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ANALYTICAL QUALITY BY DESIGN ASSISTED DEVELOPMENT AND VALIDATION OF AN UPLC METHOD FOR SIMULTANEOUS ESTIMATION BILASTINE AND MONTELUKAST IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: A simple, sensitive, accurate and precise UPLC method was developed and used to validate simultaneously the mixture of Bilastine and Montelukast. The experimental design approach can be useful to optimize the separation and to help out in the development and better understanding of the interaction of several chromatographic factors on separation quality.

Methods: A CCD design was employed to locate the optimum organic solvent volume, flow rate and pH for separation by mapping the chromatographic response surface. The qualities of the fitted polynomial models were examined on the basis of the coefficient of determination of R^2 value. The true optimum condition position was recognized by employing Derringer's desirability function, where responses were simultaneously optimized. The final step was to predict the response and design space from the polynomial equation.

Results: As a result of using C_{18} the optimum chromatographic conditions, a high-quality resolution response appears. Percent recoveries were found to be close to 100% with low variability.

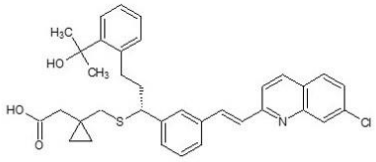
Conclusion: The optimized assay condition was validated according to ICH guidelines to confirm specificity, linearity, accuracy and precision. The method possibly will be adopted for routine analysis in industry.

Keywords: UPLC, Bilastine, Montelukast, Optimum flow rate, chromatographic conditions.

INTRODUCTION

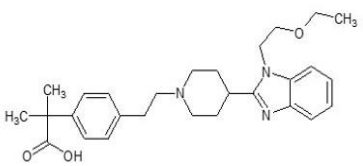
Bilastine is chemically 2- [4- [2- [4- [1- (2-ethoxyethyl) benzimidazole-2-yl] piperidine-1-yl] ethyl] phenyl]-2- methylpropane acid. Empirical formula is $C_{28}H_{37}N_3O_3$ and a molecular weight of 463.61 g/mol. It is a non-sedating antihistamine drug which is used in the allergic rhino conjunctivitis and chronic urticaria [1].

Fig 1 - Structure of Bilastine



Montelukast sodium is chemically (R- (E)) -1- (((1- (3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl)-3(2- (1-hydroxy-1-ethylethyl) phenyl) propyl) thio) methyl) cyclopropane acetic acid, monosodium salt [2]. Montelukast utilized for the treatment of asthma in kids and grown-ups. It is a strong specific inhibitor of leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor cysLT1 [3-6].

Literature survey reveals that montelukast are official in IP, USP and BP however, Bilastine individually or a combination with montelukast is not official in any Pharmacopoeia. Various analytical methods like HPLC (Chandra Umesh *et al* 2021) [7], Derivative UV-Spectroscopy and HPLC (Riya Mistry *et al* 2021) [8], UV Spectrophotometry (R. Mohan Raj, *et al* 2021) [9], In-vitro dissolution testing by HPLC (Umesh Chandra *et al* 2021) [10] and stability indicating HPLC (V. PADHIYAR, *et al* 2021) [11] methods were reported in the literature for the determination of Bilastine and montelukast combination in pharmaceutical dosage forms. HPLC method for the determination of montelukast and its degradation products in pharmaceutical formulation using an experimental design (Ahmed B. Eldin et al 2011) [12], Determination of montelukast combined with other drug (fexofenadine) in pharmaceutical dosage form by HPTLC (Hitesh Vekaria et al 2012) [13] method has been reported. Determination of Bilastine in pharmaceutical dosage form by HPLC (Chinmayee Kishor Padte et al 2021) [14], UPLC (Rambabu Katta et al 2020) [15] and UV spectrophotometric method by experimental design for Robustness has been reported (Andressa Tassinari da Silva et al 2017) [17]. Conversely, development of an ultra-performance



