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QUALITY BY DESIGN (QBD) APPROACH TO DEVELOP STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT FOR BILASTINE AND MONTELUKAST

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Keywords:

Bilastine, Montelukast, QbD, RP-HPLC, Stability Study, Method development, Validation

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ABSTRACT: Background and Objectives: As per requisition of current regulatory requirements, the simple, rapid and sensitive method by 3^3 factorial QbD approach was established and validated for Bilastine (BST) and Montelukast (MNT) by RP-HPLC. **Method:** A simple RP-HPLC method has been developed and validated with different parameters such as linearity, precision, repeatability, LOD, LOQ, accuracy as per International Conference for Harmonisation guidelines (Q2R1). Statistical data analysis was done for data obtained from different aliquots Runs on Agilent Tech. Gradient System with Autoinjector, UV (DAD) & Gradient Detector. **Results:** Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 100mm; 5µm), a 20µl injection loop and UV730D Absorbance detector at 226nm wavelength and running chemstation 10.1 software and drugs along with degradants were separated *via* acetonitrile: water 0.1% OPA (45:55), of pH 3 as mobile phase setting flow rate 0.8 ml/min at ambient temperature. The developed method was found linear over the concentration range of 10-50 /ml for BST and 5-25 µg/ml for MNT, while detection and quantitation limit were found to be 0.4825 µg/ml and 0.2144µg/ml as LOD and 2.2653 µg/ml and 0.07 µg/ml respectively for Bilastine and Montelukast. **Conclusion:** There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, robust, accurate, and stable indicating method was developed with high degree of practical utility.

INTRODUCTION: The concept of "Quality by Design" (QbD) was defined as an approach that covers a better scientific understanding of the critical process and product qualities, designing controls and tests based on the scientific limits of understanding during the development phase, and using the knowledge obtained during the lifecycle of the product to work on a constant improvement environment.

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QbD describes a pharmaceutical development approach referring to formulation design and development and manufacturing processes to maintain the prescribed product quality. Guidelines and mathematical models are used to ensure the establishment and use of the knowledge on the subject in an independent and integrated way [1-3].

Bilastine is a new, well-tolerated, non-sedating H_1 receptor antihistamine. Clinical studies have shown that Bilastine is as efficacious as other non-sedating antihistamines in allergic rhinoconjunctivitis and chronic urticaria in individuals from 12 and 18 years of age, respectively [4]. Chemically it is, 2-[4-[2 - [4 - [1 - (2 - ethoxyethyl) benzimidazol – 2 -yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropanoic acid **Fig. 1.**

Montelukast is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series with the chemical name 2-[1-[1(R) - [3 - [2(E) - (7-chloroquinolin – 2 - yl) vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl) phenyl] propyl sulfanyl methyl] cyclo-propyl] acetic acid sodium salt **Fig. 2**. It is mainly used to control and prevent symptoms caused by asthma (such as wheezing and shortness of breath) and allergic rhinitis [5]. CDSCO approved the drug Combination Bilastine and Montelukast Sodium on 11 March 2020. Drug Combinations Bilastine and Montelukast Sodium are used to treat allergic rhinitis and mild to moderate asthma [6]. Literature review revealed that several methods for analyzing Bilastine and Montelukast Sodium either alone or with other drugs by RP-HPLC [7-13], UPLC [14-16], and HPTLC [17, 18] have been reported. UV Spectroscopic method19 has reported only one Method for simultaneous estimation of this combination and RP HPLC [20, 21]. But no method has been reported for simultaneous estimation of these drugs in combination using QbD-based 3^3 factorial designing.

