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Development and Validation RP-HPLC Method for Simultaneous Estimation of Bilastine and Montelukast in Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, precise, sensitive, and rapid reverse phase high-performance liquid chromatography method was developed and validated for simultaneous estimation of bilastine and montelukast in bulk as well as in tablet formulation according to ICH guidelines. The chromatographic phase consisted by methanol and acetonitrile (70:30) at pH 3 adjusted by 0.1% orthophosphoric acid. The flow rate was adjusted to 1 ml/min and ultraviolet detection was carried out at 260 nm. The retention time for of bilastine and montelukast were found to be 3 and 7 min, respectively. The detector was showed linear responses over the concentration range 25–150 µg/ml for bilastine and 5–30 µg/ml for montelukast a good correlation coefficient of 0.999. This proposed method is highly sensitive, precise, and accurate which reduces cost of analysis, hence recommended for routine quality analysis in laboratories.

Keywords: Bilastine, Montelukast, Simultaneous estimation, Validation

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Introduction

Montelukast so is (*R*,*E*)-2-(1-((1-(3-(2-(7-Chloroquinolin-2-yl)vinyl) phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl) cyclopropyl)acetic acid as shown in Figure 1. Montelukast is freely soluble in ethanol, methanol, water, and practically insoluble in acetonitrile.[1] Montelukast is a selective, potent and orally active antagonist of the cysteinyl, CysTL1, and leukotriene receptor used for the treatment of asthma in children and adults.[2-4] Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the broncho constriction otherwise caused by the leukotriene and results in less inflammation. Due to its method of operation, it is not useful for the treatment of acute asthma attacks. Again due to its very specific locus of operation, it does not interact with other allergy medications such as theophylline. The literature survey reveals several methods for montelukast such as analytical method development and validation by reverse phase high-performance liquid chromatography (RP-HPLC),[5,6] stability indicating HPLC, HPLC-coupled with electrospray ionization mass spectrometry/MS,[7] capillary electrophoresis,[8] derivative spectroscopy,[9] HPLC simultaneous estimation with other drugs,[10,11] Thin layer chromatography (TLC)-Densitometry,[12] HPLC and HPTLC,[13,14] liquid-liquid extraction method using HPLC with fluorescence detector,[15] and derivative spectrophotometry.[16]

Bilastine is 2-[4-(2-{4-[1-(2-Ethoxyethyl)-1H-benzimidazol-2-yl]-1-piperidinyl}ethyl)phenyl]-2-methylpropanoic acid as shown in Figure 2, very soluble in methylene chloride, and sparingly soluble in methanol. It is used in antihistaminic treatment.[17] Bilastine, a piperidine derivative, is a long-acting, non-sedating, second-generation histamine receptor antagonist that binds preferentially to peripheral H1 receptors. Bilastine is a new, well-tolerated, and non-sedating H1 receptor antihistamine. In the fasting state, bilastine is quickly absorbed, but the absorption is slowed when it is taken with food or fruit juice. Therefore, it is recommended that bilastine is taken at least 1 h before and no sooner than 2 h after a meal. Clinical studies sponsored by the manufacturer have shown that bilastine 20 mg once daily is as efficacious as other non-sedating antihistamines in allergic rhino conjunctivitis and chronic urticaria in individuals from 12 and 18 years of age, respectively. The literature survey for bilastine revealed several methods, such as individual determination of bilastine by HPLC,[18,19] stability indicating LC Method,[20] HPTLC,[21] spectrofluorimetry,[22] HPLC simultaneous estimation with other drugs,[23] simultaneous spectrophotometric estimation,[24] spectrophotometric absorption ratio method,[25] and HPLC-diode-array detection.[26] The literature survey reveals several methods for simultaneous determination of ebastine and montelukast by HPLC,[27-30] but this present work describes new and kinetic validated RP-HPLC method with different retention time.

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Materials and Methods

Materials

Reference standards for bilastine and montelukast were gifted by Micro Labs Pvt. Limited, Pondicherry. Pharmaceutical preparation

for combination of bilastine and montelukast was obtained from local market. Methanol and acetonitrile of analytical reagent grade and HPLC grade, orthophosphoric acid, and water of HPLC grade were purchased from Merck Ltd., India.

