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Research Article

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

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Bilastine and Montelukast in bulk and Tablet dosage form. Chromatogram was run through Std kromasil 250 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.01N Ammonium acetate: Acetonitrile taken in the ratio 70:30 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was Ammonium acetate buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 220.0 nm. Retention time of Bilastine and Montelukast were found to be 2.645min and 3.797 min. %RSD of the Bilastine and Montelukast were found to be 1.2% and 1.7% respectively. %Recovery was obtained as 99.74% and 99.65% for Bilastine and Montelukast respectively. LOD, LOQ values obtained from regression equations of Bilastine and Montelukast were 0.31, 0.09 and 0.94, 0.26respectively. %Assay was obtained as 100.88% and 99.54% for Bilastine and Montelukast respectively. Regression equation of Montelukast is y =25730x + 4354, y = 20467x + 3190 of Bilastine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that it can be adopted in regular Quality control tests in Industries.

Key Words: Montelukast, Bilastine, HPLC

INTRODUCTION

Montelukast is a leukotriene receptor antagonist (LTRA) used for the treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. Bilastine is a novel new generation antihistamine. It is a selective histamine H1 receptor antagonist. Bilastine binds and prevents activation of H1 receptor, it reduces the development of allergic symptoms due to release of histamine from mast cells. Literature review revealed that there were few analytical methods reported for Montelukast

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and bilastine in RP-HPLC. An extensive literature search didn't reveal any method for simultaneous estimation of Montelukast and Bilastine in API and pharmaceutical dosage form by RP-HPLC. Therefore, an attempt has been made to develop and validate simple, precise, accurate, economical RP-HPLC method as per ICH guidelines for the estimation of Montelukast and Bilastine in Bulk and Pharmaceutical Dosage Form.

MATERIALS AND METHODS

Chemicals and Reagents: Acetonitrile (HPLC grade), Methanol, Phosphate buffer, Potassium dihydrogen ortho phosphate buffer, Ortho phosphoric acid, Distilled water (HPLC grade) were purchased from Rankem, India. Montelukast and Bilastine combination dosage form(tablet) were purchased from local market. All active pharmaceutical ingredients (APIs) of Montelukast and Bilastine reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions

Electronics Balance -Denver, PH meter-BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG InstrumentsT60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Montelukast and Bilastine solutions. The mobile phase used was Ammonium acetate: Acetonitrile (70:30) at a flow rate of 1ml/min, samples were analyzed at 220 nm detector wavelength and at an injection volume of 10 µL using Kromasil C18 (4.6 mm x 250mm,5 µm) with run time of 6min.

Method

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions:

5mg of montelukast,10mg of bilastine was accurately weighed and was transferred into 50ml volumetric flasks and 3/4th volume of diluent was added to the flask and sonicated for 10min.Volume was made up with diluent. (100 µg/ml of montelukast and 200 µg/ml bilastine)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into 10 ml volumetric flask and volume made up with diluent. (10 µg/ml of Montelukast and 20 µg/ml of Bilastine)

Preparation of Sample stock solutions: 10 tablets were accurately and average weight equivalent to 1 tablet was transferred into a 100ml volumetric flask,50ml of diluent was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (100 µg/ml of montelukast and 200 µg/ml bilastine)

Preparation of Sample working solutions (100% solution):1ml of sample stock solution was filtered and was transferred to 10ml volumetric flask and volume was made up with diluent. (10 µg/ml of Montelukast and 20 µg/ml of Bilastine)

Preparation of Buffer:

0.1% Ortho Phosphoric Acid (OPA) Buffer: 1ml of ortho phosphoric acid was taken and was diluted to 1000ml with water (HPLC grade)

Buffer:0.01N Ammonium acetate: 0.77gm Ammonium acetate was accurately weighed and in 1000ml volumetric flask and to it about 900ml of milli-Q water was added and degassed to sonicate and finally volume was made up with water to get 0.01N Ammonium acetate buffer.

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Stock solutions of Montelukast and Bilastine is taken into 6 different volumetric flasks and diluted to 10 ml with diluents. Linearity solutions are prepared such that 025, 0.5, 0.75,1,1.25, 1.5ml.

Accuracy: Preparation of Standard stock solutions: Accurately weighed 5mg of Montelukast, 10mg of Bilastine and transferred into 50ml volumetric flasks, $3/4^{th}$ of diluents was added to these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution.

