RESEARCH ARTICLE

Stability Indicating Quality by Design-based Development and Validation of Bilastine and Montelukast by Ultra Performance Liquid Chromatography Method

Swarupa Bandi, Sreedevi Adikay*

[1]*Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.*

Received: 21th October, 2022; Revised: 18th November, 2022; Accepted: 20th December, 2022; Available Online: 25th December, 2022

ABSTRACT

Background: The current work, which was conducted under the quality by design (QbD) paradigm, aims to establish the optimization of ultra-performance liquid chromatography (UPLC) using the design of experiments and response surface theory, such as central composite design (CCD), in order to achieve a good separation and resolution.

Methods: The mobile phase was composed of methanol (20:80% v/v) and 0.1% tri fluro amine (TFA) buffer, and chromatographic separation was performed on column phenomenex C18 (50 mm x 4.6 mm x 2.5 m) at a flow rate of 0.4 mL/min. Montelukast (MON) and bilastine (BIL) were both detected at 260 nm. The suggested method was approved in accordance with International Council for Harmonization (ICH) recommendations.

Results: The created technique successfully separates both BIL and MON with a chromatographic resolution of 6.4. The technique was linear for BIL and MON concentrations of 0.5–3.0 µg/mL and 0.25–1.5, respectively, and recovery rates ranged from 99–100%. The drug compounds had distinct degradation products that were identified in stress patterns.

Conclusion: For the quantification of BIL and MON in the combination tablet, a precise, simple, reliable, and accurate UPLC method was designed. According to the method validation, it was possible to successfully separate two medicines from their degradants utilizing aQbD techniques.

Keywords: Bilastine, Central Composite Design, Forced Degradation, Method Validation, Montelukast, ultra-performance liquid chromatography.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.4.14

How to cite this article: Swarupa B., Sreedevi A. Stability indicating Quality by Design-based Development and Validation of Bilastine and Montelukast by Ultra Performance Liquid Chromatography Method. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):426-432.

Source of support: Nil.

Conflict of interest: None

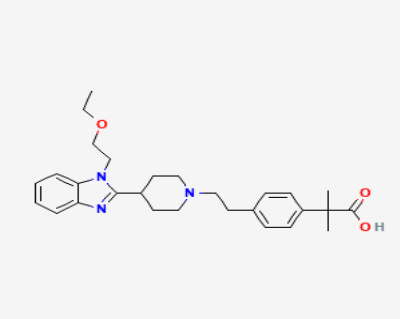
INTRODUCTION

A peripheral histamine H1-antagonist known as bilastine (BIL) is used to treat chronic spontaneous urticaria and seasonal allergic rhinitis. It is an antagonist of the histamine H1 receptor with a specificity of 64 nM. Histamine and other chemicals are released when cells degranulate during an allergic reaction.[1] BIT lessens the development of allergy symptoms brought on by the release of histamine from mast cells by interacting with and blocking the activation of the H1 receptor. In terms of chemistry, it is known as 2-[4-[2-[4-[1-(2-ethoxy ethyl) benzimidazol-2-yl] piperidin-1-yl]ethyl]phenyl] acid -2-methylpropanoic (Figure 1a).[2]

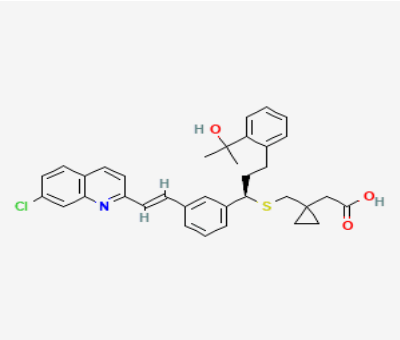
Montelukast (MON) is a leukotriene receptor antagonist that binds to CysLT type 1 receptors with greater affinity and selectivity. This helps to prevent any physiological activities of CysLTs such LTC4, LTD4, and LTE4 at the receptor that could promote asthma or allergic rhinitis.[3] Its chemical name is 2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl] propyl] sulfanylmethyl] cyclopropyl] acetic acid (Figure 1b).[4] Pharmaceutical quality by design (QbD) is a systematic procedure of development that starts with established goals and promotes product and process comprehension and control based on reliable science and quality risk management.[5] The quality project from the planning stage to the end of the product life cycle is addressed by the QbD tool.[6]

