VALIDATION OF A NEW ANALYTICAL METHOD FOR SIMULTANEOUSLY ESTIMATING MONTELUKAST AND BILASTINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Arjun Singh[*], Ankit Diwan, Kuldeep Ganju
Sagar Institute of Pharmacy and Technology, Bhopal, Madhya Pradesh, India
*Corresponding Author's E mail: mpharmsiptec@gmail.com

ABSTRACT

A new reversed phase high performance liquid chromatography (RP-HPLC) method was developed for simultaneously estimating the concentration of montelukast and bilastine in formulation and it was validated according to ICH guidelines. The chromatogram was found to be satisfactory on C-18 (4.6×150mm, 5μ Thermosil column) using mobile phase composed of isoproylalcohol: phosphate buffer (pH 4.5) (80:20) at a flow rate of 1.0 ml/min and the detection wavelength of 260 nm. The retention time of monelukast and bilastine was found to be 1.81 and 3.62 min respectively. The system suitability parameters proved that the proposed method is suitable for estimation of both the drugs under study. The number of theoretical plates for separation was found to be 13569 and 15576 with a tailing factor of 1.084 and 1.285 respectively for montelukast and bilastine. The linearity for the drugs was studied from 10 to 50 µg/ml concentrations. The precision of the method was good and the recovery of drugs was found to be within the prescribed limits of acceptance (50-150%) The LOD and LOQ were found to be 0.003 µg/mL and 0.012 µg/mL respectively for montelukast and 0.09 µg/ml and 0.3 µg/ml respectively for bilastine. The proposed RP HPLC method was found suitable for the estimation of montelukast and bilastine in dosage forms (tablet) and is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

Keywords: Montelukast, Bilastine, ICH guidelines, Dosage form, HPLC.

INTRODUCTION

Bilastine is a novel new-generation antihistamine that is highly selective for the H_1 histamine receptor, has a rapid onset and prolonged duration of action1. It has a chemical formula of $C_{28}H_{37}N_3O_3$. Montelukast is a member of the leukotriene receptor antagonist (LTRA) category of drugs with molecular formula $C_{35}H_{36}ClNO_3S$ and is indicated for the prophylaxis and chronic treatment of asthma [1].
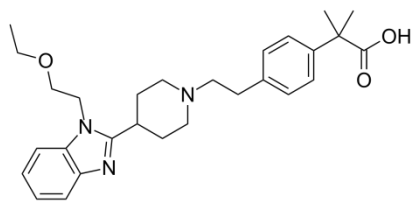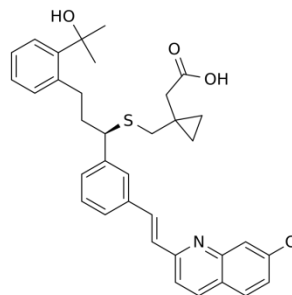
(1)   (2)

The United States Pharmacopoeia (USP) official method for estimation of montelukast involves a 21 min long gradient HPLC analysis using trifluoroacetic acid, water and acetonitrile as the solvents [2]. Bilastine has been approved to be tentatively approved for inclusion in Indian Pharmacopoeia (IP) and the method of estimation involves 68:32 ratio of phosphate buffer (pH 7.0) and acetonitrile as the mobile phase [3]. Several reports of individual estimation of montelukast [4,5,6] and bilastine [7,8] have been found using various techniques and various matrices including dosage form and biological matrices. The fixed combination of bilastine and montelukast has been approved very recently for the treatment of allergic rhinitis in adults. Using keywords simultaneous estimation of montelukast and bilastine yielded only two results in pubmed. One of these methods was quality by design HPLC method [9] while the other was high performance thin layer chromatography (HPTLC) based method [10]. A few other methods for the simultaneous estimation of these two drugs were found in google search [11, 12,13]. Hence it was envisioned to develop and validate a suitable method for the simulatenous estimation of Montelukast and Bilastin in dosage form.

MATERIALS AND METHODS

Chemicals

Montelukast sodium pure drug (>98.0% HPLC) and Bilastine pure drug (>98.0% HPLC) were purchased from Yarrow Pharmaceuticals, Mumbai. Methanol and water (HPLC grade) were obtained from Merck. Orthophosphoric acid (analytical grade) was obtained from Qualigens. Tablet formulation (Bilafav M (20 mg Bilastine and 10 mg Montelukast) manufacture by Cipla Ltd) was procured from local market and was used for in the present study.