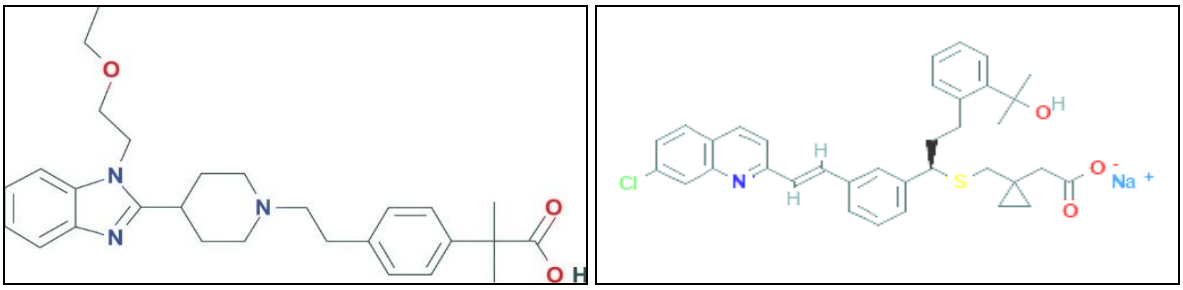

FIG. 1: STRUCTURE OF BILASTINE AND MONTELUKAST

Chemicals and Reagents: Bilastine was obtained as gift sample from Synokem Micro Labs Ltd. India while Montelukast Sodium was obtained as a generous gift from Healthcare LLP Ahmedabad, India. Pharmaceutical formulation was purchased from local market (Brand: Bilagio M tablet labelled claim Bilastine 10 mg and Montelukast 20 mg make Synokem Micro Labs Ltd). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade Acetonitrile, Methanol and Ortho Phosphoric Acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

Instruments: The analysis of the drug was carried out on Agilent Tech. Gradient System with Autoinjector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 100mm; 2.5µm), a 20µl injection loop and UV730D Absorbance detector, and running chemstation 10.1 software.

RP-HPLC Optimised Chromatographic Condition using QbD: Column C_{18} (100 mm× 4.6mm); particle size packing 5µm; detection wavelength 226 nm; flow rate 0.8 ml/min; temperature 26 °C ambient; sample size 20 µl; mobile phase Acetonitrile: water (OPA 0.1% PH 3.2) (45:55); run time 15 min. The retention time for Bilastine and Montelukast were found at 3.385min and 4.625 min respectively **Fig. 2**.

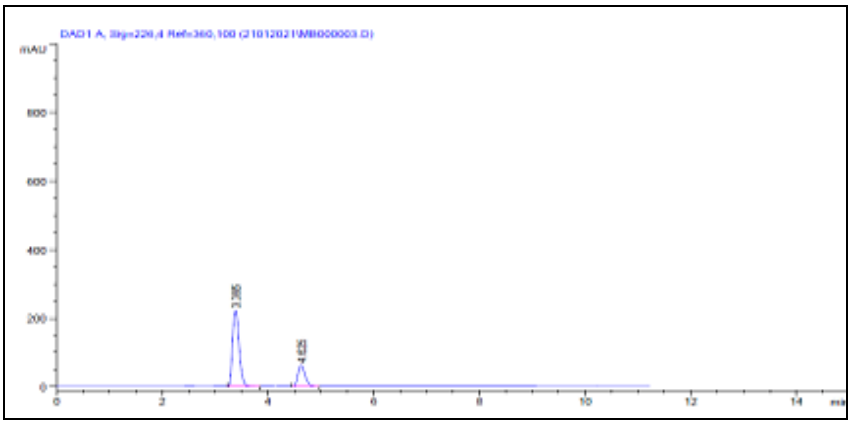

FIG. 2: CHROMATOGRAM OF STANDARD BILASTINE AND MONTELUKAST AT 226nm

Preparation of Standard Solution: All solutions were prepared on a weight basis. Solution concentrations were also measured on weight basis to avoid using an internal standard Pharmaceutical formulation available in the market in the proportion of 1: 2.

Stock Preparations: Standard stock solution was prepared by dissolving 5 mg MNT, and 10 mg BST in 10 ml clean dry volumetric flask, and dilution was up to the mark with methanol to obtain the final concentration of MNT (500 µg/ml) and BST (1000 µg/ml). All the stock solutions were filtered through a 0.45 µm membrane filter.

Detection of λ_{max}: The sample solution has been prepared and scanned in the UV region of 200-400 nm, and the spectrum showed the maximum absorbance at 226 nm **Fig. 3.**

