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Development and Validation of RP-HPLC based Analytical method for Simultaneous Estimation of Montelukast and Bilastine in Tablet Dosage Form

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ABSTRACT

A new RP-HPLC method was developed for the estimation of bilastine and montelukast in tablets and it was validated as per ICH guidelines. The chromatogram for was found to be satisfactory on symmetry C-18 (4.6×150mm, 5µ Thermosil column) using phosphate buffer (pH 4.5) and acetonitrile in the ratio of 30:70 v/v at a flow rate of 0.8 ml/min. The retention time of montelukast and bilastine were found to be 7.03 min and 9.50 min respectively. The system suitability parameters proved that the proposed method is suitable for simultaneous estimation of bilastine and montelukast. Tailing factor for the peak was found to be 1.003 and 1.258 for montelukast and bilastine respectively and the theoretical plates for separation were found to be 3409 and 7365 respectively montelukast and bilastine. The method was found to be linear in the range of 10-50µg/ml for both the drugs. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD was found to be 0.003 µg/ml for montelukast and 0.09 µg/ml for bilastine while the LOQ ws found to be 0.012 µg/ml for montelukast and 0.3 µg/ml for bilastine.

Key words: Montelukast, Bilastine, RP-HPLC, simultaneous estimation, validation, analytical method

1. INTRODUCTION

Bilastine (figure 1) is a novel new-generation antihistamine that is highly selective for the H_1 histamine receptor, has a rapid onset and prolonged duration of action.[1] It has a chemical formula of $C_{28}H_{37}N_3O_3$. Montelukast (figure 2) 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.[2]

Several methods for individually estimating of bilastine and montelukast have been reported over years by several researchers.[3-9] The fixed combination of bilastine and montelukast has been approved very recently for the treatment of allergic rhinitis in adults. Hence, no method related to the estimation of this combination has been reported till date.

The objective was the present work was to develop a simple high performance liquid chromatography (HPLC) method for simultaneous estimation of bilastine and montelukast in tablets and validate the method as per ICH guidelines.

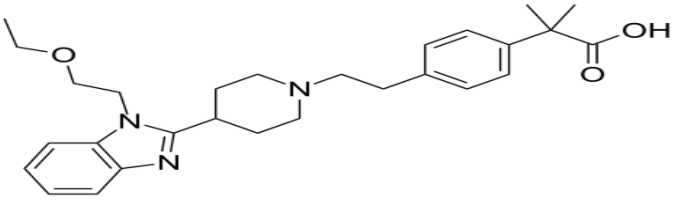

Figure 1. Chemical structure of bilastine

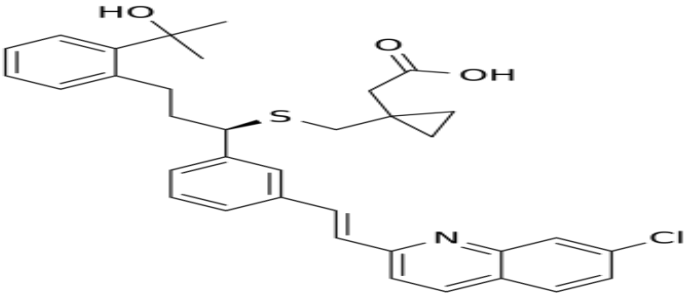

Figure 2. Chemical structure of montelukast

2. MATERIALS AND METHODS

Bilastine and montelukast pure drugs were purchased from Yarrow Pharmaceuticals, Mumbai. All solvents used were of HPLC grade while the other reagents were of analytical grade. HPLC (Shimadzu, LC-10), electronic balance (Wensar), pH meter (Labtronics, LT-53), sonicator (Biotechnics) and double beam UV Visible spectrophotometer (Labtronics, LT-2201) were used in the study.

2.1 Determination of Solubility

The qualitative solubility of bilastine and montelukast was observed by dissolving a very small quantity of the individual drug in 1 mL of different solvents (water, methanol, acetonitrile).

2.2 Determination of Working Wavelength[10]

Individual solution of 100 µg/mL of bilastine and monelukast was prepared in acetonitrile and scanned using a double beam UV-Visible spectrophotometer from 190 to 400 nm. The overlay spectra was obtained using software and the isosbestic point (point at which both the spectra meet and have same absorbance) was selected as the working wavelength for the simultaneous estimation.