Equipment

The simultaneous estimation was accomplished using a Shimadzu LC10 HPLC system equipped with UV detector (Shimadzu, SPD10), 20µL capacity manual loop injector and the processing of the results

was done using Surwit N2000 software. A thermosil column (C18, 5μ particle size, 4.6 x 150 mm) was used for chromatographic separation.

Chromatographic conditions

An isocratic mixture of isopropyl alcohol 800 ml and 200 ml phosphate buffer (pH 4.5) was used as the mobile phase for elution of the components of the drug sample. The solvent mixture was degassed in ultrasonic water bath for 15 minutes and filtered through 0.45μ filter by vacuum filtration. Methanol was used as the diluent for preparation of the working standard solutions of the drug. The detection wavelength was set to 260 nm and the analysis was performed at flow rate of 1.0 mL per minute with an injection volume of 20μL.

Preparation of Standard Solution

Montelukast (10 mg) and Bilastine (20 mg) were accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 5 ml of methanol was added and sonicated to dissolve the drugs completely and the volume was made up to the mark with the same solvent to obtain the standard stock solution. 1 ml of the stock solution was pipetted out from the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with methanol to obtain the working standard solution. A series of dilutions of the working standard were prepared from the working standard solution. These solutions were also analyzed using the developed chromatographic conditions to study the various validation parameters like accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability.

Development and Validation of method

Conditioning of the column was done by initial washing of the column with distilled water followed by methanol and finally saturating the column with the mobile phase at flow rate of 1 ml/min for 20 min to obtain a stable base line.

Method Development

Several trials of solvent combinations were made to obtain the best possible combination to elute out the drugs within reasonable retention time.

Method Validation

The RP-HPLC method for simultaneous estimation of Montelukast and Bilastine was validated according to the Q2B(R1) ICH guidelines for linearity, LOD, LOQ, precision, accuracy, robustness, and system suitability [14,15].

System suitability

The working standard solution was injected six times into HPLC system as and the chromatographic study was performed as per the developed and optimized conditions. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Linearity

Dilutions of the working standard were prepared at five different and each level of dilution was injected into the chromatographic system in six replicates and the peak area and retention time was measured. A calibration plot of peak area against concentration was used to determine linearity.

LOD and LOQ

LOD and LOQ of the method were determined using method of standard deviation of the calibration curve to slope of the calibration curve ratio using the following calculations

$$LOD = \frac{3.3\,\sigma}{S} \qquad \text{--------- (1)}$$

$$LOQ = \frac{10\,\sigma}{S} \qquad \text{--------- (2)}$$

Where, σ is the standard deviation of the calibration curve; S is the slope of the calibration curve.

Accuracy

The regular spiking method was used for assessing the accuracy of the method. The working standard solution of concentration 20µg/ml of Montelukast and 40 µg/ml of Bilastine was analyzed using the developed method, and the solution was spiked with three concentrations of the drug (50%, 100% and 150%) of the target concentration and the mean % recovery was assessed. The percentage relative standard deviation was calculated.

Precision

The precision of the method was validated in terms of repeatability (intra-day precision) and inter-day repeatability (intermediate precision). The working standard solution was injected in six replicates in the HPLC system and the peak area for all six injections in HPLC was measured. Similar procedure was repeated for all the concentrations of the linearity range. The % RSD for the area was calculated.

Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system to study identification specificity, while blank interference was studied by injecting the diluent. The interference of excipients was also assessed for by injecting placebo solution.

Robustness

In order to evaluate the robustness of the method, deliberate changes in the flow rate and mobile phase composition were made and the drug was assayed using the proposed conditions.

Analysis of tablet formulation

Twenty tablets were crushed and powder equivalent to 10 mg Montelukast and 20 mg Bilastine was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 35 ml of methanol was added and sonicated to dissolve the drug completely and the volume was made up to the mark with the same solvent. 5 ml of this solution was pipetted out in to a 50 ml volumetric flask and diluted up to the mark with mobile phase. An aliquot of this solution was injected into HPLC system.

20 µl of the sample solution was injected into the HPLC system and the peak area and retention times obtained for Montelukast and Bilastine were measured using the optimized chromatographic conditions.