The literature suggests that a number of analytical techniques, including UV Spectroscopy,[7-10] high-performance liquid chromatography (HPLC),[1-16] high-performance thin layer chromatography (HPTLC),[17] and ultra performance liquid chromatography (UPLC)[18,19] for the simultaneous determination of BIL and MON, have been reported.

**Author for Correspondence: sreedevi@spmvv.ac.in*



(a)



(b)

Figure 1: (a) Structure of Bilastine (b) Montelukast

Except for spectrophotometry, none of these technologies were applied for analytical QbD in the published literature. The current goal is to improve the UPLC technique using a QbD methodology. In order to simultaneously estimate BIL and MON in pharmaceutical dosage form utilizing the QbD methodology, it was therefore intended to produce and validate a unique, linear, and accurate UPLC method.

MATERIALS AND METHODS

Materials

BIL and MON powder (purity 100.01 and 99.98% respectively) were procured from Glenmark, Mumbai, India. All chemicals were of HPLC grade (Merk India Ltd, Mumbai, India) and HPLC water acquired from Milli Q. Montek BL Tablet labelled to contain 20 mg of BIL and 10 mg of MON from Sun Pharma Pharmaceuticals LTD.

Instruments and Software

The analysis was carried out using the UPLC system; Agilent1290 Infinity II LC System (Agilent Infinity lab suppliers), and Photodiode Array (PDA) Detector. UV-vis. spectrophotometer, model UV-1700. The utilized software was Empower 2.0 version.

Chromatographic Conditions

Column: Phenomenex C18 column, (50x4.6 mm), (2.5 µ),
Mobile Phase: 0.1% tri fluro amine (TFA): Methanol (80:20 v/v),
Flow rate: 0.5 mL/min
Detector: PDA detector at 260 nm.

Standard Stock Solution Preparation

Standard stock solutions of BIL and MON were separately prepared in a concentration of 500 mg/mL for both. A 1-mL and 0.5 mL of each stock solution were transferred into a 10 mL volumetric flask and adjusted with diluent as methanol to obtain BIL and MON in the concentration of 100 µg/mL and 50 µg/mL, respectively.

Sample Solution Preparation

Accurately weighed and transferred 102 mg of sample into a 10 mL clean dry volumetric flask, added diluent, sonicated it for up to 30 minutes, and centrifuged for 30 minutes to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through a 0.45 µ injection filter. Further pipetted 1-mL of sample solution into a 10 mL volumetric flask and diluted up to the mark with diluent (100 ppm of BIL and 50 ppm of MON.

Method Optimization

The highest selectivity with the shortest analytical time is achieved through chromatographic optimization. All findings were evaluated during the experiment design, and the primary parameters for the optimization phase were chosen. The selected variables fit the criteria for the critical quality features in the initial requested analysis conditions. A percentage of the organic phase (A) and flow rate (B) was chosen as the independent variables, and the resolution of MON (Rs), the retention time of BIL (Rt BIL), and the retention time of MON (Rt MON) were chosen as the responses (Table 1).

A central composite design (CCD) with 13 tests made up the quadratic model that was suggested for process optimization. 25% Methanol and a 0.45 mL/min flow rate were used in the experiment's center points. The model for a two-factor experimental design can be written as $Y= \beta0 + \beta1\ X1 + \beta2\ X2 + \beta12\ X1\ X2 + \beta11\ X22 + \beta22$ where Y is the response to be modeled, 0 is the coefficient constant, 1 and 2 are linear coefficients, and 12 represents the coefficient of interaction between the two components. A and B are coded as the levels of independent variables high (+), low (-), and center point, respectively, and 11 and 22 are quadratic coefficients computed from the observed experiments value of Y from experimental runs (0). Table 2 lists the total range of the two parameters utilized to create the 13 analytical trials and the corresponding observed results.