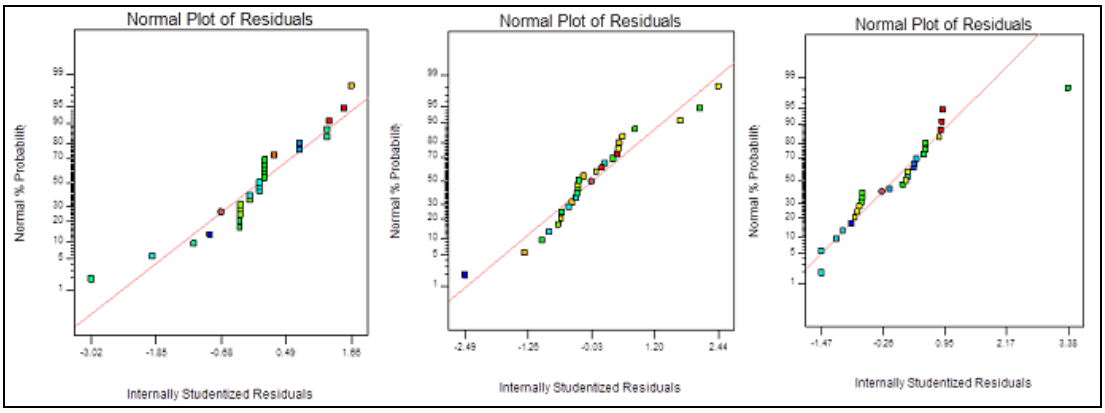

FIG. 3: NORMAL PLOT OF RESIDUALS FOR RT AND PLOT OF PREDICTED *VS.* ACTUAL DATA BY THE VALUE OF 4.06 TO 5.31

QbD Approach to Analysis: The application of QbD in HPLC method development commences with establishing analytical objectives based on sound science to ensure consistent method performance characteristics are achieved [21].

The use of QbD for an analytical method commences with defining the target analytical profile in which the pre-defined objectives for method performance must be appropriately validated and documented [22, 23]. Thus the objective of this work was to perform experimental design using Design Expert Software, leading to develop a simple, rapid, and sensitive method by QbD approach and validated as per ICH Guidelines (Q2R1) for Bilastine and Montelukast and its stability-indicating method by RP-HPLC. Further statistical data analysis and numerical and graphical optimization are needed to develop Analytical Design Space (ADS).

MATERIALS AND METHODS:

Calibration Curve: A calibration curve was constructed succeeding replicate (n=6) analysis of five standards of 10, 20, 30, 40, 50 µg/ml of Bilastine and 5, 10, 15, 20, 25 µg/ml of Montelukast. The peak height ratio of drugs was calculated and plotted AUC versus concentration, after which least-squares linear regression analysis of data was undertaken to establish the equation for the best fit line and the correlation coefficient (R^2) to authorize linearity. Samples were injected, peaks were recorded at 226 nm, and the graph plotted the drug concentration versus peak area as shown in **Table 1-2** and **Fig. 4 - 5.**

TABLE 1: LINEARITY DATA FOR BILASTINE

Method	Conc µg/ml	Peak area(µV.sec) 1	Peak area(µV.sec) 2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UHPLC Method	10	544.07	549.28	546.6750	3.6840	0.6739
	20	1063.1300	1069.3070	1066.2185	4.3678	0.4097
	30	1693.9500	1700.1800	1697.0650	4.4053	0.2596
	40	2192.1100	2192.3200	2192.2150	0.1485	0.0068
	50	2771.2600	2770.0400	2770.6500	0.8627	0.0311
	Equation			Y=55.74 x -17.62		
	R^2		0.999			

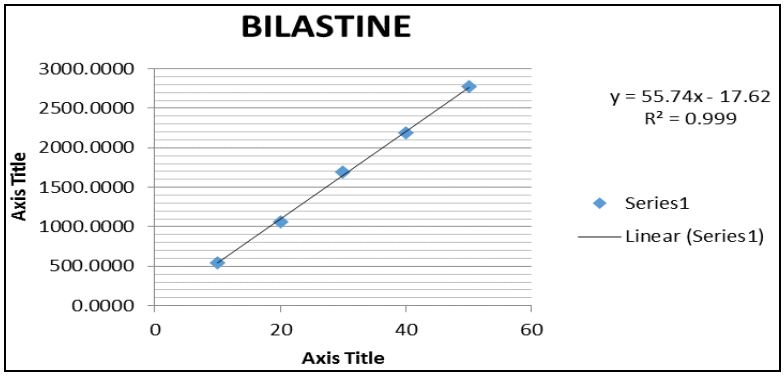

FIG. 4: CALIBRATION CURVE OF BILASTINE

TABLE 2: LINEARITY DATA FOR MONTELUKAST

Method	Conc µg/ml	Peak area(µV.sec) 1	Peak area(µV.sec) 2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UHPLC Method	5	209.3	209.4	209.3500	0.0707	0.0338
	10	437.5300	440.0600	438.7950	1.7890	0.4077
	15	696.5700	694.9700	695.7700	1.1314	0.1626
	20	902.7500	901.2000	901.9750	1.0960	0.1215
	25	1142.9600	1144.2700	1143.6150	0.9263	0.0810
	Equation			46.634 X – 21.612		
	R^2			0.999		