Chromatographic Conditions

The analysis of drugs was carried out on a Shimadzu LC isocratic system, prominence, and equipped with an manual sampler and photodiode-array detection (PDA) detector was used for the analysis. The data were recorded using LC-solution software. Hypersil C18 (10 um, 150 × 4.6 mm) was used for the analysis. A ultrasonicator was used for degassing of the mobile phase. In this RP-HPLC method, separation was carried out using a mobile phase consisting of HPLC methanol and acetonitrile (70: 30) at pH 3 adjusted by 0.1% orthophosphoric acid. The mobile phase was filtered using a 0.45 µm nylon membrane filter. The analysis was carried out room and the flow rate was 1 ml/min. The Optimized chromatographic conditions were shown in Table 1.

Preparation of stock and working standard solutions

Weighed accurately 100 mg of bilastine and montelukast and transferred to separate volumetric flask of 100 ml. Sufficient amount of mobile phase was added and drugs were dissolved to give a stock solution of 1 mg/ml each; then, it is was sonicated for 10 min. Working standard solutions were prepared to get the linearity range from 10–250 µg/ml to 5–30 µg/ml, respectively.

Preparation of Test Solution

Weighed accurately powdered tablet equivalent to 20 mg of bilasitne and montelukast, transferred to volumetric flask, and mixed it well with 20 ml of mobile phase to prepare 1000 µg/ml. From above, solution is further subjected for dilution get a solution containing 25 µg/ml of bilastine and montelukast each. The solutions were chromatographed using the HPLC conditions described above and the concentrations of bilastine and montelukast were calculated.

System Suitability

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed RP-HPLC method permits the determination of bilastine and montelukast in sample drug have different retention times. System suitability data are given in Table 2.

Determination of Purity

The amounts of bilastine and montelukast per tablet were calculated by extrapolating the value of area from the calibration curve using ultraviolet (UV) detection at is absorptive point of 268 nm. Procedure was repeated 6 times with the same tablet formulation. Moreover, obtained results are tabulated in Table 3.

Method Validation

The present method of analysis was validated according to the recommendations of ICH-1996[31] and USP-30 for the parameters

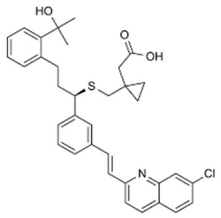

Figure 1: Chemical structure for montelukast sodium

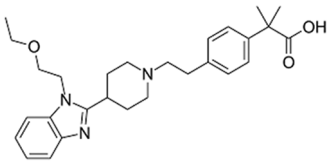

Figure 2: Chemical structure for bilastine

Table 1 : Optimized chromatographic conditions

Parameters	*Conditions*
Stationary phase	Hypersil C18 (10 um, 150×4.6 mm)
Mobile phase	Methanol and Acetonirile (70:30 v/v)
Flow rate (ml/min)	1
Run time (min)	10
Column temperature (°C)	Room temperature
Injection volume (µl)	10
Detection wavelength (nm)	260 nm
Retention time of bilastine (min)	3
Retention time of and montelukast (min)	7

Table 2: System suitability parameters

S. No.	*System suitability parameters*	*Bilastine*	*Montelukast*
1	Retention time (Min)	3	7
2	Theoretical plates	5302.5	3545.4
3	Area under curve	659.13	690.34
4	Tailing factor	0.96	1.16

such as specificity, system suitability, accuracy, linearity, precision, robustness, limit of detection (LOD), and Limit of quantification (LOQ).

Specificity

It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resoluted from any other peak by resolution of minimum 2. This could be done injecting placebo and compare it with that of standard and placebo spiked with standard and sample; then, peak purity was ascertained by use of PDA.

Linearity

The response for the detector was determined to be linear over the range of 10–250 µg/ml for. The response for the detector was determined to be linear over the range of 5–30 µg/ml for montelukast linearity curves which were constructed using relative peak area to avoid very high value for intercept.

Table 3: Assay of bilastine and montelukast sodium

S. No.	Amount present (mg)		Amount found in mg		% Label claim	
	Bilastine	Montelukast	Bilastine	Montelukast	Bilastine	Montelukast
1	20	20	19.906	19.902	99.10	99.03
SD					1.235	0.894
% RSD					1.246	0.903

*All the results were average of 6 readings, (*n*=6)

Precision

The precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision was determined by repeatability, interday and intraday experiments.