Table 1: Factors selected in CCD

Factor	*Name*	*Type*	*Minimum*	*Maximum*	*Coded Low*	*Coded High*	*Mean*	*Std. Dev*
A	Mobile Phase	Numeric	17.93	32.07	-120	+130	25	4.08
B	Flow Rate	Numeric	0.3793	0.5207	-10.40	+10.50	0.4500	0.0408

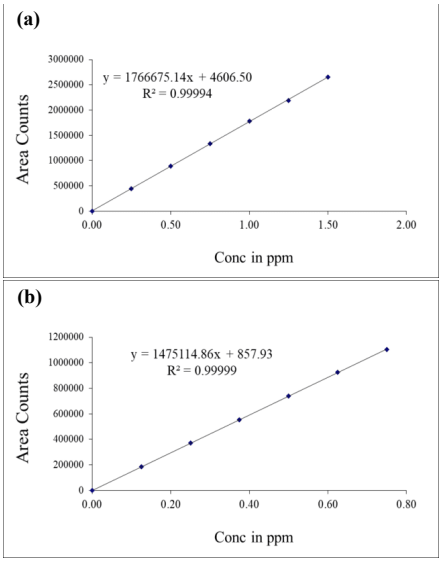


Figure 2: (a) Bilastine calibration curve (b) Montelukast calibration curve.

Method Validation

In accordance with International Council for Harmonization (ICH) Q2 (R1) guidelines, the created approach was validated.[20]

Linearity and Range

Five common BIL and MON solutions, with concentrations ranging from 25 to 150 µg/mL and 12.5 to 75 µg/mL, were made. Each experiment was carried out in triplicate, and chromatograms were used to determine the peak area. The graph was created by plotting peak areas of BIL and MON against their respective concentrations (Figure 2 a, b).

Limit of Detection (LoD) and Limit of Quantification (LoQ)

LoD and LoQ of the drugs were calculated using equations according to ICH guidelines. LoD = 3.3 σ/s and LoQ = 10 σ/s were found. Where σ is the SD of the response and S is the slope of the calibration curve.

Accuracy

At three levels—50% (5 and 2.5 µg/mL BIL and MON), 100% (10 and 5 µg/mL BIL and MON), and 150% (15 and 7.5 µg/mL BIL and MON) accuracy tests are conducted.

Precision

A 6 replicate sets were used to study repeatability. System precision, method precision, and intermediate precision data were acquired using BIL and MON. Determine the %RSD.

Robustness

The robustness was checked by small but deliberate changing in chromatographic conditions like organic phase (20 ± 5 mL), flow rate (0.4 ± 0.05 mL/min), and wavelength (260 ± 13 nm). The %RSD is within the limit for all parameters.

Analysis of Marketed Formulation

An aliquot of 0.4 µL from the sample solution was injected under a chromatographic condition and peak area was measured and % assay was calculated from the regression equation. The response was an average of six determinations.

Forced Degradation Studies

Studies on stress degradation were conducted using samples and in accordance with ICH recommendations. The drug sample was subjected to forced degradation conditions including thermal (at 105°C for 74 hours), peroxide (3% v/v H_2O_2 at ambient temperature for 15 minutes), acidic (0.1 N HCl reflux at 60°C for 15 minute), basic (0.1 N NaOH reflux at 60°C for 15 minute.), and photolytic (254 nm for 24 hours). The samples were further diluted with methanol and determined the separation of BIL and MON from degraded products. Up to 24 hours, all the solutions are injected every 6 hours.