2.3 Instrument Used For Method Development

Shimadzu binary HPLC system (LC-10) equipped with Rheodyne injector (20 µL loop), SPD 10 UV detector, hypersil C18 column (4.6 x 150 mm) with 5 µ particle size and Surwit N 2000 data acquisition software was used for the development and validation of the method.

2.4 Preparation of Phosphate Buffer Solution, 0.025 M Standard[11]

Accurately weighed 3.40 g of potassium dihydrogen phosphate and 3.55 g of anhydrous disodium hydrogen phosphate, both previously dried at 110º to 130º for 2 hours, were dissolved in sufficient HPLC water to produce 1000 ml.

2.5 Preparation of Standard Solution

A mixed standard stock solution of bilastine and montelukast was prepared by dissolving 20 mg and 10 mg respectively of bilastine and montelukast in 10 mL methanol. 2.0 mL of this stock solution was withdrawn and diluted with methanol (8 mL) to obtain a solution containing 400 µg/mL and 200 µg/mL of bilastine and montelukast. 1.0 mL of the above solution was further diluted with methanol to produce working standard solution containing bilastine (40 µg/mL) and montelukast (20 µg/mL). The solution was sonicated for 10 min and filtered through 0.45 µ membrane filter before use.

2.6 Tablet Sample For Analysis

Ten tablets were weighed to determine the average weight. The tablets were crushed and finely powdered in a mortar using pestle. Tablet powder equivalent to 20 mg bilastine and 10 mg montelukast was accurately weighed and transferred to volumetric flask and dissolved in 10 mL methanol by sonication for 30 min. The solution was filtered through Watman filter paper and 2.0 mL of this solution was diluted suitably to obtain concentration of bilastine (40 µg/mL) and montelukast (20 µg/mL). The solution was sonicated for 10 min and filtered through 0.45 µ membrane filter syringe filter before use.

2.7 Optimized Conditions

Mobile phase consisted of phosphate buffer (pH 4.5) and acetonitrile in the ratio of 30:70 v/v and chromatographic conditions include:

Column: Octadecylsilane (ODS) (4.6 x 150mm, 5µm, Thermosil)
Flow rate: 0.8 ml per min
Wavelength: 225 nm
Injection volume: 20 µl
Run time: 15 min

2.8 Validation of Method[12]

The method was validated according to ICH Q2B guidelines for accuracy, precision, linearity, limit of detection, limit of quantification and robustness.

2.9 System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

2.10 Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system. The chromatograms were recorded. A study to establish the interference of blank was conducted by injected the mobile phase into HPLC system.

2.11 Linearity

The working standards were prepared at different concentrations by diluting with the diluents. The dilutions were injected in to the HPLC system and analyzed as per the optimized conditions.

2.12 Accuracy

Accuracy of the method was determined by recovery studies. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels (50%, 100%, 150%) of the target assay concentration. The amounts added, amounts estimated and the individual recovery and mean recovery values were calculated

2.13 Precision

2.13.1 Repeatability

The working standard solution was injected in six replicates in the HPLC system and the peak area was measured. The % RSD for the area was calculated.

2.13.2 Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was evaluated on different days by different analysts.

2.14 Limit of Detection And Quantification

2.14.1 Montelukast

A 0.003µg/ml solution was injected to the HPLC system and the LOD was calculated using the signal to noise ratio. For determination of LOQ 0.012µg/ml solution was used.

2.14.2 Bilastine

A 0.09µg/ml solution was injected to the HPLC system and the LOD was calculated using the signal to noise ratio. For determination of LOQ 0.3µg/ml solution was used.

2.15 Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous batches by differing physical parameters like flow rate and mobile phase composition which may differ but the responses would be still within the specified limits of the assay.

3. RESULTS AND DISCUSSION

The solubility analysis was done qualitatively and it was found that bilastine was soluble in water, methanol and acetonitrile whereas montelukast was insoluble in acetonitrile. The overlay spectrum of monelukast and bilastine was obtained using the software attached to the UV spectrophotometer and the isosbestic point (225 nm) was selected as the working wavelength for the simultaneous estimation of both the drugs. The chromatogram was obtained using the optimized conditions (figure 3-5).