RESULTS AND DISCUSSION

The analytical method using isopropyl alcohol and phosphate buffer (pH 4.5) in the ratio of 80:20 in symmetric peaks of both the drugs at the flow rate of 1 ml/min. The maximum absorption of both the drugs in mixture solution was observed at 260 nm, and this wavelength was chosen for its determination. Montelukast was found to elute out from the sample at **1.825 minutes** while Bilastine eluted at **3.623 minutes** (Retention time, R_t) (Figure 1).
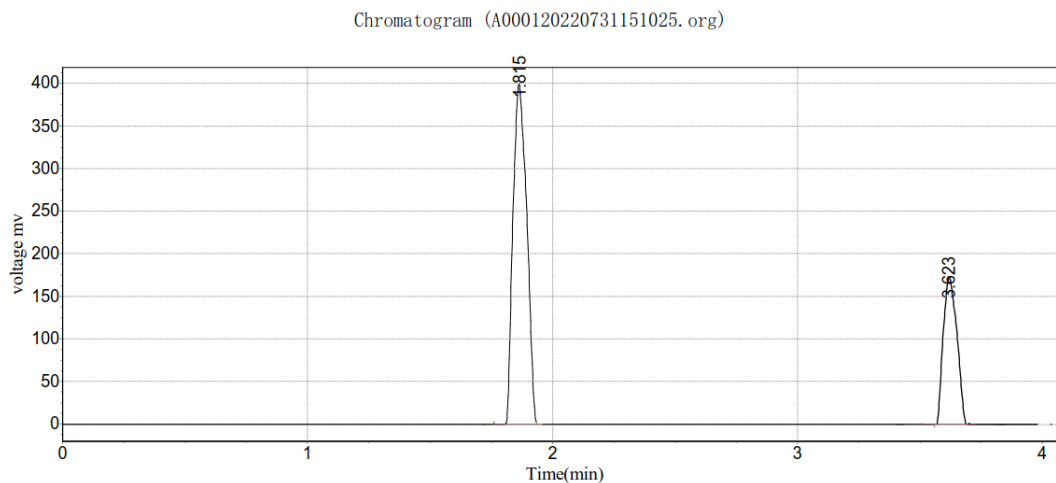
Figure 1. **Chromatogram of Montelukast and Bilastine**

System Suitability

Six replicate injections of the mix standard were made to HPLC system and the % RSD of retention time and the number for theoretical plates was observed. The % RSD of retention time was less than 2% and the number of theoretical plates was found to be sufficiently satisfactory. Hence the selected system parameters were found to be suitable for the simultaneous estimation of these drugs (Table 1).

Table 1. System suitability of the method

System suitability parameters	Montelukast		Bilastine	
	Mean	RSD	Mean	RSD
Peak Area (AUC x 10^6)	1.619	0.629	1.129	1.505
Retention Time	1.81	0.265	3.62	0.057
No. of Theoretical plates	13569			15576
Tailing Factor	1.084			1.285