RESULTS AND DISCUSSION

With the use of Design Expert 12 version software CCD of the experiment was chosen, and all suggested trials were run randomized with the CCD. Table 2 displays the developed model and the outcomes of potential combination trials. The

Table 2: DoE for the optimized chromatographic conditions

Std	*Run*	*Factor 1 A: Mobile phase*	*Factor 2 B: Flow Rate*	*Response 1 RT1*	*Response 2 RT2*	*Response 3 Resolution*
7	1	25.0	0.38	1.33	2.05	4.28
5	2	17.9	0.45	1.81	2.59	7.20
1	3	20.0	0.40	1.74	2.53	6.41
9	4	25.0	0.45	1.13	1.99	4.17
8	5	25.0	0.52	1.02	1.70	3.84
11	6	25.0	0.45	1.13	1.98	4.12
12	7	25.0	0.45	1.13	1.98	4.08
2	8	30.0	0.40	0.94	1.51	3.32
3	9	20.0	0.50	1.46	2.22	5.57
13	10	25.0	0.45	1.12	1.98	4.16
4	11	30.0	0.50	0.82	1.35	3.26
10	12	25.0	0.45	1.13	1.97	4.14
6	13	32.1	0.45	0.79	1.25	3.25

Table 3: ANOVA results for Responses

Source	*Sum of Squares*	*df*	*Mean Square*	*f-value*	*p-value*	
RT1						
Model	1.18	5	0.2368	13493.14	< 0.0001	significant
A-Mobile Phase	1.04	1	1.04	59007.85	< 0.0001	
B-Flow Rate	0.0861	1	0.0861	4904.14	< 0.0001	
Lack of Fit	0.0001	3	0.0000	5.27	0.0711	not significant
RT2						
Model	1.95	5	0.3891	5975.62	< 0.0001	significant
A-Mobile Phase	1.80	1	1.80	27644.78	< 0.0001	
B-Flow Rate	0.1187	1	0.1187	1823.43	< 0.0001	
Lack of Fit	0.0003	3	0.0001	1.97	0.2605	not significant
Resolution						
Model	17.68	5	3.54	1293.64	< 0.0001	significant
A-Mobile Phase	15.09	1	15.09	5519.95	< 0.0001	
B-Flow Rate	0.2902	1	0.2902	106.15	< 0.0001	
Lack of Fit	0.0140	3	0.0047	3.65	0.1216	not significant

Table 4: Recovery data of BIL and MON

% Recovery Azilsartan					*% Recovery Cilnidipine*			
Recovery sample name	*BIL amount added (mg)*	*BIL amount recovered (mg)*	*%Recover*	*Mean*	*MON amount added (mg)*	*MON amount recovered (mg)*	*% Recovery*	*Mean*
50% -1	5	5	100.0		2.50	2.49	99.6	
50% -2	5	5	100.0	100.1	2.50	2.48	99.2	99.6
50% -3	5	5.02	100.4		2.50	2.5	100.0	
100% -1	10	10	100.0		5	4.99	99.8	
100% -2	10	10	100.0	100.0	5	4.95	99.0	99.3
100% -3	10	10	100.0		5	4.96	99.2	
150% -1	15	14.89	99.3		7.5	7.51	100.1	
150% -2	15	14.86	99.1	99.2	7.5	7.55	100.7	100.4
150% -3	15	14.87	99.1		7.5	7.54	100.5	
Mean ± SD 99.8 ± 0.493					Mean ± SD 99.8 ± 0.569			
%RSD 0.49					%RSD 0.57			