liquid chromatographic (UPLC) method for simultaneous estimation of Bilastine and montelukast in combined dosage Formby experimental design (CCD) has not been reported till date. The objective of our present study was to develop simultaneous multiple response optimizations using the Derringer's desirability function for the determination of Bilastine and montelukast in bulk and pharmaceutical dosage form by UPLC method using experiment Central composite design (CCD) approach for quantitative analysis and to validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Pharmaceutically pure samples of Bilastine and montelukast were obtained as a gift sample from Shree Icon Pharmaceutical Laboratories, Vijayawada, Andhrapradesh. A combination of Bilastine and montelukast tablet formulations (Bilargic M) was procured from the local market. HPLC grade of methanol, Acetonitrile, Triethylamine (TEA), water (milli Q or Equivalent) and orthophosphoric acid (AR grade), were purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

Analysis was performed with a Agilent UPLC2010 CHT separation module equipped with LC solution software, Pump LC2010 binary and PDA detector. The wavelength was set at 281 nm. Compounds were separated on a Kinetex column (100 × 4.6 mm i.d., 2.6µm particle size). The mobile phase was methanol and Buffer (pH 2.0). The flow rate was 0.6ml/min and the total run time was 3 minutes. Samples were injected using Rheodyne injector with 5µL loop and detection was carried out at 281nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µ nylon filter. Chromatography was performed in column by using ambient temperature

PREPARATION OF MOBILE PHASE

750 ml of Triethylamine buffer (pH 2.0) and 250 ml of methanol were mixed and degassed in ultrasonic water bath for 5min. Then it was filtered through 0.45µ pore filter under vacuum and transferred into a 1000ml volumetric flask.

PREPARATION OF WORKING STANDARD STOCK SOLUTION

About 10mg of Montelukast and 20 mg of Bilastine were weighed accurately and transferred into a 100 ml volumetric flask. 10ml of the mobile phase was added and sonicated for 15 min. and the volume was made up to 100 ml with the mobile phase. Then it was made up to

the volume with diluent to get a concentration of 100 µg/ml for Montelukast and 200 µg/ml for Bilastine.

PREPARATION OF SAMPLE SOLUTION

Twenty tablets of (Bilargic -M) were accurately weighed and crushed into fine powder. The powder equivalent to 10 mg was weighed and transferred into a 100 ml volumetric flask. About 50 ml of mobile phase was added, shaken for 5 minutes and then sonicated for 10 minutes with intermediate shaking. After that the volume was finally made up to the mark with 100 ml of mobile phase. Then the resulting solution was filtered through by using 0.22µ filter. Then it was suitable dilution made to get required concentration.

EXPERIMENTAL DESIGN

The compositional parameters optimization study was used by Central composite design (CCD). The interaction effect, main effects and quadratic effects of the factors on the retardation factor (R_f) of both drugs were evaluated. In response surface methodology, CCD is useful for exploring quadratic response surfaces and second order polynomial models is constructing without the need to use a complete three-level factorial experiment.

The experimental design approach can be useful to optimize the separation and to help out in the development of better understanding of the interaction of several chromatographic factors on separation quality. In this research study, based on preliminary experiments and prior knowledge from the literature the important chromatographic factors were selected and central composite design (CCD) experiment was used for optimization. A CCD design was employed to find the volume of organic solvent, mobile phase pH, optimum flow rate for separation by mapping the chromatographic response surface. Composite Design is used to provide for three independent variables, a partial factorial design is combined with five replicates of centre points and five axial points at an extreme level. The second order model was fitted to the experimental results. The coefficient of determination R^2 were evaluated to found the qualities of the fitted polynomial models. Derringer's desirability function was applied to recognize the position of the true optimum condition and the responses were simultaneously optimized. The response and design space from the polynomial equation was predicted, this was the final step. Response surface methodology (RSM) is a mathematical and statistical technique valuable for analysing problems where several independent variables like column temperature, pH, flow rate, etc. affect dependent variables or responses (e.g.,

resolution, tailing of peak, run time). This technique is used to simultaneously optimize the levels of these variables to attain the best system performance. RSM enables definition of quadratic models that accurately explain the response for all values of the chromatographic conditions in the experimental region. Quadratic regression model coefficients calculation, variable for each design must be studied, at least at three distinct levels and consequently, the optimization study was performed by using CCD.