Accuracy

Accuracy was calculated by addition of standard drugs to pre-analyzed sample at three different concentration levels (50%, 100%, and 150%) and computing percentage recoveries. Standard limit of % recovery study is 98–102% as per ICH guideline. From the studies, it was concluded that % recovery study of bilastine and montelukast complies with standard limit of ICH guideline.

Robustness

The robustness is the capacity of method to remain unaffected by small but deliberate changes in chromatographic conditions. Robustness was studied by testing the influence of small changes in column temperature (±5°C), change in flow rate (±10%), and changes in mobile phase composition (70:30).

Quantification limit

LOD and LOQ for the optimized method were performed as per ICH guidelines. LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analytes in standards that can be reproducibly measured with acceptable accuracy and precision. LOD and LOQ were separately determined at a signal to noise ratio (S: N) of 3: 10 and which was based on calibration curve. The standard deviation of y intercept and slope of the regression line were used. The LOD and LOQ were calculated using following formulas LOD = 3.3 × D/S and LOQ = 10 × D/S

Where, S = Slope of regression line, D = Standard deviation of y – intercept on the regression line.

Results and Discussion

The proposed RP-HPLC method required fewer reagents and materials, standard solution of bilastine, and montelukast were injected to get a chromatogram. There was clear resolution between bilastine and montelukast with retention time of 3 and 7 min, respectively. Chromatogram of bilastine and montelukast is shown in Figure 4.

Specificity

In general, the specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 260 nm to demonstrate the specificity of the optimized method. A comparison of the retention times of bilastine and montelukast in sample solutions and in the standard solutions was exactly the same. Figures 3–5 showed that there were no interferences at the retention times for bilastine and montelukast due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in tablet formulation.

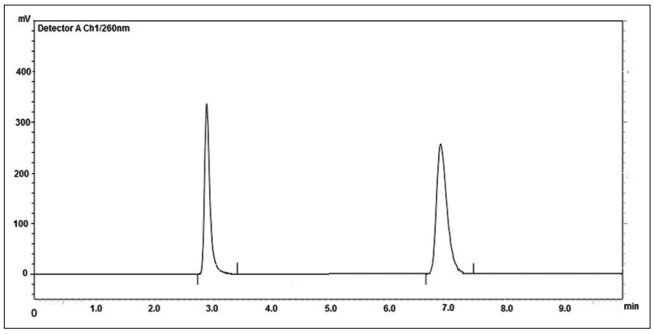

Figure 3: Reverse phase high-performance liquid chromatography chromatogram of bilastine and montelukast standard drug

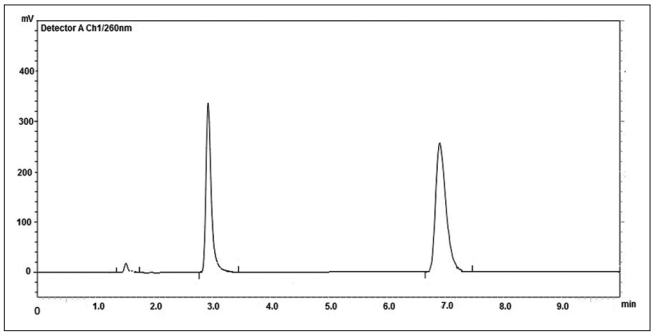

Figure 4: Reverse phase high-performance liquid chromatography chromatogram of bilastine and montelukast sample drug

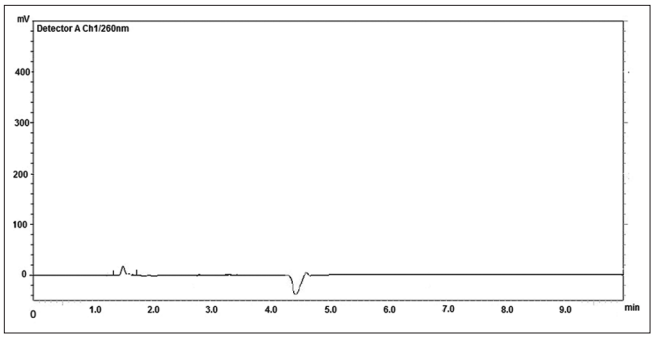

Figure 5: Reverse phase high-performance liquid chromatography chromatogram of chromatogram of placebo

Linearity (calibration curve)

The response for the detector was determined to be linear over the range of 10–250 µg/ml for bilastine, as shown in Figure 6 and data are shown in Table 4. The response for the detector was determined to be linear over the range of 5–30 µg/ml for montelukast, as shown in Figure 7 and data are shown in Table 5.