Table 5: Degradation studies

Sample exposure condition	*% Degradation*		*Purity angle*		*Purity threshold*	
	BIL	*MON*	*BIL*	*MON*	*BIL*	*MON*
Acidic, 1-mL of 1N HCL 60˚C, 15 minutes	17.9	16.1	4.148	1.246	10.274	5.776
Alkali, 1-mL of 1N NaOH 60˚C, 15 minutes	13.6	15.8	4.156	3.451	10.255	21.742
Peroxide, 1-mL 3% H2O2, room temperature,15 minutes	12.1	15.4	4.285	1.261	10.745	5.776
Reduction, 1-mL 3%NaHSO4, 15 minutes	12.3	16.8	4.147	1.249	10.719	5.717
Hydrolysis, 1-mL water, 15 minutes	13.6	5	4.144	1.242	10.282	5.784
Thermal 105˚C, 72 hours	7.3	12.7	4.148	1.249	10.119	5.717
Photodegradation, 6 hours in sunlight	8.3	7.3	4.148	1.252	10.121	5.718

resolution and retention times of both drugs were shown to be influenced by two parameters. The quadratic ANOVA data in Table 3 that illustrates the model chosen is significant (*p-value* < 0.05) to identify the impact of factors affecting all three variables.

According to the quadratic ANOVA data response 1 (Table 3), the organic modifier percentage and flow rate significantly impact the resolution between BIL and MON. The results of the quadratic ANOVA for response 2 (Table 3) reveal that the flow rate and percentage of organic modifiers have a highly