METHOD VALIDATION (ICH GUIDELINES Q2A 1994, Q2B 1996)

LINEARITY

The linearity of analytical method is the ability to elicit test results that are directly proportional to the analyte concentration in samples within a given range [17,18]. About 10mg of Montelukast and 20 mg of Bilastine were weighed accurately and transferred into a 100 ml volumetric flask. 10 ml of mobile phase was added and sonicated to dissolve. Then it was made up to the volume with the same. The concentrations 100 µg/ml for Montelukast and 200 µg/ml for Bilastine respectively were obtained. The above standard stock solutions were pipetted out 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5ml separately, transferred in to series of six 10 ml standard flask and diluted with diluent. The final concentration of the solutions were in the range of 2.5-15 µg/ml for Montelukast and 5-30 µg/ml for Bilastine. 5µl solutions of each concentration were injected and chromatograms were recorded. Calibration curves were constructed using peak area against concentration.

LIMIT OF DETECTION

This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosage containing low drug levels and placebos. LOD was calculated by using the following formulae

$$LOD = 3.3 \times \text{std. dev} / \text{slope}$$

The limit of detection was calculated by using the average value of slope and the standard deviation of intercept.

LIMIT OF QUANTIFICATION

This is the lowest concentration in a sample that can be detected and quantified. LOQ was calculated by using the following formula. LOQ = 10 x std. dev / slope.

Preparation of calibration curve from the serial dilutions of standard was repeated for three times. Limit of quantification was calculated by using the value of the slope and the standard deviation of intercept.

PRECISION (SYSTEM PRECISION AND METHOD PRECISION)

The precision of an analytical method is the degrees of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample in a same day (Repeatability). Aliquots of standard stock solution of Montelukast and Bilastine (1.0ml of 100µg/ml of Montelukast and 1.0 ml of 200µg/ml of Bilastine) were transferred into a 10 ml standard flask and made up to the mark with mobile phase. 5µl solutions of each concentration were injected and chromatograms were recorded. The procedure was used for method precision and system precision study. The peak areas were measured and the %RSD was calculated.

ACCURACY

The ICH defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted as reference values and the values found. The accuracy of the method was checked by spiking the sample with reference compound. It was evaluated in triplicate at the concentration levels (50%, 100% and 150%) of the target test concentrations (100µg/ml of Montelukast, and 200µg/ml for Bilastine). 5µl solutions of each concentration were injected and the chromatograms were recorded.

Montelukast and Bilastine API were undergone forced degradation study under acid and base hydrolysis as well as oxidative, photolytic stress conditions. Only thermal degradation of drug substance was carried out in solid state. 10 µg/ml for montelukast and 20 µg/ml for Bilastine solutions were prepared by using standard stock solution. Finally, 5µl solutions were injected and chromatograms were recorded. A chromatogram by UPLC was performed to take peak area after 24 hours for observing degradation study.

Acid hydrolysis of drug substance in solution state was conducted with 0.1N HCl at room temperature for 24 hours. Base hydrolysis of drug substance in solution state was conducted

with 0.1N NaOH solution at room temperature for 24 hours. For oxidative stress, sample solutions of drug substance in 0.1% H_2O_2 were kept at room temperature for 24 hours. For thermal stress, solid samples of drug substance and drug products were kept at sunlight for 24 hours.

