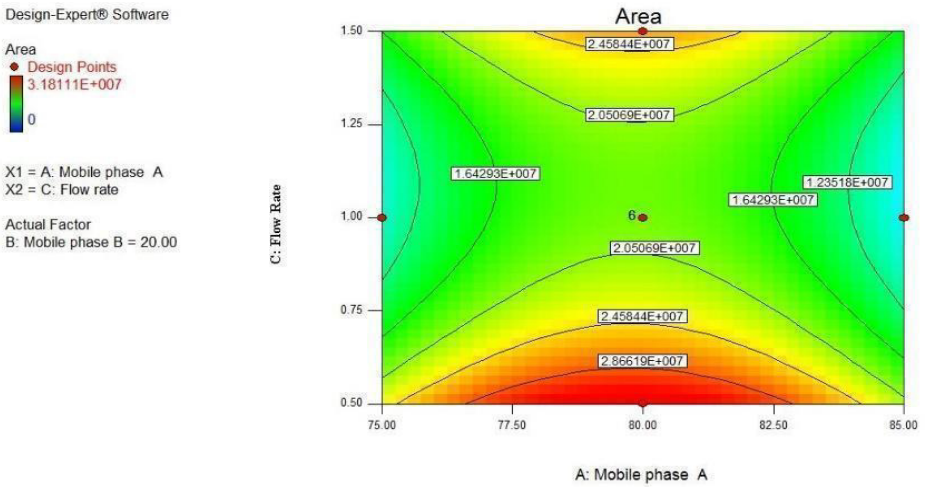
80.00
82.50
85.00
A: Mobile phase A

(b)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1.64293E+007
1.23518E+007
1.64293E+007
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2.45844E+007
2.86619E+007
C: Flow Rate
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75.00
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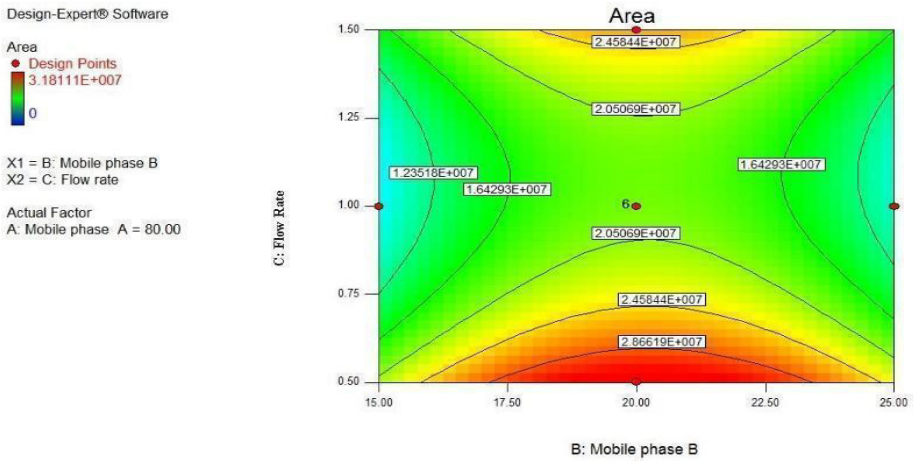
(C)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1.64293E+007
1.64293E+007
6
2.05069E+007
2.45844E+007
2.86619E+007
C: Flow Rate
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15.00
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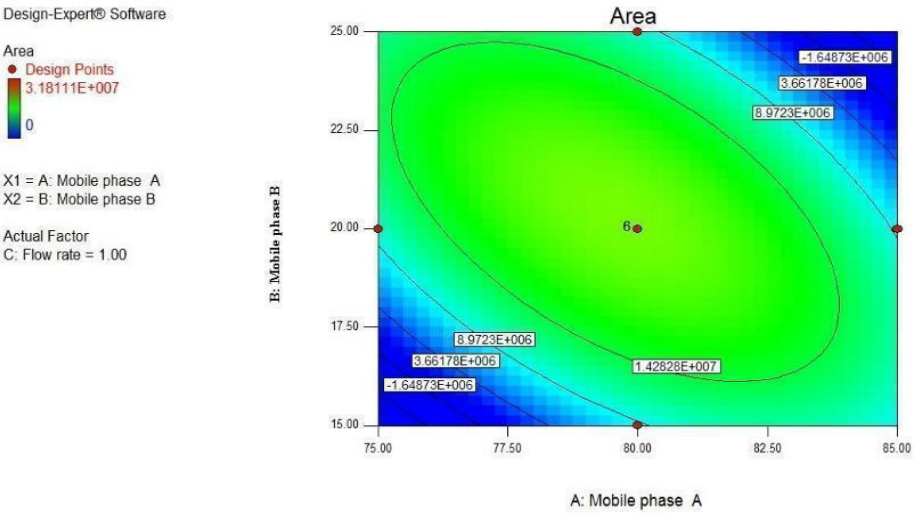
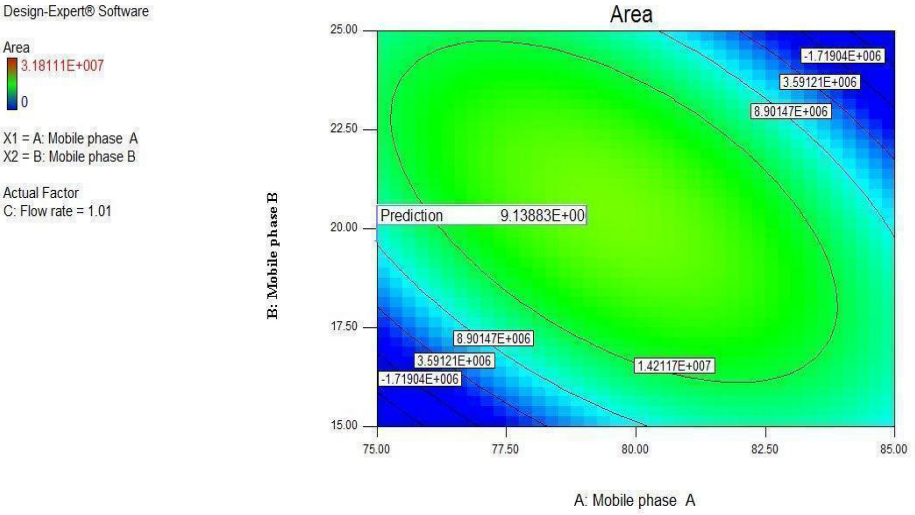
22.50
25.00
B: Mobile phase B