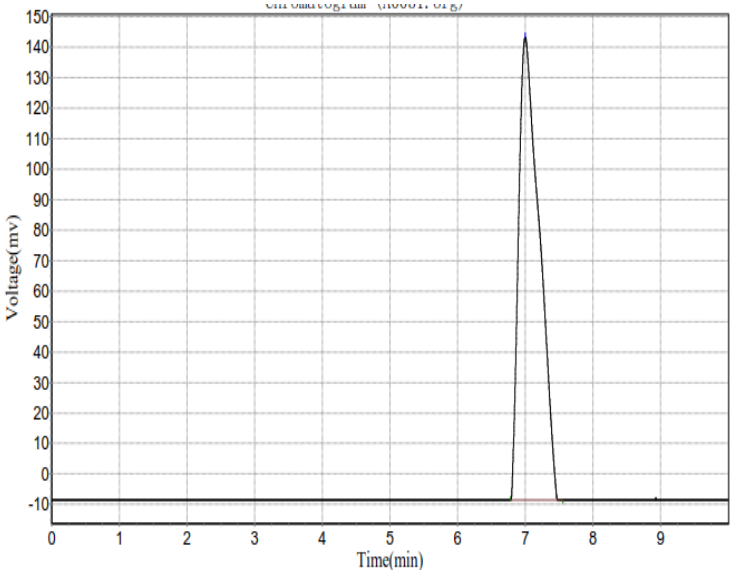

Figure 1. Chromatogram of Montelukast

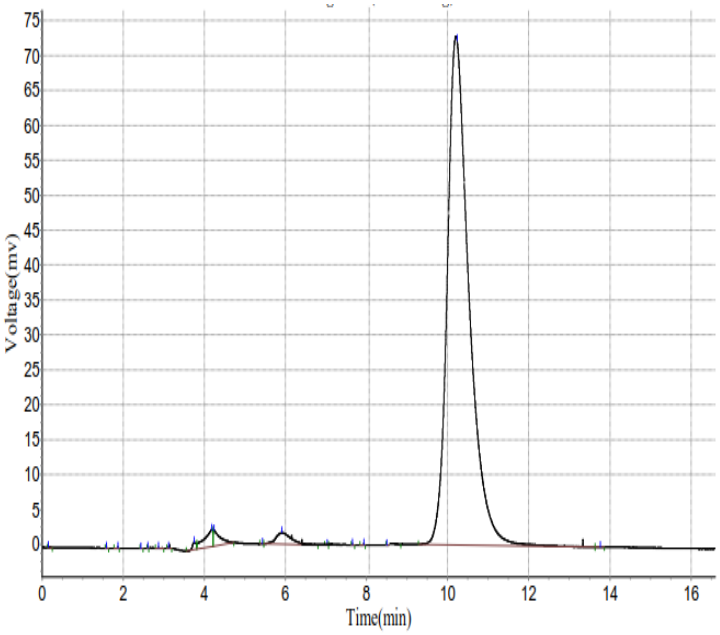

Figure 2. Chromatogram of Bilastine

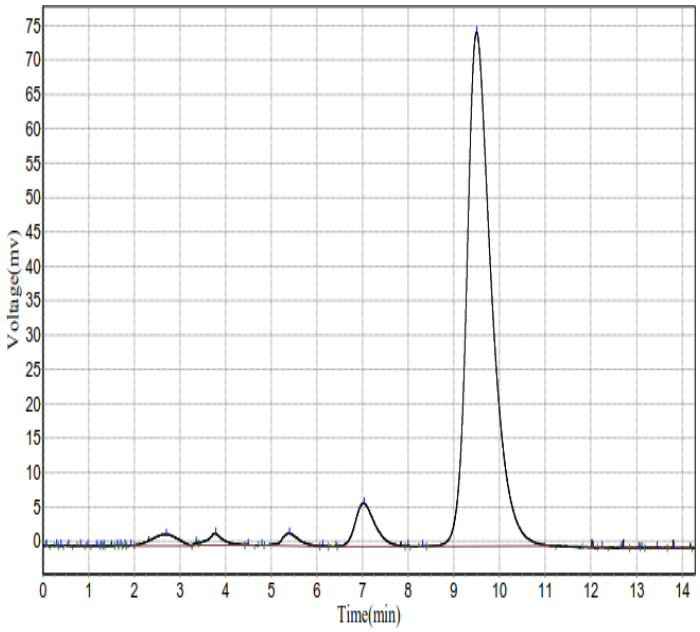

Figure 3. Chromatogram of Montelukast and bilastine combination

3.1 Validation of The Method

3.1.1 System Suitability

The % RSD of retention time was less than 2% and tailing factor and number of theoretical plates were found to be satisfactorily within the limits for both montelukast and bilastine (table 1). Hence the selected system parameters were found to be suitable for the simultaneous estimation.