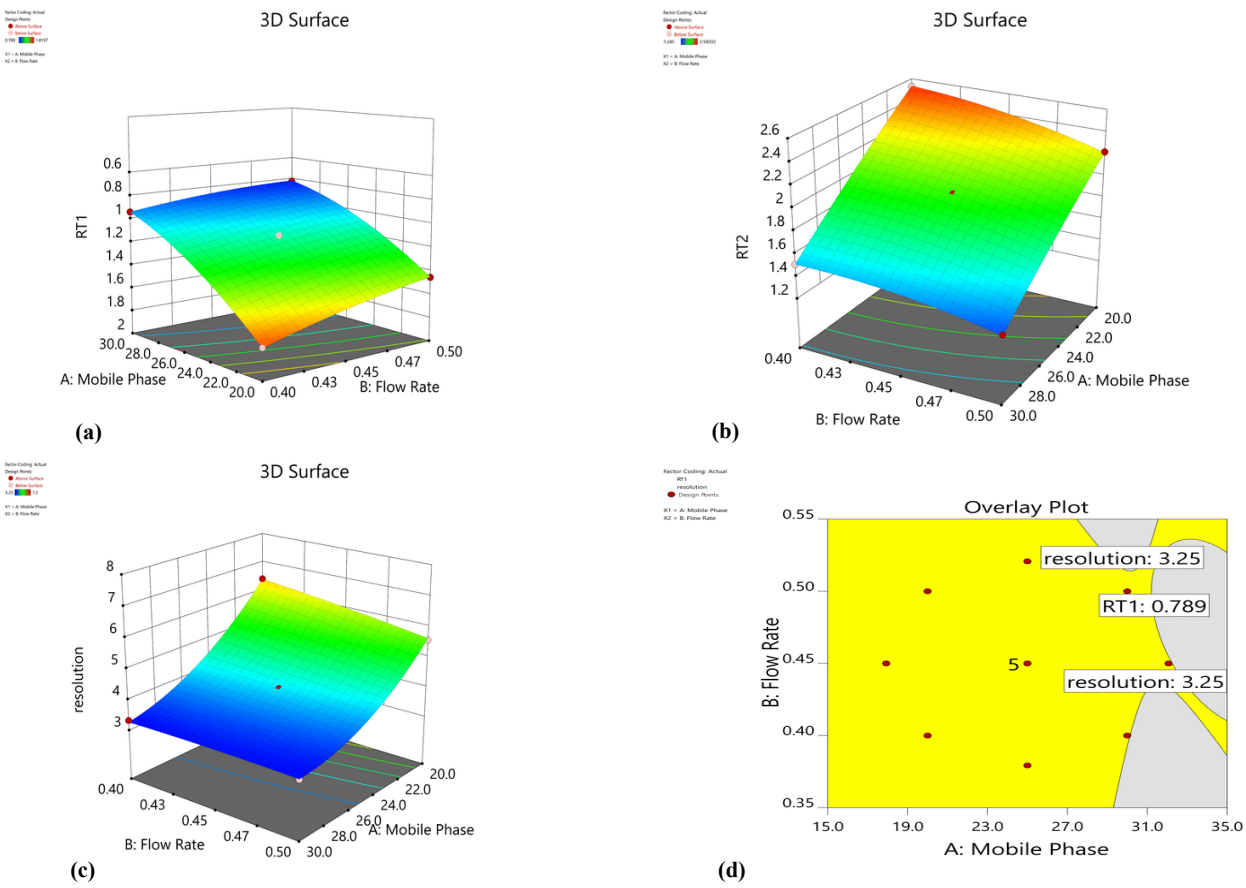


Figure 3: 3D response surface plot (a) Resolution Resolution, (b) The Retention Time of BIL, and (c) The Retention Time of MON, (d) Overlay Plot of Optimized Condition

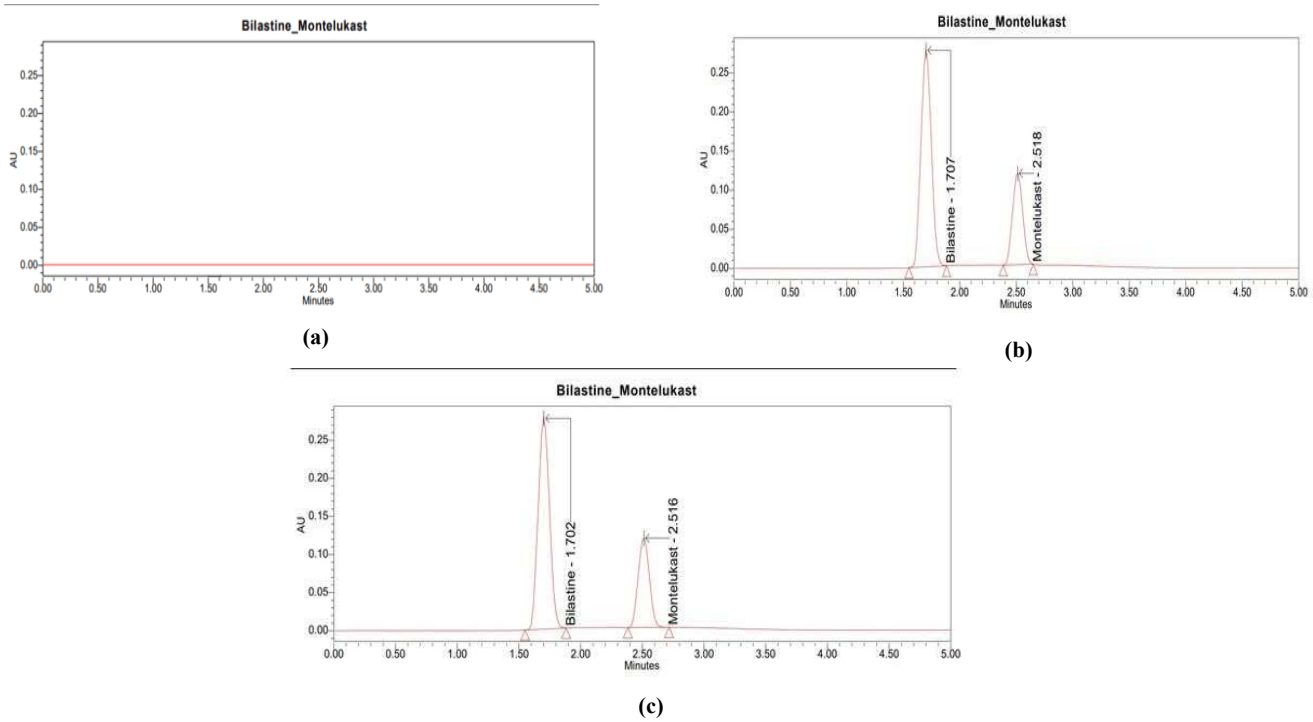


Figure 4: (a) Chromatogram of blank (b) Standard BIL 100µg/mL and MON 50 µg/mL (c) Sample BIL 100 µg/mL and MON 50 µg/mL (c).