RESULTS AND DISCUSSION

Solvent type (acetonitrile or methanol), Column chemistry (C_{18}), flow rate and solvent strength were then differing to estimate the finest chromatographic set up that produce quality separation. The mobile phase conditions were optimized such that the first eluting component does not interfere with the peaks of solvent and excipient. Other criteria like analysis time, resolution for eluted peaks, assay sensitivity and noise were also considered. Therefore, Kinetex column (100 × 4.6 mm i.e., 2.6µm particle size) and mobile phase consisted of Triethylamine buffer: methanol (pH 2.5) was tried to examine initial separation conditions. It is important to investigate the curvature term using factorial design with centre points before starting on optimization procedure. ANOVA generated 2^Kfactorial design showed that curvature was significant for all the responses (Rt, Rs, plate count) since p value was less than 0.05. This implied that quadratic model should be considered to model the separation process. In order to obtain the second order predictive model, central composite design (CCD) a design type under response methodology was employed. CCD was chosen due to its flexibility and it could be applied to optimize an UPLC separation by gaining better understanding of factor's main and interaction effects. Based on preliminary experiment and prior knowledge from literature as well as certain instrumental limitations the factors were selected for optimization.

Stationary phase C_{18} column and mobile Phase consisted of Triethylamine buffer: methanol (pH 2.5) was employed based on preliminary experiments. The volume of triethylamine buffer in the mobile phase was fixed at (75%) and only methanol content was varied. The mobile phase flow rate could also moderately influence selectivity in UPLC analysis. Therefore, the key factors selected for optimization process were methanol concentration (A), flow rate (B) and Buffer pH (C). Table 1 showed the levels of each factor studied for finding out the optimum values and responses. In table 1 the ranges of each factor used were methanol concentration (15-25%v/v), flow rate (0.4-0.6ml/min) and buffer pH (2.0- 3.0).

Table 1: Central composite arrangement and responses

As response variables, the resolution between two peaks Bilastine and montelukast (Rs), the retention time of the last peak montelukast (Rt) and USP plate count for first peak Bilastine were selected. For an experimental design with the three factors, including linear, quadratic and cross terms, the model can be expressed as $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32}$ where Y is the response to be modelled, β is the regression coefficient and X_1, X_2 and X_3 represent factors A, B and C respectively. Statistical parameters obtained from ANOVA for the reduced models are given in table 2.

Run	Space type	Factor 1 A: Organic solvent (%)	Factor 2 B: Flow rate (ml/min)	Factor 3 C: pH	Response 1 Rt (min)	Response 2: Rs (min)	Response 3: Plate count
1	Centre	20	0.5	2.5	1.887	4.08	8745
3	Centre	20	0.5	2.5	1.887	4.08	8745
6	Centre	20	0.5	2.5	1.887	4.08	8745
8	Centre	20	0.5	2.5	1.887	4.08	8745
12	Centre	20	0.5	2.5	1.887	4.08	8745
17	Centre	20	0.5	2.5	1.887	4.08	8745
2	Axial	20	0.3318	2.5	2.021	3.89	8626
4	Axial	20	0.5	2.3409	2.054	3.95	8556
7	Axial	28.409	0.5	2.5	1.541	4.12	8896
14	Axial	20	0.5	1.6591	1.724	4.02	8745
15	Axial	20	0.6681	2.5	1.681	4.11	8898
20	Axial	11.591	0.5	2.5	2.526	4.27	8823
5	Factorial	25	0.4	3.0	1.975	4.12	8521
9	Factorial	15	0.6	2.0	2.387	4.56	8896
10	Factorial	15	0.4	3.0	2.612	3.18	8745
11	Factorial	25	0.4	2.0	1.962	3.54	8623
13	Factorial	15	0.6	3.0	2.458	3.57	8771
16	Factorial	15	0.4	2.0	2.587	3.27	8989
18	Factorial	25	0.6	2.0	1.691	4.28	8966
19	Factorial	25	0.6	3.0	1.841	4.02	8497

Table 2: Reduced Response Surface Models and Statistical parameters obtained from ANOVA

The insignificant terms ($p > 0.05$) were eliminated from the model through backward elimination process to obtain a simple and realistic model. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modelling the adjusted R^2 which takes the number of regressor variables into account, is usually selected (Parajo JC, et al., 1992) [19]. The adjusted R^2values were well within the acceptable limits of $R^2 \geq 0.80$ (Lundstedt T, *et al.*, 1998) [20], which revealed that the experimental data showed a good fit

with second order polynomial equations. For all the reduced models p value < 0.05 was obtained, implying these models were significant. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio. A ratio greater than 4 is desirable (Beg Q, et al., 2003) [21]. The ratio was found to be in the range from 6. 5715 to 10.2912 which indicated an adequate signal and therefore the model was significant for the separation process. The coefficient of variation (C.V) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if it is less than 10%.