(d)



(e)



(f)

Fig 15: (a), (c), (d), and (e) contour plots, (b) Response surface plot showing the effect of (X1=mobile phase ratio) and (x2= flow rate) on a response (y1=response is) and (y2= retention time)

Table 24: Actual Design of Set as per Influential Factors (Bilastine in combination)

Std	Run	Block 1	Factor 1 Mobile Phase A	Factor 2 Mobile Phase B	Factor 3 Flow Rate	Area V
14	1	Block 1	80	20	1.5	211647
3	2	Block 1	75	25	0.5	11601345
20	3	Block 1	80	20	1	244703
15	4	Block 1	80	20	1	244703
4	5	Block 1	85	25	0.5	0
11	6	Block 1	80	15	1	0
1	7	Block 1	75	15	0.5	0
2	8	Block 1	85	15	0.5	11601345
16	9	Block 1	80	20	1	244703
10	10	Block 1	85	20	1	0
7	11	Block 1	75	25	1.5	1175641
13	12	Block 1	80	20	0.5	244703
18	13	Block 1	80	20	1	244703
6	14	Block 1	85	15	1.5	4409566
17	15	Block 1	80	20	1	244703
19	16	Block 1	80	20	1	244703
5	17	Block 1	75	15	1.5	0
12	18	Block 1	80	25	1	0

8	19	Block 1	85	25	1.5	0
9	20	Block 1	75	20	1	0

Table 25: Design summary for Montelukast

Design Summary			
Study type	Response surface		
Initial design	Central composite	**Runs**	20
Design model	Quadratic	**Blocks**	No blocks