Linearity

The stock solutions of Montelukast and Bilastine were diluted to obtain solutions (10 - 50 µg/mL) and the calibration curve was plotted (Figure 2 and 3). A linear line was obtained at the entire range, regression analysis was performed and the calibration curve equation $y = 255.6.x + 89.51$ with $R^2 = 0.999$ was obtained for Montelukast and $y = 17045x -3342$ with R2 = 0.997 was obtained for Bilastine.
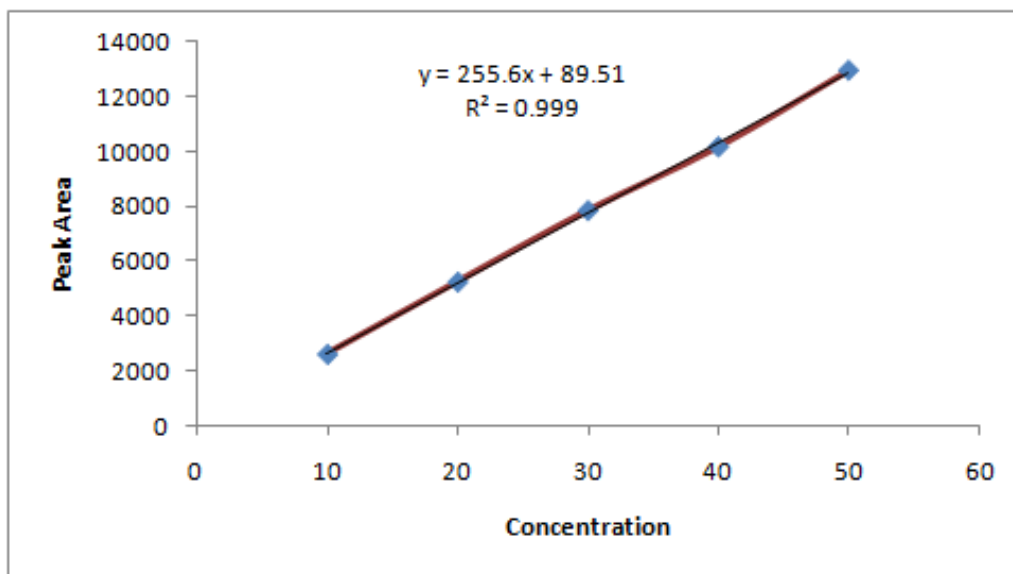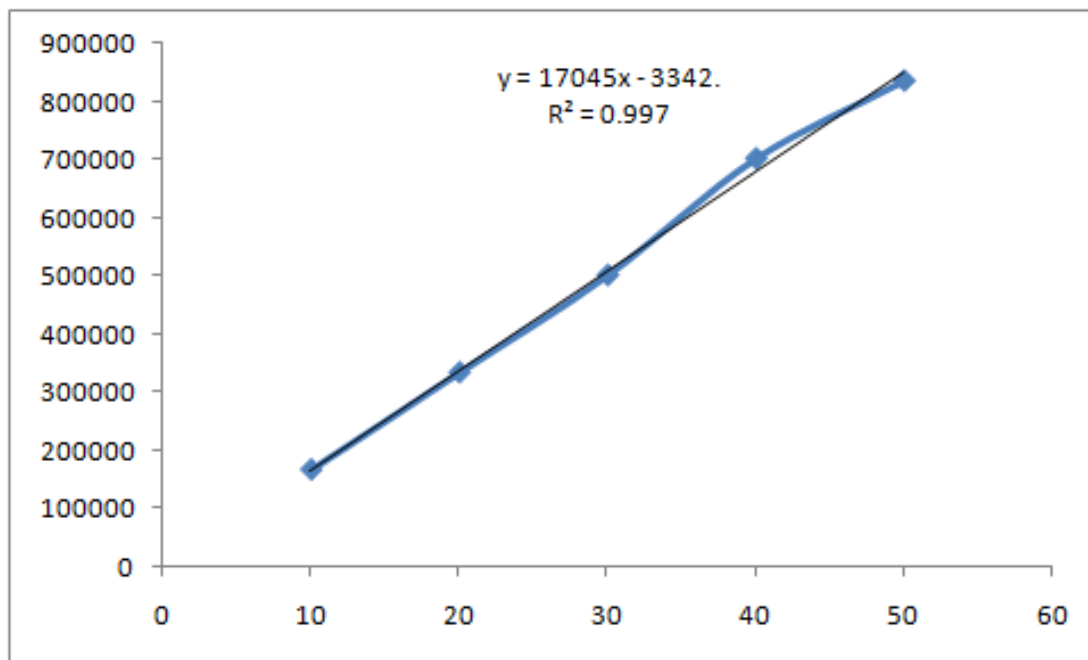
Figure 2. Linearity graph for Montelukast

Figure 3. Linearity graph for Bilastine

Limit of Detection

The calibration curve analysis was used to obtain the LOD and LOQ of the method. The values of LOD and LOQ were found to be 0.003 µg/mL and 0.012 µg/mL respectively for montelukast and 0.09 µg/ml and 0.3 µg/ml respectively for bilastine. Hence it could be said that the method was sensitive.

Accuracy

The accuracy of the method was determined by spiking a preanalyzed solution of 20µg/mL Montelukast and 40 µg/ml Bilastine with corresponding 50, 100 & 150 % strength solutions and analyzing the spiked solutions. Accuracy of the method was determined by recovery analysis of spiked samples. Pre-analyzed sample was spiked with 50%, 100% and 150% of standard drug solution. The spiked sample was recovered within a range of 98-102% suggesting that the method was accurate in estimating the concentration of the drugs of mixture and the % RSD was found to be 0.740 within the specifications (less than 2) (Table 2).