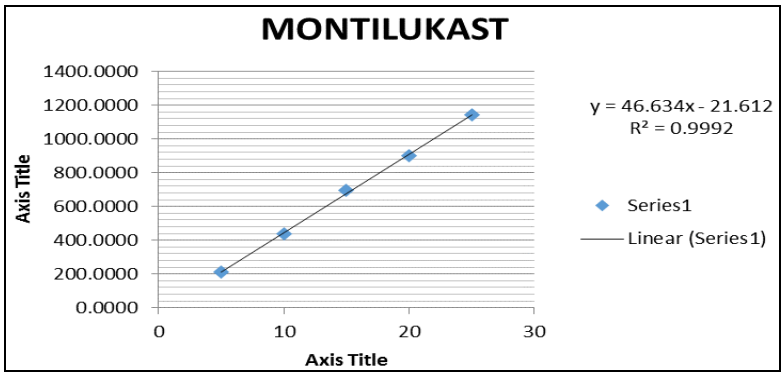

FIG. 5: CALIBRATION CURVE OF MONTELUKAST

Precision: Intra-day (repeatability) precision was established following analysis of replicate samples (n=3) at three concentrations indicative of low, medium, and high levels within the linear range *viz*., 20, 30, 40 µg/ml of Bilastine and 10, 15, 20 µg/ml of Montelukast. Analysis was performed over a short period of time on the same day. Inter-day precision or reproducibility was assessed at low, medium, and high concentrations on three consecutive days. The percent relative standard deviation (% RSD) was used to assess intra- and inter-day precision. An upper limit of 2% was used to confirm precision in our laboratory. The precision of an analytical method is usually expressed as standard deviation or relative standard deviation. **Table 3** and **4** describes the Intraday, Interday, and repeatability of the method.

TABLE 3: RESULTS OF PRECISION STUDIES (INTRA-DAY AND INTER-DAY)

Method	Drug	Conc (µg/ml)	Interday Precision Mean± SD	Interday Precision %Amt Found	Intraday Precision Mean± SD	Intraday Precision %Amt Found
	BST	20	1073.6278 ± 0.96	97.85	1070.72 ± 5.65	97.62
Rp- HPLC		30	1696.7094 ± 0.88	102.51	1696.55 ± 1.86	102.50
Method		40	2202.7469 ± 2.86	99.58	2201.80 ± 0.79	99.54
	MNT	10	441.6759 ± 5.65	99.3536	21.42 ± 0.96	100.0000
		15	683.4896 ± 0.28	100.8000	33.35 ± 0.24	102.0500
		20	42.7595 ± 0.14	98.8000	43.01 ± 0.15	99.3800

*Mean of each 3 reading for RP-HPLC method

TABLE 4: RESULTS OF REPEATABILITY STUDY

Method	Conc. of BST and MNT (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC BST Method	30	1701.64	30.78	102.63
	30	1695.54		
	Mean	1698.60		
	SD	4.31		
	%RSD	0.16		
HPLC MNT Method	20	42.47	19.78	98.90
	20	43.11		
	Mean	42.80		
	SD	0.45		
	%RSD	0.16		

Accuracy: Recovery studies were performed to validate the accuracy of the developed method. To pre-analyze tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added, and then its recovery was analyzed. Statistical validation of recovery studies is shown in **Table 5** and **Table 6.**

TABLE 5: RESULT OF RECOVERY DATA FOR MNT AND BST

Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
BST	80 %	20	16	36.15±0.014	16.15±0.014	100.96±0.055
	100 %	20	20	39.86±0.061	20.58±0.06	99.32±0.30
	120 %	20	24	43.93±0.0076	20.58±0.0076	99.71±0.031
	80 %	10	8	18.04±0.11	7.96±0.11	99.51±1.39
MNT	100 %	10	10	20.01±0.01	20.58±0.017	100.12±0.17
	120 %	10	12	21.99±0.047	20.58±0.04	99.94±0.38

*mean of each 3 reading for RP-HPLC method.