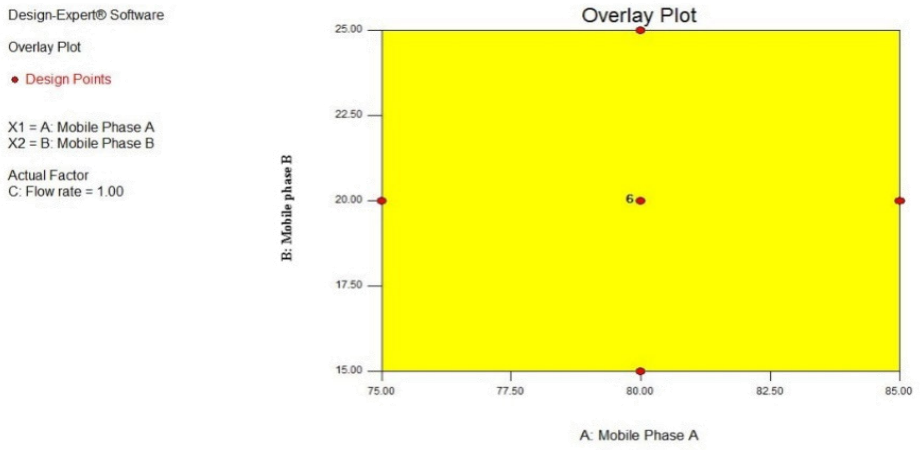
Factor	Name	Unit	type	Low actual	High actual	Low coded	High coded	Mean	SD
A	Mobile phase A	v/v %	Numerical	75	85	-1	1	80	3.535534
B	Mobile phase B	v/v %		15	25	-1	1	20	3.535534
C	Flow rate	mL/min		0.5	15	-1	1	1	0.353553

Response	**Name**	**Unit**	**obs**	**Analysis**	**Min**	**Max**	**Mean**	**SD**	**Ratio**	**Trans**	**Model**
Y1	Area	Volts	20	Polynomial	0	1160134	153562	348790	N/A	None	No model Chosen

Table 26: ANOVA for response surface quadratic model for Montelukast

Response	**1**	**Area**				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Probe> F**	
Model	1.89846E+14	9	2.1094E+13	3.945465253	0.0217	Significant
A-Mobile Phase A	1.04583E+12	1	1.04583E+12	0.19561359	0.6677	
B-Mobile Phase B	1.04583E+12	1	1.04583E+12	0.19561359	0.6677	
C-Flow rate	3.11542E+13	1	3.11542E+13	5.827135004	0.0364	
AB	1.03593E+14	1	1.03593E+14	19.37621876	0.0013	Significant
AC	1.30728E+12	1	1.30728E+12	0.244516987	0.6316	
BC	1.30728E+12	1	1.30728E+12	0.244516987	0.6316	
A^2	3.69294E+12	1	3.69294E+12	0.690735754	0.4253	
B^2	3.69294E+12	1	3.69294E+12	0.690735754	0.4253	
C^2	5.29041E+12	1	5.29041E+12	0.989528766	0.3433	
Residual	5.34639E+13	10	5.34639E+12			
Lack of Fit	5.34639E+13	5	1.06928E+13			
Pure Error	0	5	0			
Cor Total	2.4331E+14	19				