Table 2. Accuracy data for the method

Drug Studied	Conc. of pre-analyzed sample µg)	Conc. of drug added (µg)	Mean Recovery (µg)	% Recovered (mean)
	20	10	30.09	100.31
Montelukast	20	20	39.46	98.66
	20	30	49.52	99.04
	40	20	59.64	99.40
Bilastine	40	40	80.06	100.08
	40	60	99.94	99.94

Precision

The precision (% RSD) of the developed analytical method was evaluated by calculating intra-day and inter-day coefficient of variation. Precision depicts the ability of the method produce the same results irrespective of the instrument used, or day of analysis or even the analyst performing the analysis (Table 3 and 4).

Table 3. Precision of the analytical method for Montelukast

Concentration (20 µg/ml)	Intermediate Precision		Repeatability	
	Retention time	Peak Area	Retention time	Peak Area
Rep 1	1.815	773869	1.815	773956
Rep 2	1.807	773918	1.807	773948
Rep 3	1.815	773885	1.815	773941
Rep 4	1.817	773899	1.817	773963
Rep 5	1.807	773943	1.807	773974
Rep 6	1.807	773651	1.807	773993
Mean	1.811	773962.500	1.811	773962.500
SD	0.004802777	105.9781424	0.004802777	18.85470763
%RSD	0.265151471	0.01369293	0.265151471	0.002436127

Table 4. Precision of the analytical method for Bilastine

Concentration (10 µg/ml)	Intermediate Precision		Repeatability	
	Retention time	Peak Area	Retention time	Peak Area
Rep 1	3.623	701887	3.623	701930
Rep 2	3.627	701896	3.627	701364
Rep 3	3.623	701922	3.623	701901
Rep 4	3.621	701905	3.621	701922
Rep 5	3.625	701919	3.625	701944
Rep 6	3.623	701896	3.623	701918
Mean	3.624	701829.833	3.624	701829.833
SD	0.002065591	13.90563435	0.002065591	228.6485659
%RSD	0.05700279	0.00198134	0.05700279	0.032578918

The results reveal that the % RSD in both the repeatability and intermediate precision studies was less than 2%, thereby ascertaining that the developed method will produce consistent results.

Specificity

The absence of peak of drugs in the chromatogram of blank and placebo confirmed the specificity of the method in separating the drugs from the sample matrix (Figure 4).
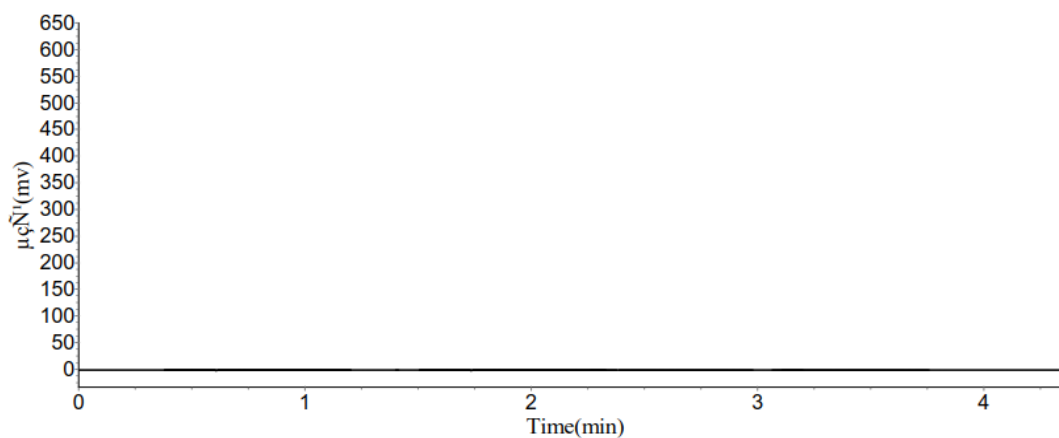
Figure 4. **Blank chromatogram**

Robustness

A few deliberate changes in flow rate were made for studying its effect on the results obtained by the method. The method was able to adjust to the changes with no significant change in the retention time of the eluted components (Table 5).