TABLE 6: STATISTICAL VALIDATION OF RECOVERY STUDIES MNT AND BST

Method	Level of Recovery (%)	Drug	% RSD	Standard Deviation*	Mean % Recovery
Rp-HPLC Method	80%	BST	0.014	0.014	100.96
		MNT	0.061	0.06	99.32
	100%	BST	0.0076	20.0076	99.71
		MNT	0.11	0.11	99.51
	120%	BST	0.01	0.017	100.12
		MNT	0.047	0.04	99.94

*Denotes average of three determinations for RP-HPLC method.

Robustness: To evaluate robustness, a few parameters were deliberately varied. The parameters include a variation of flow rate and percentage of methanol as described in **Table 7.**

TABLE 7: ROBUSTNESS EVALUATION OF THE HPLC METHOD

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD	Amount of detected (mean ±SD)	%RSD
		For Montelukast		For Bilastine	
Chromatogram of flow change 0.7 ml	40+20	359.78±2.06	0.57	4573.28±0.25	0.07
Chromatogram of flow change 0.9 ml	40+20	1423.35±0.84	0.65	3452.93±0.26	0.09
Chromatogram of comp change wavelength change 300 nm	40+20	283.9±1.49	0.53	4271.6±0.19	0.07
Chromatogram of comp change wavelength change 302 nm	40+20	338.97±2.69	0.79	3628.11±0.98	0.03
Chromatogram of mobile phase change 74+26 ml	40+20	311.0±2.20	0.71	3933.0±0.4	0.10
Chromatogram of mobile phase change 76+24 ml	40+20	313.35±2.34	0.75	1710.49±0.23	0.07

Forced Degradation Studies: Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like the exposure of sample solution to Acid, Base, Hydrogen peroxides (H_2O_2), and Neutral conditions. Investigations were done for the degradation products in different conditions and are shown in **Table 8.**

Procedure for Bilastine and Montelukast Degradation:

Acid Hydrolysis: The acid hydrolysis performed using 0.1N HCl at 70 ºC for 1st hr and 2nd h for both Bilastine and Montelukast indicated degradation.

The major degradation products for Bilastine and Montelukast were observed at relative retention time (RRT) for 1st and 2nd h.

Alkaline Hydrolysis: The alkaline hydrolysis condition was performed using 0.1N NaOH at 70 ºC for 1st h and 2nd h of both Bilastine and Montelukast. The major degradation products for Bilastine and Montelukast were observed at relative retention time (RRT) for 1st and 2nd h.

Oxidation: In the oxidation condition with 3% H_2O_2 for 1st hr and 2nd h, both Bilastine and Montelukast show oxidative stress degradation peaks in the chromatogram.

Neutral: There was no major degradation observed for both Bilastine and Montelukast, and hence they were not sensitive to light at 70 ºC for 1st h and 2nd h.

TABLE 8: FORCED DEGRADATION

Sample Exposure condition	Total Number of products with their Rt	MNT		BST	
		Degradation remained (10 µg/ml)	Recovery (%)	Degradation remained (20 µg/ml)	Recovery (%)
Acidic, 1N, 1 h	4 (2.851,3.387, 4.139,4.946)	8.85	88.56	18.83	94.18
Basic, 1N, 1 h	5 (2.842, 3.325, 3.407, 4.595, 5.923)	8.14	81.48	16.85	84.29
Per oxide, 30%, 1 h	5 (2.639, 2.954, 3.363, 4.250, 4.586, 6.991)	8.56	85.67	15.94	79.73
Heat, 50 °C, 1 h	2 (3.363, 4.561)	9.10	91.05	18.80	94.01

Application of Analytical Methods: To determine the content of MNT and BST in marketed tablets (Brand Name: Bilagio label claim 10 mg of Montelukast and 20 mg Bilastine), 20 tablets powder weighed 5.96 gm and an average weight of powder was calculated in 0.298 gm. Tablets were triturated and powder equivalent to weighed in 298 mg. The drug was extracted from the tablet powder with 10 ml of methanol. To ensure complete extraction, it was sonicated for 15 min. 0.1 ml of supernatant was then diluted up to 10 mL with the mobile phase. The resulting solution was injected in HPLC, and the drug peak area was noted**.**

RESULTS AND DISCUSSION: Such analytical methods are, in fact, an indicator of a quality product and the robustness of that product for the duration on the lifecycle of that product. The main goal of any HPLC method is to separate and quantitate analyte(s) of interest from any impurity and/or excipients. Initially, it is important to establish the critical quality attributes (CQA) of a system that may impact the quality of the analytical method. Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study; all the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version). State-Ease Inc., Minneapolis, MN, USA).