significant impact on the retention time of BIL. The % organic phase and flow rate demonstrate a highly significant effect on the retention time of MON, according to the quadratic ANOVA data response 3 (Table 3). 3D response surface plots that represent the significant influence of critical factors on chosen responses have been created for the investigation of the overall effect of all critical elements (Figure 3) displayed the overlay plot of all results.

Using a 3D-response surface plot, the ultimate chromatographic parameters for the developed technique were chosen based on peak symmetry and retention time. By utilizing a mobile phase composition of 80:20% v/v (0.1% TFA buffer: methanol) at a flow rate of 0.4 mL/min and monitoring the elutes at 260 nm for the final UPLC analysis, the optimum separation of BIL and MON was produced on the phenomenex C18 column (50x4.6 mm), (2.5 µ) (Figure 4).

The analytical process system suitability parameters of the optimized technique were validated in accordance with the ICH Q2 (R1) recommendations, and it was observed that they met the required ranges for acceptance. The concentrations of BIL and MON were linearly related to the analyte peak area and varied from 0.5 to 3 and 0.25 to 1.5 µg/mL, respectively. The mean r^2 for the standard curves for BIL and MON was 0.99999 and 0.99989, respectively, indicating acceptable linearity. The study of repeatability validates precision. The BIL and MON data were indicated as % RSD below 2, which demonstrated good precision of the proposed technique of both system and method precision. While the LoD and LoQ data for BIL were 0.0415 and 0.126 µg/mL, respectively, the computed limits of detection and quantification for MON were 0.0073 and 0.0226 µg/mL, respectively. The LoD and LoQ values represent the sensitivity of the suggested UPLC procedure. A percentage recovery study was used to determine accuracy, and findings for BIL and MON ranged from 99.2 to 100.1% and 99.2 to 100.4%, respectively. The % recovery statistics demonstrated the accuracy of the developed approach. (Table 4).

A% RSD of less than 2 was obtained after purposefully altering the organic phase composition, flow rate, and injection volume; this result indicates a positive and effective reaction to the suggested technique. % assay of BIL and MON was in good agreement with the label's promise. BIL and MON were discovered to have percent assays of 99.8 and 99.8%, respectively. To determine how precisely and specifically the analyte of interest is measured in the presence of other circumstances, stability tests on BIL and MON were conducted. The effects of thermal, photolytic, peroxide, acidic, and basic stress were examined and the results were depicted in Table 5.

CONCLUSION

The QbD methodology was used to develop and validate an easy, fast, robust, and precise stability indicating the UPLC method. The organic phase and flow rate were the two factors in an experiment of the design that successfully separated the two drugs. Design Expert 12.0 software produced the experimental central composite design for the key method parameters. A 3D response surface plot demonstrating the simultaneous action of both drugs was produced after the analysis of all factor influences on responses. The findings of the verified approach show that BIL and MON have symmetric peak shapes, acceptable resolution, and suitable retention times. Drug substances were subjected to stress conditions, and fewer than 20% of the drug acquired degradation. Thus, the developed approach appears suitable for quality control in the pharmaceutical industry.