- The Model F-value of 3.95 implies the model is significant. There is only a 2.17% chance that a "Model F-value" this large could occur due to noise.
- The "Probe> F" values less than 0.0500 indicate that model terms are significant. In this case, C and AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.
- If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.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X2 = B: Mobile Phase B
Actual Factor
C: Flow rate = 1.00
Overlay Plot
B: Mobile phase B
A: Mobile Phase A
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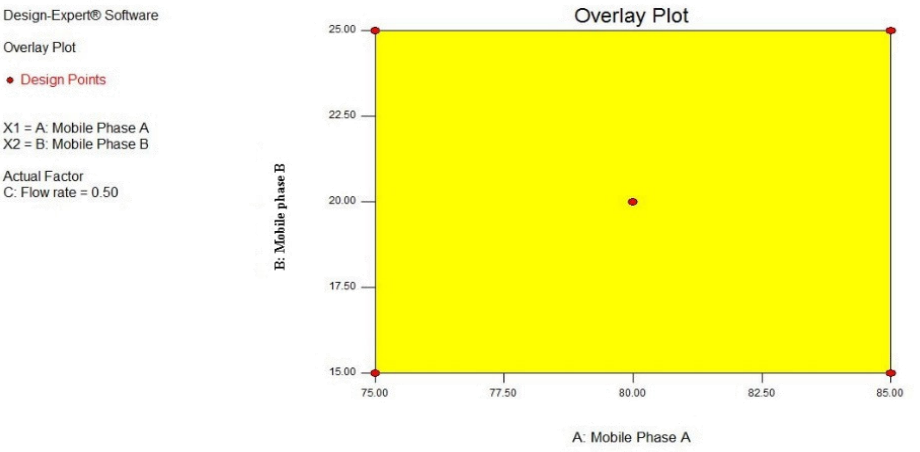
20.00
17.50
15.00
75.00
77.50
80.00
82.50
85.00
6

(a)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Overlay Plot
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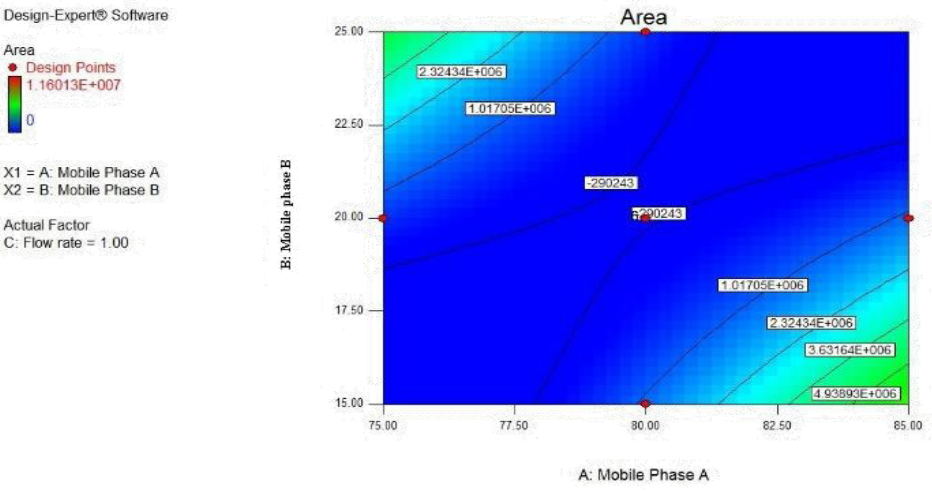
20.00
17.50
15.00
75.00
77.50
80.00
82.50
85.00

(b)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X2 = B: Mobile Phase B
Actual Factor
C: Flow rate = 1.00
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15.00
75.00
77.50
80.00
82.50
85.00
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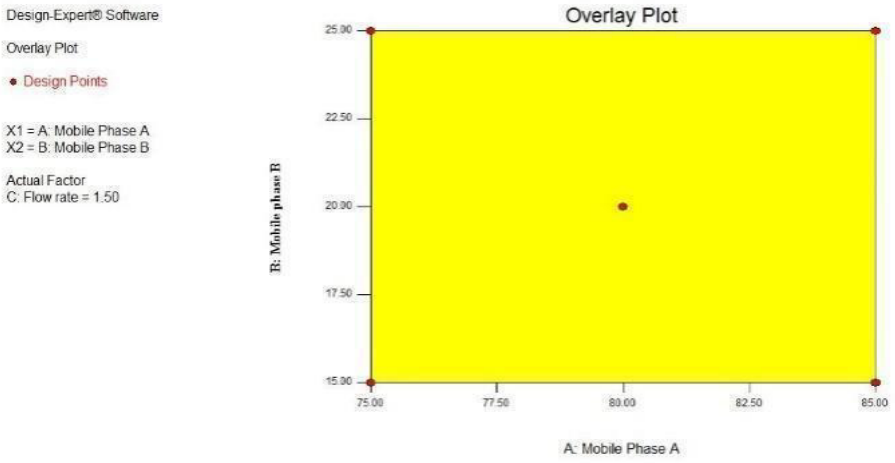
(c)