Response	Regression model	Adjusted R^2	Model P-value	(%) C.V	Adequate precision
Rt	+1.88-0.3098*A-0.0974*B+0.0596*C-0.0064*AB+0.0084*AC+0.0229*BC-0.1072*A^2+0.0426*B^2+0.0561*C^2	0.8028	< 0.0001	7.64	10.2912
Rs	+4.09+0.0826*A+0.1970*B-0.0643*C-0.1300*AB+0.1750*AC-0.2175*BC-0.0103*A^2-0.0792*B^2-0.0845*C^2	0.8317	< 0.0001	5.79	6.9702
Plate count	+8745.23-49.15*A+51.95*B-92.10*C+48.25*AB-25.25*AC-31.00*BC+38.99*A^2+4.52*B^2-34.91*C^2	0.8126	< 0.0001	12.17	6.5715

In table 2 the interaction terms with the largest term coefficient among the fitted model was AB (+ 48.25) of plate count model. The positive interaction between A and B was statistically significant (< 0.0001) for plate count model. The study revealed that changing the concentration of methanol from low to high resulted in the plate count of Bilastine at the flow rate of low and high levels. In order to gain a better understanding of the results the predicted models were presented in the form of 3D response surface plot (figure 3, 4 & 5).

Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models, were chosen for the axes of the response surface plots. Perturbation plots provide silhouette views of the response surface plots, where it showed how the response changes as each factor moves from a chosen reference point, with all other factors held constant at the reference value. The steepest slope or curvature indicated the sensitiveness of the response to a specific factor**.** Figure 1 c showed that methanol concentration (factor A) had most important effect on plate count following the factor B (flow rate). The rest of the factors had significant effect on Rt and *R*s**.** Figure 1a showed that Rt values increased as the level of buffer pH increased and that Rt values decreased as the level of methanol concentration increased. The value of resolution (*Rs*) increased with increasing levels of A and B. Analysis of the perturbation plots and response plots of optimization models revealed that factor A, B and C had significant effect on the separation of the analytes. Derringer's desirability function was employed for global optimization of three responses and to select different optimal conditions for the analysis of formulation in the present study. The identified criteria for the optimization were resolution between the peaks, capacity factor and elution time. The Derringer's desirability function, D, is defined as the geometric mean,

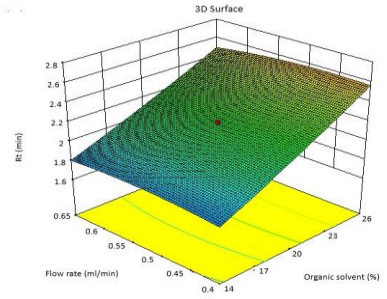


Fig 4: 3D Response Surface Plot for Rs

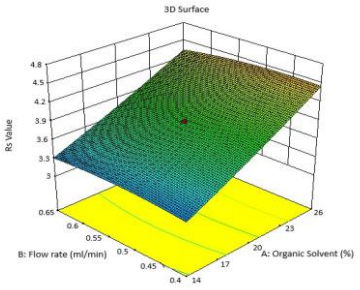


Fig 4: 3D Response Surface Plot for Rs

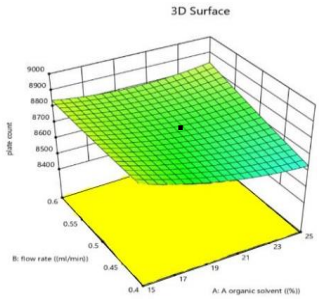


Fig 5: 3D Response Surface Plot for Plate Count

weighted or otherwise of the individual desirability functions. The expression that defines the Derringer's desirability function is:

$$D = [d_1^{p2} \times d_2^{p2} \times d_3^{p2} \times \ldots\ldots\ldots \times d_n^{pn}]^{1/n}$$

where pi is the weight of the response, n the number of responses and di is the individual desirability function of each response. Desirability function (D) can take values from 0 to 1. Weights can range from 0.1 to 10. Weights lower than 1 give less importance to the goal, whereas weights greater than 1 give more importance to the goal. The criteria for the optimization of each individual response were shown in table 3.