Application of Design of Experiments for Method Optimization Design of Experiments (DOE): The use of the experiment is to ensure flexibility to facilitate continuous product improvement while avoiding the need for costly post-approval changes following market authorization. In compliance with prerequisites, 3 randomized response surface designs with a full fraction design were used with 17 trial runs to study the impact of three factors on the three key response variables.

In this design, 3 factors were evaluated, each at 3 levels, and experimental trials was performed at all 3 possible combinations. The mobile phase composition (X1), Wavelength (X2), and flow rate (X3), were selected as independent variables, and retention time (RT), Area Under Curve (AUC), and Resolution (Rs) were selected as dependent variables. Prediction of the optimum composition was carried out using overlay plotting, brute Force method, and numeric approach of desirability function. The resulting data were fitted into Design Expert 10 Software and analyzed statistically using analysis of variance (ANOVA) and F-Test.

Fig. 3 indicates the normal plot of residuals for retention time with other chromatographic parameters. The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, wavelength, and mobile phase composition on dependent variables, as shown in **Fig. 6**. The probable trial runs using 3^3 full fraction designs are shown in **Table 4**. Further ANOVA and F-test with variables are shown in **Table 9-13**. Moreover, degradation peaks of API were shown in **Fig. 7-10** from acidic, alkaline, peroxide, and Heat.