(d)

Fig16: (a), (b), (c) Response surface plot showing the effect of (X1=mobile phase ratio) and (x2= flow rate) on response (y1=response is) and (y2= retention time) (d) Contour plot

5. DISCUSSION

A new simple, sensitive, accurate, and precise [19,20] HPLC method has been developed and validated with different parameters for Bilastine and Montelukast. The chromatograms were developed using a mobile phase of Methanol: Buffer (85:15) with a flow rate of 1 ml/min. C18 Column was used as a stationary phase, with particle size 10µm. The detection was carried out at 249 nm and 293 nm wavelengths for Bilastine and Montelukast, respectively. The method was validated according to ICH guidelines for System suitability, linearity, precision, accuracy, LOD, and LOQ [21,22] Estimation of Bilastine and Montelukast UV- Spectroscopy. Estimating Bilastine and Montelukast [23,24] by RP- HPLC has been done despite the case of UV- spectroscopic method solubility being the important parameter. The solubility parameter was studied, and methanol and water were selected as the solvent; it gave a maximum absorbance and a good spectral pattern when compared with other solvents. The linearity for Bilastine and Montelukast was found to be in the range of 05-30 µg/ml at the maximum absorbance of 249 nm and 293 nm. Percentage recovery and linearity studies were also carried out. The above method gave a good recovery value and was linear. The method's precision was studied, and the standard deviation was determined. Inter-day and intraday precision were also carried out, and % RSD was calculated. The UV method has been developed to quantify Bilastine and Montelukast in bulk. The validation procedure confirms that this is an appropriate method[25] for their quantification in the formulation. It is also used in routine quality control of this entire compound's formulation. Observing the validation parameters, such as accuracy, precision, and linearity, shows that the developed methods can be employed to analyze the bulk of Bilastine and Montelukast.[26] The results obtained from the validation parameters met the ICH requirement and obeyed Beer's law. The method was validated according to ICH guidelines for System suitability, linearity, precision, accuracy, LOD, and LOQ. The marketed formulation was also analyzed by this method, an assay of Bilastine and Montelukast was performed, and % purity was determined. The method was linear in a concentration range of 200-600µgm/ml (R^2 = 0.999) for Bilastine and Montelukast. During the recovery study, Bilastine and Montelukast were used in a concentration of 50%, 100%, and 150%. For Bilastine, the % amount recovered was 100.23%, 102.06%, and 95.33%, respectively. For Montelukast, the % amount recovered was 97.07%, 104.05%, and 96.31%, respectively. During the study of intraday precision[27], both the drugs were used in a concentration of 200 µg m/ml, 400 µg m/ml, and 600 µg m/ml, were analyzed on the same day, and % RSD was found to be 0.53 and 1.08 respectively. Using the same concentration, the Interday precision of method [28] was studied, and the % RSD was found to be 0.47 and 1.11, respectively. Repeatability [29,30] or system suitability tests were carried out on standard solutions of Bilastine and Montelukast. The retention time of Bilastine and Montelukast was found to be 3.4 and 7.04, respectively.[31] The % RSD was 1.25 and 1.33 for Bilastine and Montelukast, respectively. LOD of Bilastine and Montelukast was found to be 0.493 and 0.693, respectively. LOQ [32,33] of Bilastine and Montelukast was found to be 1.49 and 2.10, respectively. An assay of the marketed formulation was also performed, and % the amount of Bilastine and Montelukast recovered was 102.06% and 104.05%, respectively. The current study shows that the developed method is simple, sensitive, accurate, and precise and can be used for routine analysis of both drugs, i.e., Bilastine and Montelukast.[34] The responses obtained after carrying out trail runs were entered into DOE software, and the contour plots and a 2D graph of retention time and area were plotted. Response surface design was utilized for method development to evaluate the effect of mobile phase ratio (x1) and flow rate(x2) on response Area (y1) and Retention time (y2). The software suggested a total of 20 runs. And software suggested the optimized ratio of the mobile phase is (80:20), which is closer to our actual experimental ratio, i.e. (85:15) [35-37]. Experimental data obtained for both trials approximately matches the data provided by DOE software which shows the authenticity of the chromatographic condition. The method optimizations for Bilastine and Montelukast compare with the actual v/s predictions by design expert software. The values of probe>F less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. The value of probe>F for Bilastine is 0.0067, and for Montelukast, 0.0013 can be found.[38- 39]