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Montelukast, Bilastine solutions respectively were transferred to 10ml volumetric flasks and volume made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two Standard stock solution was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml each of Montelukast, Bilastine solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Montelukast (10ppm) and Bilastine(20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Assay of Montelukast and Bilastine Combination Dosage Form (BillargicM): Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable-HPLC method for simultaneous estimation of Montelukast and Bilastine in Bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1. The final analysis was performed by using 70% 0.01N Ammonium acetate:30%Acetonitrile, at a flow rate of 1ml/min, samples were analyzed at 220nm detector wavelength and at an injection volume of 10µL using Kromasil C18 (4.6, 250mm, 5µm) with run time of 6min.The proposed method was optimized to give sharp peaks with good resolution, the optimized chromatogram was obtained as shown in(Figure-2).

Validation:
Linearity was established at six different concentrations of Bilastine (15-30µg/ml) and Montelukast (2.5-15µg/ml) each were injected in duplicate manner. Average areas were determined and linearity equations obtained for Montelukast was y=20467x+3190.4 and of Bilastine was y=25730x + 4354. Correlation coefficient obtained was 0.999 for two drugs. The Linearity calibration curves were plotted as shown in Figure-4&5. Retention time of Montelukast was 4.106 min and Bilastine was 2.875 min. No interfering peaks in blank and placebo were found in this method. So this method holds its specificity.

Three levels of accuracy samples 50%, 100%, 150% were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % recovery was obtained as 100.67% and 99.65% for Bilastine and Montelukast respectively was shown in (Table-2&3).

%RSD for system precision for Bilastine was 1.2% and for Montelukast was 1.7%. The % RSD for repeatability for Bilastine was 0.4% and for Montelukast was 0.9%. The % RSD for intermediate precision for Bilastine and Montelukast was 1.4%. Since % RSD is less than 2 the system precision was passed in this method shown in (Table-4).

The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curves. The Detection limit value for Bilastine was 0.31 and for Montelukast was 0.09. The Quantitation limit value for Bilastine was 0.94 and for Montelukast was 0.26 as given in (Table-5). Robustness conditions like Flow minus(0.9ml/min), Flow plus (1.1ml/min), Mobile

phase minus (75B:25A), Mobile phase plus (65B:35A), Temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. (Table-6)

System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. (Table-7).Bilastine and Montelukast pure drug (API) was obtained from Spectrum Pharma research solutions. BillargicM bearing the label claims Montelukast 40mg and Bilastine 8mg. Assay was performed with the formulation. Average % Assay for Bilastine and Montelukast obtained was 100.88% and 99.54% respectively the result was shown in (Table-8) and the chromatogram of standard drugs and pharmaceutical dosage form were shown in (Figure 6&7) respectively.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Bilastine and Montelukast. Chromatographic conditions used are Kromasil C18 (4.6,250mm,5µm). Mobile phase 0.01NAmmonium acetate: acetonitrile in the ratio of 70:30 and flow rate was maintained at 1ml/min, detection wavelength was 220nm, column temperature was set to 30^0C. Retention time of Bilastine and Montelukast were found to be 2.875min and 4.106 min. %RSD of the Bilastine and Montelukast were and found to be 1.2% and 1.7% respectively. %Recovery was obtained as 99.74% and 99.65% for Bilastine and Montelukast respectively. LOD, LOQ values obtained from regression equations of Bilastine and Montelukast were 0.31, 0.09 and 0.94, 0.26respectively. %Assay was obtained as 100.88% and 99.54% for Bilastine and Montelukast respectively. Regression equation of Montelukast is y =25730x + 4354, y = 20467x + 3190 of Bilastine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