Table 1. System suitability parameters

System suitability parameters	Bilastine	RSD	Montelukast	RSD
Retention Time	9.50	0.008	7.032	0.016
Tailing Factor	1.28		0.99	
No.of Theoretical plates	7365.33		3460	

3.1.2 Linearity

The linearity range was found to be from 10 to 50 µg/mL for both the drugs (Table 2).

Table 2. Linearity

Concentration (µg/ml)	AUC (Bilastine)	AUC (Montelukast)
10	4240.1	2613.86
20	8463.98	5240.14
30	12502.1	7841.49
40	16760.9	10155.4
50	21111.2	12936
Correl Coeff (r^2)	0.999	0.999
Slope (m)	420.3	255.6
Intercept (c)	3.917	89.51

3.1.3 Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The spiked sample was recovered within a range of 98-102% justifying the accuracy of method in estimating the concentration of the drugs of mixture (Table 3).

Table 3. Accuracy

Conc. of montelukast in tablet sample □g/ml)	Conc. of montelukast added to final (□g/ml)	% Recovered (mean)	Conc. of bilastine in tablet sample □g/ml)	Conc. of bilastine added to final (□g/ml)	% Recovered (mean)
20	10	100.317	40	20	99.408
20	20	98.665	40	40	100.083
20	30	99.048	40	60	99.941

3.1.4 Precision

The precision of the method depicts its ability to reproduce the results irrespective of the day of analysis, the analyst or even the instrument used for analysis. The results of repeatability and intermediate precision are reported in table 4 and table 5 respectively.

Table 4. Repeatability of the developed method

Concentration (□g/ml)	Montelukast (20 µg/mL)		Bilastine 40 (µg/mL)	
	Retention time	Peak Area	Retention time	Peak Area
Mean (n =6)	7.030	5241.667	9.560	16889.167
SD	0.044657213	33.67887567	0.157304058	240.2943334
%RSD	0.635267859	0.64252227	1.645468624	1.422771994

3.1.5 Robustness

The deliberate changes in flow rate and mobile phase composition were made in order to study the effect of the same 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. Intermediate precision

Concentration (□g/ml)	Montelukast (20 µg/mL)		Bilastine 40 (µg/mL)	
	Retention time	Peak Area	Retention time	Peak Area
Mean (n =6)	7.002	5243.167	9.579	17069.167
SD	0.053560246	35.99120263	0.050017664	135.5011685
%RSD	0.764982453	0.686440179	0.522150468	0.793835875

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 produced consistent results.

3.1.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the signal to noise ratio method. The average base line noise obtained from the blank run (mobile phase injection) was found to be 54 µV whereas the signal obtained from LOD solution (0.25% of target assay concentration for montelukast and 0.3% assay concentration for bilastine) was found to be 152 and 155 µV respectively.

S/N = 152/54 = 2.81 (for montelukast)
S/N = 155/54 = 2.87 (for bilastine)

The S/N Ratio value shall be 3 for LOD solution.
The signal obtained from the LOQ solution (1.0% of target assay concentration) was found to be 571 µV.

S/N = 521/54 = 9.64 (for montelukast)
S/N = 524/54 = 9.70 (for bilastine)

The S/N Ratio value shall be 10 for LOQ solution.

3.2 Application of The Method To Marketed Formulation

The developed and validated method was applied for the analysis of the marketed formulation of bilastine and montelukast and the results obtained are presented in table 6. The percentage assay of montelukast and bilastine were found to be 100.3 % and 99.45 % respectively

Table 6. Results of assay of marketed formulation

Brand name & label content	Amount found (mg)*		Percentage recovery	
	Montelukast	Bilastine	Montelukast	Bilastine
Bilafav M tablet	10.03	19.89	100.3	99.45

* Average of three replicate values

4. CONCLUSION

The investigation resulted in the development of a new RP – HPLC method for the simultaneous estimation of bilastine and montelukast in tablet formulations. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis. The method could be easily used with accurate results for routine estimation of the above combination in dosage forms.

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