REFERENCES

1. Bilastine drug bank (cited 2022 aug 20). https://go.drugbank.com/drugs/DB11591.
2. Bilastine pubchem (cited 2022 aug 20). https://pubchem.ncbi.nlm.nih.gov/compound/Bilastine#section=Names-and-Identifiers.
3. Montelukast drug bank (cited 2022 Aug 20). https://go.drugbank.com/drugs/DB00471
4. Montelukast pubchem (cited 2022 aug 20). https://pubchem.ncbi.nlm.nih.gov/compound/Montelukast#section=Names-and-Identifiers
5. Juran JM. Juran on quality by design: the new steps for planning quality into goods and services. New York: The Free Press; 1992.
6. ICH, Q8(R2), Harmonized Tripartite Guideline, Pharmaceutical Development. Available from: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.
7. Prajapati P, Tamboli J, Surati P, Mishra A. Risk assessment-based enhanced analytical quality-by-design approach to eco-friendly and economical multicomponent spectrophotometric methods for simultaneous estimation of Montelukast sodium and Bilastine. Journal of AOAC International. 2021 Sep;104(5):1453-63.
8. Thakur S. Spectroscopy-based development and validation of analytical technique for simultaneous estimation of bilastine montelukast.
9. Da Silva AT, Brabo GR, Marques ID, Bajerski L, Malesuik MD, Paim CS. UV spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness. Drug Analytical Research. 2017 Dec 28;1(2):38-43.
10. Sowjania G, Sastri T. UV Spectrophotometric method development and validation for simultanious determination of fexofenadine hydrochloride and Montelukast sodium in tablets. World J Pharm Pharm Sci. 2018 Jul 11;6(10):780-9.
11. Shinde A, Gajeli GB, Ubale S, Matole V. Simultaneous hplc method development and validation of bilastine and montelukast in bulk and formulation. Research Journal of Pharmacy and Technology. 2021 Nov 1;14(11):6061-5.
12. Amarendra CV, Anusha K, Muneer S. Method development and validation of new RP-HPLC method for the estimation of Bilastine in pharmaceutical dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(8):2297-315.
13. Gandhi BM, Rao AL, Rao JV. Method development and validation for simultaneous estimation of Montelukast sodium and Desloratadine by RP-HPLC. American Journal of Analytical Chemistry. 2015;6(08):651.
14. Sunkara R, Ajitha M. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Montelukast and Bilastine in Bulk and Pharmaceutical Dosage Form: https://doi. org/10.54037/WJPS. 2022.100109. World Journal of Pharmaceutical Sciences. 2022 Jan 2:90-7.
15. Vyas P, Thakur VS, Basedia D, Dubey B. Development

and Validation of RP-HPLC based Analytical method for Simultaneous Estimation of Montelukast and Bilastine in Tablet Dosage Form. Current Research in Pharmaceutical Sciences. 2022 Apr 6:68-73.

16. Nikalje AP, Andhale SM. Simultaneous Estimation of Bilastine and Montelukast in Bulk by Rp-hplc and Assessment of Its Applicability in Marketed Tablet Dosage Form. Journal of Pharmaceutical Research International. 2022 Jan 24:8-25.
17. Shah DA, Patel PA, Chhalotiya U. Thin-layer chromatographic–densitometric method of analysis for the estimation of montelukast and bilastine in combination. JPC–Journal of Planar Chromatography–Modern TLC. 2021 Aug;34(4):289-95.
18. Katta R, Murty NN, Ramasrinivas GN. Rao. Stability indicating method development and validation for the determination of Bilastine and its impurities by UPLC method. International Journal of Pharmaceutical Sciences and Research. 2020;11(3):1312-21.
19. Firdous S, Rizwan SH. Analytical Method Development And Validation For The Estimation Of Bilastine In Bulk And Pharmaceutical Dosage Form By Uplc. World J. Pharm. Life Sci.. 2020;6:138-43.
20. ICH Expert Working Group. ICH Q2 (R1) Validation of analytical procedure: Text and methodology: ICH Harmonized tripartite guideline. InInternational Conference on Harmonization of technical requirements for registration of phar-maceuticals for human use. Geneva 2005.