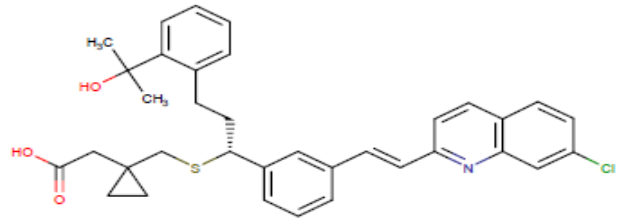

Figure-1: Chemical structure of Montelukast

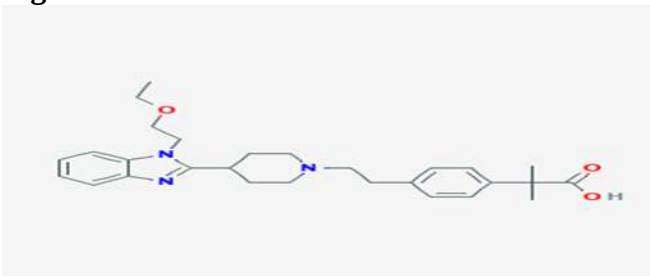

Figure-2: Chemical structure of Bilastine

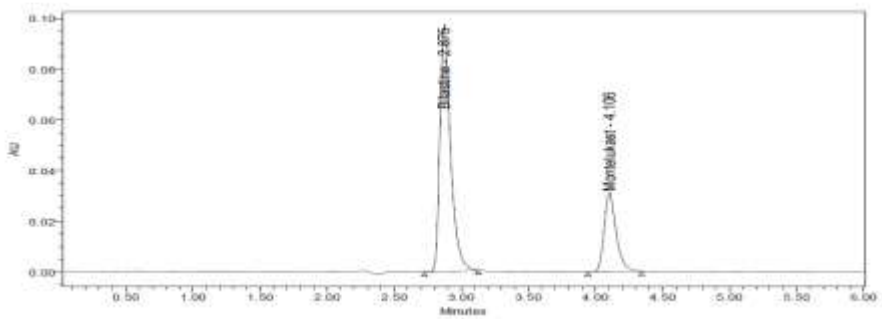

Figure-3: Optimized Chromatogram of Montelukast and Bilastine

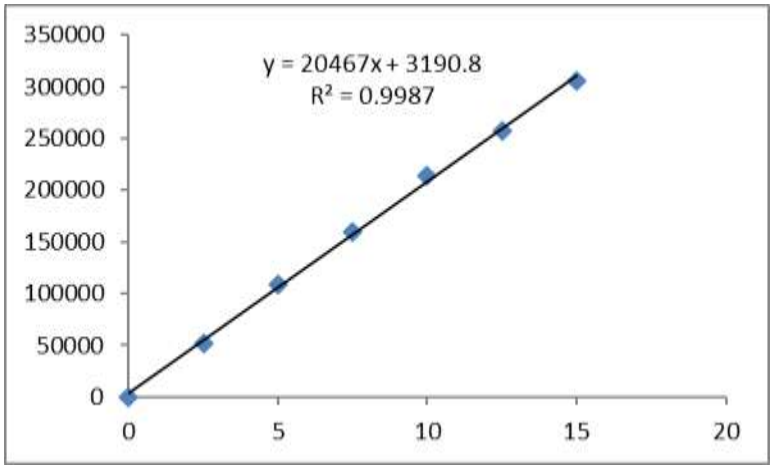

Figure-4: Linearity curve of Montelukast

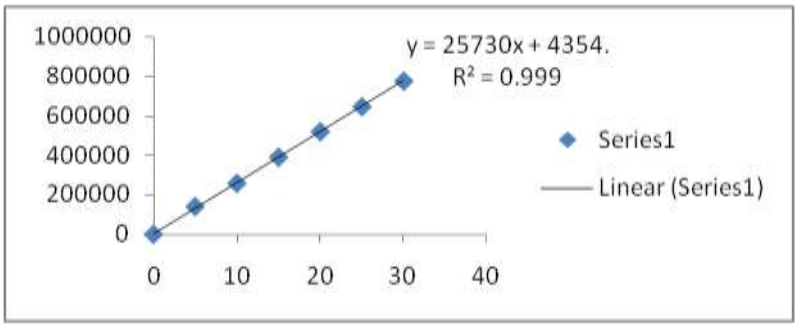

Figure-5: Linearity curve of Bilastine

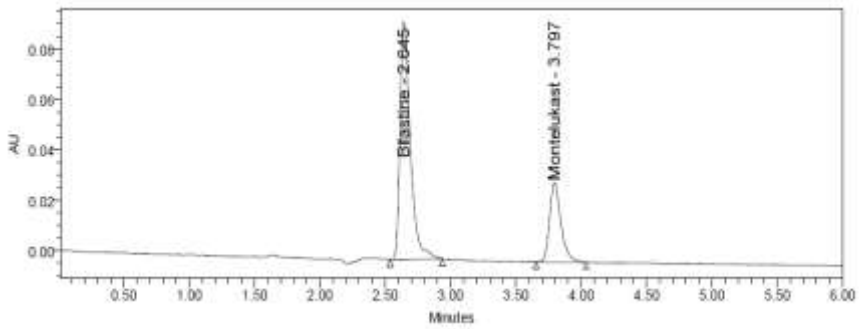

Figure-6 Standard Chromatogram of Montelukast and Bilastine

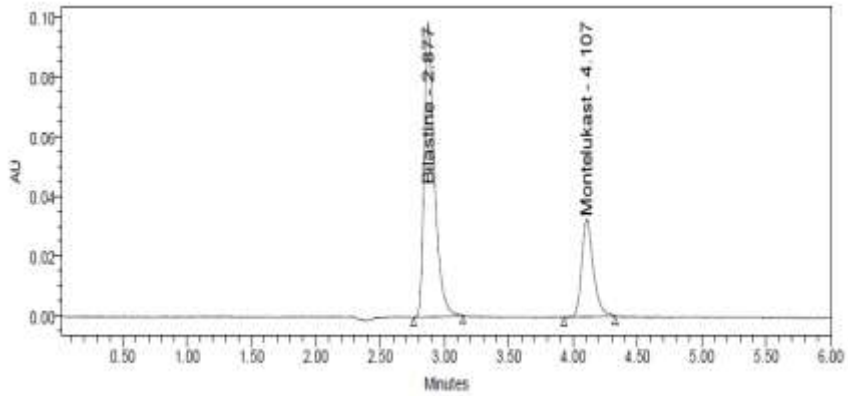

Figure-7 : Sample Chromatogram of working solutions

Table-1: Optimized Chromatographic Conditions

Parameters	Conditions
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	70% 0.01N Ammonium acetate:30% Acetonitrile
Flow rate	1ml/min
Column	Kromasil C18(4.6x250mm,5µm)
Detector wavelength	220nm
Column temperature	30^0C
Injection volume	10µL
Run time	6 min
Diluent	Water:Acetonitrile(50:50)
Retention Time	Bilastine 2.875 min and Montelukast 4.106 min
Theoritical plates	Bilastine - 6605, Montelukast- 10640

Table-2: Accuracy results of Bilastine

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	10	9.9	98.8	100.57%
	10	10.0	99.7	
	10	9.9	99.0	
100%	20	20.0	99.8	
	20	19.9	99.5	
	20	20.0	100.2	
150%	25	25.1	100.6	
	25	25.1	100.6	
	25	24.9	99.4	

Table-3 Accuracy results of Montelukast

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	5	5.01	100.23	99.65%
	5	4.96	99.19	
	5	4.98	99.58	
100%	10	9.90	99.02	
	10	10.00	100.01	
	10	9.93	99.25	
150%	15	15.28	101.86	
	15	14.75	98.34	
	15	14.90	99.36	

Table-4 Precision results of Montelukast and Bilastine

S.No	Montelukast		Bilastine	
	Repeatability	**Intermediate precision**	**Repeatability**	**Intermediate precision**
1.	201638	195046	515370	519363
2.	199495	202393	518685	518398