Each of the concentration was injected in triplicate to get reproducible response calibration curves which were constructed by plotting peak area verses concentration in µg/ml. Each reading was average of three determinations. They were represented by the linear regression equation. Y Bilastine = 11174x + 228.75, r^2 = 0.9997

Y Montelukast = 44175x + 35245, r^2 = 0.9998

Slopes and intercepts were obtained using regression equation (Y = mx + c) and least square treatment of the results used to confirm linearity of the method developed.

Accuracy

Accuracy was calculated by addition of standard drugs to pre-analyzed sample at three different concentration levels (50%, 100%, and 150%) and computing percentage recoveries. Standard limit of % recovery study is 98–102% as per ICH guideline. From the studies, it was concluded that % recovery study of bilastine and montelukast complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 6 and % RSD is 0.2261 and 0.3570 of bilastine and montelukast, respectively, which is within the acceptable limit (<2.0).

Intraday Precision

Sample stock solution containing 50, 150, and 250 µg/ml of bilastine and 10, 20, and 30 µg/ml montelukast was prepared from their respective solution. Analysis was performed in triplicate; the result of intraday precision studies was shown in Table 7.

Interday Reproducibility (Method Ruggedness)

Three replicates of a different concentration of sample solution are used for each determination. 1st day: 3 replicates, on a 2nd day: 3 replicates, then on 3rd day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions. The result of interday reproducibility and percentage amount obtained is shown in Table 8.

Quantification Limit

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of bilastine and montelukast found to be 3.99 µg/ml and 0.98 µg/ml respectively. The LOQ of bilastine and montelukast found to be 12.109 µg/ml and 2.994 µg/ml respectively. The result obtained is shown in Table 9.

Stability of Analytical Solution

The stability of analytical solutions was established by injecting the standard solution and sample solution at different interval of time up to 8 h (0, 4, and 8 h) at room temperature (25°C). The % differences of peak area of standard solution and sample solution that were injected at periodic intervals found to be the specified limit. The values are presented in Tables 10 and 11.

Table 4: Statistical data of calibration curves of bilastine

S. No.	Stock solution taken in ml	Concentration (µg/ml)	Average Peak area (n=3)
1	0.1	10	112751
2	0.5	50	563712
3	1.0	100	1129836
4	1.5	150	1644186
5	2.0	200	2239673
6	2.5	250	2803359

Regression coefficient=0.9997

Table 5: Statistical data of calibration curves of Montelukast

S. No.	Stock solution taken in ml	Concentration (µg/ml)	Average Peak area (n=3)
1	0.05	5	234538
2	0.1	10	455142
3	0.15	15	709710
4	0.2	20	922797
5	0.25	25	1157345
6	0.3	30	1356010