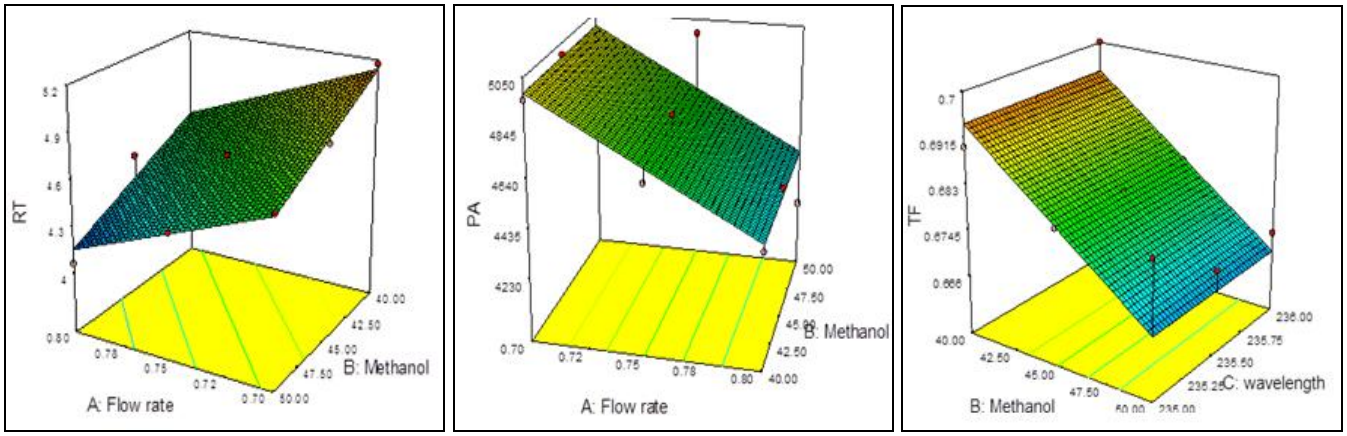

FIG. 6: CONTOUR PLOT FOR FLOW RATE, MOBILE PHASE COMPOSITION, AND WAVELENGTH

TABLE 9: PROBABLE TRIAL RUNS USING 33 FULL FRACTION DESIGNS

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
		A:Flow rate	B:Methanol	C:Wave-length	RT	PA	TP	TF
		ml/min	%	nm				
9	1	0.67	45	235.50	4.31	5175.68	7920	0.60
10	2	0.83	45	235.50	3.488	4225.46	7156	0.63
20	3	0.75	45	235.50	3.853	4563.82	7552	0.83
5	4	0.70	40	236.00	4.48	5500.42	6494	0.96
8	5	0.82	50	236.00	3.45	4545.43	5811	0.96
7	6	0.70	50	236.00	4.01	4344.53	6299	0.96
4	7	0.80	50	235.00	3.46	4212.09	6399	0.96
1	8	0.70	40	235.00	4.62	4879.86	6414	0.87
19	9	0.75	45	235.50	3.88	4592.91	7165	0.62
2	10	0.80	40	235.00	3.98	4192.40	5318	0.90
12	11	0.75	53.41	235.50	3.61	4752.48	7452	0.62
11	12	0.75	36.59	235.50	4.08	4709.13	6361	0.61
3	13	0.70	50	235.00	3.9	5252.10	5989	0.95
13	14	0.75	45	234.66	3.93	4390.99	7175	0.62
18	15	0.75	45	235.50	3.94	4664.20	7349	0.62
16	16	0.75	45	235.50	3.960	4464.09	7284	0.63
15	17	0.75	45	235.50	3.957	4703.10	7274	0.63
17	18	0.75	45	235.50	3.941	4765.02	7288	0.62

TABLE 10: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 1: RT)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.95	7	0.2783	211.69	< 0.0001	significant
A-Flow rate	1.10	1	1.10	833.33	< 0.0001	
B-Methanol	0.0005	1	0.0005	0.4083	0.5464	
C-Wavelength	0.0000	1	0.0000	0.0124	0.9149	
AB	0.0144	1	0.0144	10.93	0.0163	
A^2	0.1479	1	0.1479	112.53	< 0.0001	
B^2	0.0882	1	0.0882	67.09	0.0002	
C^2	0.0810	1	0.0810	61.60	0.0002	
Residual	0.0079	6	0.0013			
Cor Total	1.96	13				

TABLE 11: ANOVA FOR REDUCED LINEAR MODEL (RESPONSE 2: PA)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.294E+06	1	3.294E+06	57.63	< 0.0001	significant
A-Flow rate	3.294E+06	1	3.294E+06	57.63	< 0.0001	
Residual	6.858E+05	12	57151.71			
Cor Total	3.979E+06	13				

TABLE 12: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 3: TP)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.120E+07	7	1.600E+06	288.01	< 0.0001	significant
A-Flow rate	6.682E+06	1	6.682E+06	1202.92	< 0.0001	
B-Methanol	40072.40	1	40072.40	7.21	0.0363	
C-Wavelength	358.64	1	358.64	0.0646	0.8079	
AB	60031.13	1	60031.13	10.81	0.0167	
A^2	7.371E+05	1	7.371E+05	132.68	< 0.0001	
B^2	7.252E+05	1	7.252E+05	130.54	< 0.0001	
C^2	5.848E+05	1	5.848E+05	105.27	< 0.0001	
Residual	33330.78	6	5555.13			
Cor Total	1.123E+07	13				

TABLE 13: ANOVA FOR REDUCED QUADRATIC MODEL (RESPONSE 4: TF)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0040	7	0.0006	646.40	< 0.0001	Significant
A-Flow rate	0.0020	1	0.0020	2334.61	< 0.0001	
B-Methanol	0.0000	1	0.0000	0.0000	1.0000	
C-Wavelength	0.0000	1	0.0000	0.0000	1.0000	
BC	0.0002	1	0.0002	228.17	< 0.0001	
A^2	0.0004	1	0.0004	427.66	< 0.0001	
B^2	0.0003	1	0.0003	298.35	< 0.0001	
C^2	0.0003	1	0.0003	298.35	< 0.0001	
Residual	5.259E-06	6	8.765E-07			
Cor Total	0.0040	13				