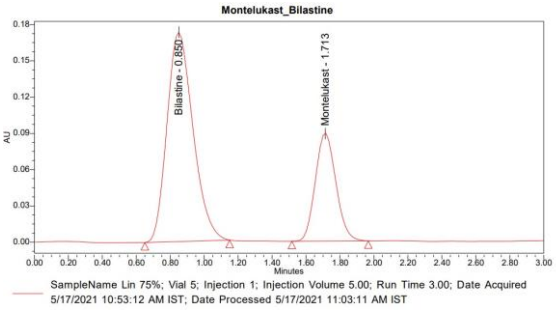


Fig 6: Global desirability response surface plot

Response	Lower limit	Upper limit	Criteria/Goal
Rt	1.541	2.612	minimize
Rs	3.18	4.56	minimize
Plate count	8497	8989	Is in range

Table 3: Criteria for the Optimization of the Individual Responses

In criteria, the responses Rt was minimized in order to shorten the analysis time and Rs was minimize to allow the base line separation of Bilastine and Montelukast. In order to separate the first eluting peak Bilastine from the solvent front plate count was in range. Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function was presented in figure 6.

From the figure 3 it could be concluded that there was a set of coordinates producing high desirability value (D = 0.843) were methanol concentration of 25.0%, buffer pH of 2.0 and flow rate of 0.4 ml/min. The optimized assay conditions were Triethylamine buffer: methanol 75:25%v/v) (pH 2.0) as mobile phase at a flow rate of 0.4 ml/min. The predicted response values corresponding to the later value of D were Rt = 1.65min, Rs = 3.30min and plate count = 8190.83. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatogram was shown in figure 7.

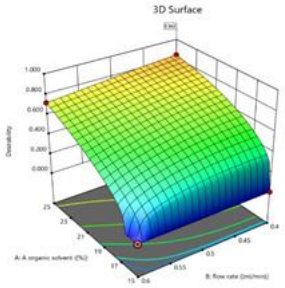


Fig 7: Optimal conditions corresponding Chromatogram

The observed difference between the predicted and experimental responses were found to be in good agreement, within a difference of 5.0% was shown in table 4.

Optimum conditions	Organic Solvent (%v/v)	Flow rate (ml/min)	Buffer (pH)	Rt	Rs	Plate count
Predictive	25.00	0.40	2.0	1.65	3.30	8190.83
Experimental	25.00	0.40	2.0	1.71	3.46	8214.47
Average error				3.65	4.84	0.28
Desirability value (D) =0.843						

Table 4: Comparison of Experimental and Predictive values of different functions under optimal conditions

METHOD VALIDATION

This work was focused on optimization of the conditions for the simple and rapid as well as low cost-effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution) and flow rate were varied to determine the chromatographic conditions giving the best separation.

LINEARITY

The linearity of the proposed method was evaluated by analysing a series of different concentrations of each compound. Six concentrations were chosen, ranging between 5-30 and 2.5-15 μgml^{-1} Bilastine and Montelukast. Each concentration was repeated three times. The linearity of the calibration graphs and adherence of the system to Beer's law was validated by the high value of the correlation coefficient 0.999 for all the determined drugs.

DETECTION AND QUANTITATION LIMITS

According to ICH recommendations, the approach based on the standard deviation of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and the method has a detection limit of 0.1and 0.2 μgml^{-1} of Bilastine and Montelukast and quantitation limit of 0.333and 0.66 μgml^{-1} of Bilastine and Montelukast respectively.