3.	202115	197206	517073	500197
4.	204074	197155	519927	514083
5.	203530	201061	521762	510236
6.	204207	198001	520117	506472
Mean	202510	198477	518822	511458
S.D	1809.6	2734.0	2303.7	7358.1
%RSD	0.9	1.4	0.4	1.4

Table-5 LOD and LOQ values of Montelukast and Bilastine

Molecule	LOD	LOQ
Bilastine	0.31	0.94
Montelukast	0.09	0.26

Table-6 Robustness values of Montelukast and Bilastine

S.no	Condition	% RSD of Bilastine	% RSD of Montelukast
1.	Flow rate(-) 0.9ml/min	0.6	1.6
2.	Flow rate(+)1.1ml/min	1.7	1.3
3.	Mobile phase(-)75B:25A	1.4	0.5
4.	Mobile phase(+)65B:35A	0.9	0.7
5.	Temperature(-)25^{0}C	1.7	1.2
6.	Temperature(+)35^{0}C	0.9	1.6

Table-7 System suitability parameters for Bilastine and Montelukast

S.no	Bilastine			Montelukast			
Inj	**RT(min)**	**USP plate count**	**Tailing**	**RT(min)**	**USP plate count**	**Tailing**	**RS**
1.	2.645	6115	1.52	3.797	9576	1.30	7.6
2.	2.691	6349	1.52	3.865	9977	1.26	7.9
3.	2.839	6680	1.47	4.067	10784	1.31	8.1
4.	2.851	6161	1.50	4.091	10363	1.29	7.9
5.	2.872	6605	1.52	4.101	10640	1.31	7.9

Table-8 Assay results of Bilastine and Montelukast

S.no	Bilastine (% Assay)	Montelukast (%Assay)
1.	100.21	99.11
2.	100.86	98.06
3.	100.54	99.34
4.	101.10	100.31
5.	101.46	100.04
6.	101.14	100.37
Avg	100.88	99.54
Stdev	0.45	0.89
%RSD	0.4	0.89

Table-9 Degradation data of Bilastine and Montelukast

S.no	Bilastine			Montelukast		
	Degradation Condition	**% Drug Degraded**	**%Drug Remained**	**Degradation Condition**	**%Drug Degraded**	**% Drug Remained**
1.	**Acid**	5.96	94.04	**Acid**	5.49	94.51
2.	**Alkali**	4.60	95.40	**Alkali**	4.47	95.53
3.	**Oxidation**	3.85	96.15	**Oxidation**	3.77	96.23
4.	**Thermal**	2.64	97.36	**Thermal**	2.30	97.70
5.	**UV**	1.26	98.74	**UV**	1.48	98.52
6.	**Water**	0.71	99.29	**Water**	0.89	99.11

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