Regression coefficient=0.9998

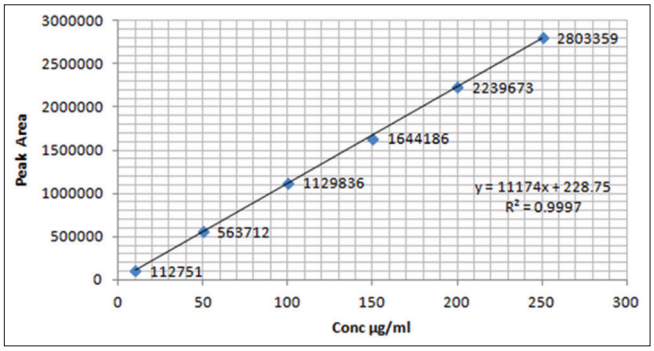

Figure 6: Calibration curve of bilastine

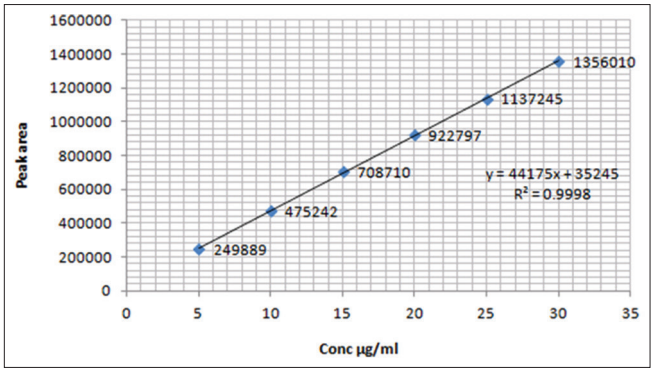

Figure 7: Calibration curve of montelukast sodium

System Suitability

The system suitability was determined by injecting of six replicates the standard solutions and analyzing each active ingredient for its

Table 6: Results of accuracy for bilastine and montelukast

Different concentration levels (%)	Bilastine				Montelukast			
	Amount of drug spiked (µg)	Conc. (sample sol) µg/ml	Amount found	% Recovery (n=3)	Amount of drug spiked (µg)	Conc. (sample sol) µg/ml	Amount found	% Recovery (n=3)
50	25	50	24.94	99.76	12.5	25	12.43	99.44
100	50	50	50.12	100.24	25	25	24.92	99.68
150	75	50	74.99	99.98	37.5	25	37.52	100.05
Average recovery				99.99				99.72
SD				0.240278				0.3073
%RSD				0.240294				0.308153

Table 7: Intraday precision data of bilastine and montelukast

S. No.	Bilastine			Montelukast		
	Peak area			Peak area		
	Sample – 1	Sample –2	Sample –3	Sample – 1	Sample – 2	Sample –3
1.	554732	1645220	2799496	449892	923435	1343683
2.	563543	1599876	2814532	468768	919841	1355431
3.	561564	1632443	2813976	459324	929839	1359834
Average	559946.3	1625846	2809335	459328	924371.7	1352983
SD	4622.885	23380.69	8525.069	9438.00064	5064.386	8349.219
%RSD	0.825594	1.438063	0.303455	2.05474098	0.547873	0.617097

Table 8: Interday reproducibility data of bilastine and montelukast

Sample No	Assay (% labeled amount)					
	Bilastine			Montelukast		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
Sample –1	98.41	99.97	99.61	98.84	102.99	100.91
Sample –2	100.01	97.30	97.30	100.06	99.67	100.76
Sample –3	99.87	100.40	100.37	99.09	99.95	100.28
Average	99.43	99.22333333	99.09333	99.33	100.87	100.65
SD	0.886115117	1.679474124	1.598885	0.644438	1.841304	0.32908965
%RSD	0.891194928	1.69262014	1.613514	0.648785	1.825423	0.32696438

Table 9: Results of LOD and LOQ

Sample	LOD (µg/ml)	LOQ (µg/ml)
Bilastine	3.99	12.109
Montelukast	0.98	2.99

LOD: Limit of detection, LOQ: Limit of quantification

Table 10: Stability of standard and sample solution of bilastine

Time interval (h)	Standard		Sample	
	Std Peak Area	% Difference	Sample Peak Area	% Difference
0	1645221	-	1645225	-
4	1645233	0.01	1645234	0.01
8	1645227	0.01	1645229	0.01

Table 11: Stability of standard and sample solution of montelukast

Time interval (h)	Standard		Sample	
	Std Peak Area	% Difference	Sample Peak Area	% Difference
0	923435	-	923437	-
4	923436	0.02	923438	0.02
8	923439	0.03	923429	0.02

peak area, peak tailing factor, resolution, number of theoretical plates, and capacity factor. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations system suitability parameters might be fall within ±2% standard deviation range during routine performance of the methods.

Discussion

In his study reported, HPLC method for simultaneous estimation of bilastine and montelukast showing linearity range at 5–35 µg/ml, 1.2 ml/min flow rate, etc., this indicating scope to minimize total cost of analysis.

Conclusion

Proposed study describes HPLC method for the simultaneous estimation of bilastine and montelukast in bulk as well as in tablet formulation. This method showed good separation of two compounds with less retention time than any method listed in reference. This method is useful to minimize total cost of analysis. The method is validated and found to be simple, sensitive, accurate, precise, and robust. Hence, proposed method is suggested for routine quality analysis of bilastine and montelukast in laboratories.

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