PRECISION

Repeatability of the method was tested by choosing three concentration levels for each compound and analysing them as described under experimental section at nominal conditions. The mean percentage relative standard deviation values for method precision of Bilastine and Montelukast were found to be 0.91% and 1.29% respectively and system precision the values of Bilastine and Montelukast were found to be 0.51% and 0.69% respectively.

ACCURACY

The mean percentage recovery values obtained for Bilastine and Montelukast were 100.4 and 100.0%, respectively. In all the cases, the results showed a fairly good accuracy of the method. Consequently, the excipients in the pharmaceutical formulations do not interfere with the analysis of the latter compounds in their formulations. The method validation parameter reports were shown table -5.

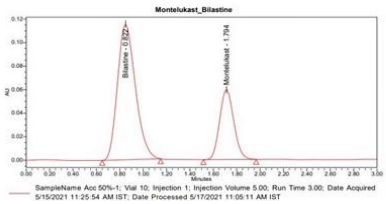


Figure 8: Accuracy Chromatographs of Bilastine & Montelukast for 50%

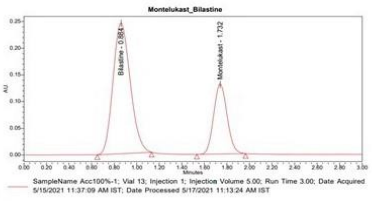


Figure 9: Accuracy Chromatographs of Bilastine & Montelukast for 100%

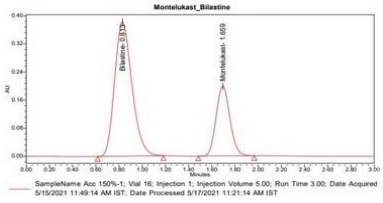


Figure 10: Accuracy Chromatographs of Bilastine & Montelukast for 150%

Parameters		Bilastine	Montelukast
Range(μgmL^{-1})		5-30	2.5-15
Y=mx + c		y = 11742x + 9025	y = 12695x + 322.50
r^2		0.9992	0.9990
Slope (m)		11742	12695
Intercept (c)		9025	322.50
LOD (μgmL^{-1})		0.1	0.2
LOQ(μgmL^{-1})		0.33	0.66
Accuracy (%)		100.4	100.00
Precision (%RSD)	Method	0.91	1.29
	System	0.51	0.69

Table 5: Validation Parameters

FORCED DEGRADATION STUDIES

The specificity was determined according to ICH guidelines by subjecting a standard solution to various stress conditions like acid, base hydrolysis, oxidative and photolytic conditions [20]

The percentage degradation report was shown in table -6. From the stability testing data represented the % degradation was found to be not more than 20%, which does meet the acceptance criteria (% limit of degradation 5-20%). So, the analytes were stable under the mentioned stress conditions.

Stress condition	% Degradation	
	Montelukast	Bilastine
Acid 0.1 N HCl	3.2	2.6
Alkaline 0.1N NaOH	2.6	3.5
Oxidative 0.1% H_2O_2	2.9	3.1
Reduction	4.1	4.6
Thermal	4.6	5.0
Photolytic degradation	3.8	4.2
Hydrolysis	5.0	2.8

Table 6: Degradation data for Analytes

CONCLUSION

An innovative UPLC method has been developed for simultaneous estimation of in marketed formulation using central composite design. Multivariate regression analysis was successfully employed to effectively screen the main effects of factors that significantly affected the resolution, column efficiency and tailing of the critical peaks. Three factors that were determined to significantly affect the peaks were then analyzed to determine their interactions and quadratic effects with the CCD in conjunction with response surface methodology. The method gave good resolution for both the drugs with a short analysis run time within 3.0 min. The developed method was validated as per ICH guidelines. It was found to be novel, simple accurate precise, sensitive and cost effective. Hence the proposed UPLC method is fitting for routine assay of in Bilastine and Montelukast pharmaceutical dosage form in quality control laboratories.

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