6. CONCLUSION

It was concluded that the proposed method was found to be rapid, precise, accurate, and sensitive. It Developed HPLC, and U. V. Spectroscopy method was advantageous in terms of time and economy as it saved the system's run time and solvents used to analyze Bilastine and Montelukast's combination formulation. Many samples can be suitably analyzed by this method. The % RSD value for intraday and interday precision was less than 2%. A % recovery value greater than 95% for this method indicates that the method is accurate and free of interference from excipients used in the formulation. % Recovery of the formulation was found to be 95-104%. The readings acquired were entered into DOE programming, and the form plots and a 2D chart of area and time were plotted. Response surface design was utilized for method development to evaluate the effect of mobile phase ratio (x1) and flow rate(x2) on response Area (y1) and Retention time (y2). The software suggested a total of 20 runs, and the optimized ratio of the mobile phase is (80:20) which is closer to our actual experimental ratio, i.e. (85:15). The experimental data obtained for both experiments and trials approximately match the data provided by the software, which shows the authenticity of the chromatographic conditions. The method was validated as per ICH guidelines, and the proposed method was determined to be specific, accurate, precise, and robust for the quantitation of Bilastine and Montelukast. It can be applied to the routine analysis of the developed tablet formulation, which combines both drugs. It meets analytical needs, as shown in its linearity and efficiency data, correlating with current screening methods.

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8. ABBREVIATIONS

RP-HPLC: Reverse Phase High-performance liquid chromatography; BIL: Bilastine; MKT: Montelukast sodium; PDA: Photo Diode Detector; QbD: Quality by Design; ICH: International conference on harmonization; Cys LT: Cysteine Leukotriene: RSD: Relative standard deviation; SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of Quantitation; Rf: Retention factor; SGLT2: Sodium-glucose co-transporter 2; API: Active pharmaceutical ingredient; UV: Ultraviolet, pH: the potential of hydrogen.

9. SUMMARY

Quality by Design (QbD) permitted the achievement of explicit quality with a foreordained and needed assurance. Another specific quick and delicate RP-HPLC technique was created and assessed to concurrently assess Bilastine (BIL) and Montelukast sodium (MKT) in mass and drug measurement structure. BIL and MKT were assessed with C18 (4.6 × 250 mm, 5-µm molecule size) with LC-10AD siphon and PDA detector. The critical stage involved methanol and ammonium acetic acid derivation support pH-3.6 in the proportion of 85:15 v/v. The Flow rate was kept up at 1.0 ml/min, and BIL and MKT were recognized separately at 249 nm and 293 nm. The HPLC technique produces straight reactions found in the 200-600 µg/ml scope. The connection coefficient was viewed as 0.9995 for BIL and 0.9991 for MKT. The LOD and LOQ for BIL were viewed as 0.493 and 1.495 µg/ml, separately, and for MKT, 0.693 and 2.100 individually. The recovery rate for BIL was 95.33 to 102.06, and MKT's was 96.31 to 104.05 individually. The scientific exhibition of the RP-HPLC strategy was approved concerning linearity, accuracy, precision, and particularity and measurement limits. UV-Spectroscopy was performed for the assessment of BIL and MKT API.

10. AUTHORS CONTRIBUTION STATEMENT

Aejaz Ahmed, Manjra Mehfuza U, Lajporiya Mubina gathered the data and all the details of the patient. Sayyed Nazifa, Patel Seema, G.J. Khan, Qazi Majaz Ahamad gave the necessary inputs required for preparing the case. All the authors have read and agreed to the whole of the manuscript.

11. CONFLICT OF INTEREST

Conflict of interest declared none.

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