Table 5. Robustness of the method

Flow rate (ml/min)	Isopropyl alcohol:Phosphate buffer (pH 4.5)	Retention Time Montelukast (min)*	%RSD	Retention Time Bilastine (min)*	% RSD
0.9 (-0.1)	80:20	1.827	0.013	3.629	0.029
1.1 (+0.1)	80:20	1.807	0.017	3.608	0.027
1.0	75:25	1.817	0.020	3.623	0.017
1.0	85:15	1.817	0.025	3.623	0.080

*Average of six replicates

Assay of marketed formulation

The validated method was applied to perform the assay of Bifalav M tablet and the results obtained are presented in table 6. The percentage concentration of Montelukast and Bilastine were found to be 100.1

% and 99.8 % respectively (Table 6). This suggests the suitability of the method for routine analysis of formulations.

Assay of marketed formulation

The validated method was applied to perform the assay of Bifalav M tablet and the results obtained are presented in table 6. The percentage concentration of Montelukast and Bilastine were found to be 100.1 % and 99.8 % respectively (Table 6). This suggests the suitability of the method for routine analysis of formulations.

CONCLUSION

The investigation resulted in the development of a new RP – HPLC method for the simultaneous estimation of Montelukast and Bilastine in formulations. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis. The method was found to be highly effective in the analysis of fixed dose combination formulation of Montelukast and Bilastine.

ACKNOWLEDGEMENT

CONFLICT OF INTERESTS

The authors declare on conflict of interests.

REFERENCES

1. https://go.drugbank.com/drugs/DB00471; assessed on 16/08/2022

2. https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/2009-10USPMontelukastSodiumProspectiveHarmonization.pdf; assessed on28/03/2023 International Organization for Standardization. Guide 8402: Quality Vocabulary. ISO, Geneva, 1994.

3. https://ipc.gov.in/images/Bilastine_version_1.0.pdf; assessed on 28/03/2023

4. Rathore AS, Sathiyanarayanan L and Mahadik KR. Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form. Pharmaceutical Analytica Acta. 2010; 1(1): 106. doi:10.4172/2153-2435.1000106

5. Singh RM, Saini PK, Mathur SC, Singh GN and Lal B. Development and validation of a RP-HPLC method for estimation of montelukast sodium in bulk and in tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2010; 72(2): 235-237

6. Rana NS, Rajesh KS, Patel NN, Patel PR, Limbachiya U and Pasha TY. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Montelukast Sodium and Ebastine in Tablet Dosage Form. Indian Journal of Pharmaceutical Sciences. 2013; 75(5): 599-602

7. Prathyusha P, Sundararajan R, Bhanu P, Annapurna M and Mukthinuthalapati. A new stability indicating RP-HPLC method for determination of Bilastine in bulk and pharmaceutical formulation. Research Journal of Pharmacy and Technology. 2020; 13(6): 2849-2853.

8. Prathyusha P and Sundararajan R. UV spectrophotometric method for determination of Bilastine in bulk and pharmaceutical formulation. Research Journal of Pharmacy and Technology. 2020; 13(2): 933-938.

9. Prajapati P, Tamboli J, Surati P and 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; 104(5): 1453-1463. doi: 10.1093/jaoacint/qsab089

10. Prajapati P, Tamboli J and Mishra A. Risk and DoE-Based Analytical Failure Mode Effect Analysis (AFMEA) to Simultaneous Estimation of Montelukast Sodium and Bilastine by HPTLC Method Using Enhanced AQbD Approach. Journal of Chromatographic Science. 2022; 60(6): 595-605. doi: 10.1093/chromsci/bmab107.

11. Chandra U, Kumar M, Sharma S, Gupta P and Garg A. Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for In-vitro Dissolution Testing of Bilastine and Montelukast Sodium Tablets. International Journal of Pharmaceutical Sciences and Drug Research. 2021; 13(3): 281-287.

12. Padhiyar V, Patani P and Tiwari N. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of montelukast sodium and bilastine from its pharmaceutical dosage form. Journal of Emerging Technologies and Innovative Research. 2021; 8(6): b865-b877

13. Vyas P, Thakur VK, Basedia D and 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; 12(1): 68-73. DOI: 10.24092/CRPS.2022.120110

14. Biswas P, Jain P, Parkhe G and Mishra B. Validated HPLC method for the estimation of Aspirin and Omeprazole in dosage form. Journal of Pharmacology and Biomedicine. 2018; 2(3): 171-180.

15. Patel A, Soni P, Yadav N and Omray LK. New cost-effective RP-HPLC method development and evaluation for quantitative estimation of Pitavastatin in pharmaceutical formulation. Journal of Pharmacology and Biomedicine. 2018; 2(3): 181-188.