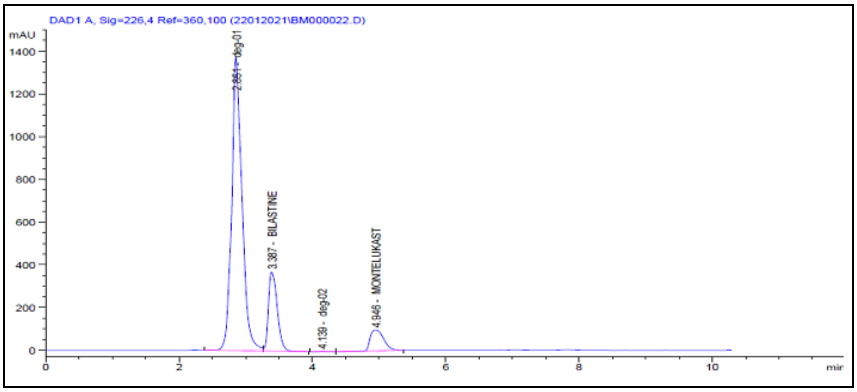

FIG. 7: ACIDIC DEGRADATION

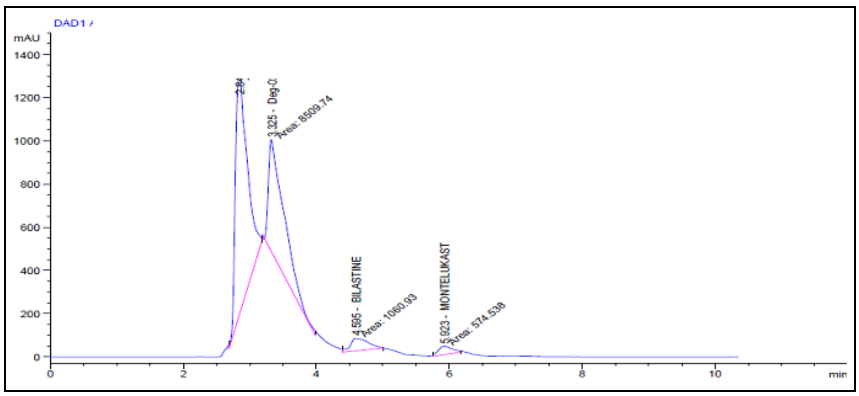

FIG. 8: ALKALINE DEGRADATION

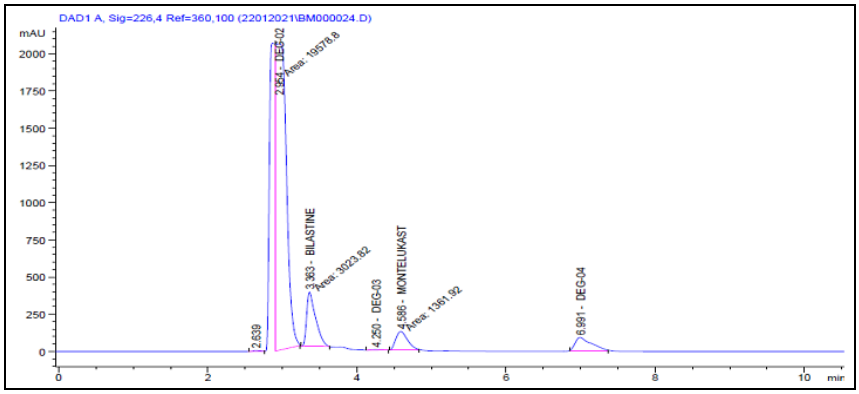

FIG. 9: PER OXIDE DEGRADATION

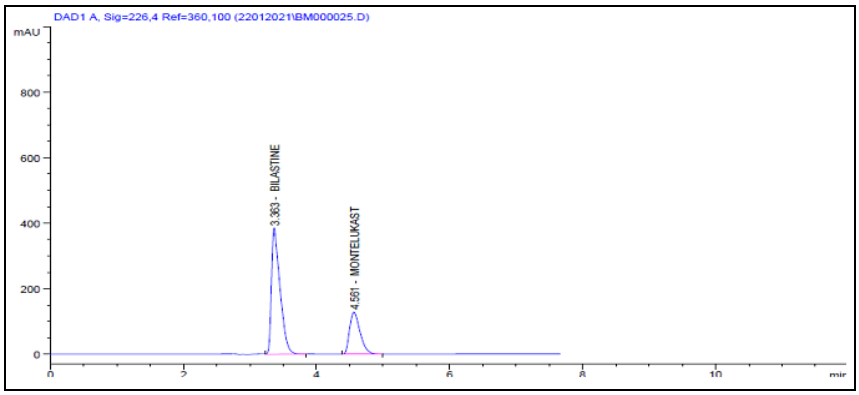

FIG. 10: HEAT DEGRADATION

CONCLUSION: A simple, rapid, reliable, robust, and optimized reversed-phase high-performance liquid chromatographic method for estimating Bilastine and Montelukast was successfully developed and validated as per International Conference on Harmonization guidelines.

The percentage of mobile phase, flow rate, and wavelength was optimized using the QbD approach, *i.e.*, 3^3 factorial design. There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, accurate, and stability-indicating method was developed with a high practical utility.

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